Veterinary Research Communications (2025) 49:4
https://doi.org/10.1007/s11259-024-10577-9

RESEARCH

Nettle (*Urtica dioica*) supplementation: impact on growth, hematology, immune response, and resilience to *Aeromonas hydrophila* in *Labeo rohita* fingerlings

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Received: 20 June 2024 / Accepted: 21 October 2024 / Published online: 15 November 2024

Abstract

The significance of plant-derived products in aquaculture lies in their potential to offer sustainable alternatives, promoting eco-friendly practices. This study investigated the impact of nettle (*Urtica dioica*) leaves powder on the growth efficiency, hemato-biochemical variables and non-specific immune system of rohu, *Labeo rohita* fingerlings. To achieve this objective, sample average weight (5.23 ± 0.34 g) were categorized into four groups, namely control, T1, T2 and T3 in triplicate and administered diets fortified with nettle in amounts of 0, 1, 3 and 5% respectively for a duration of 60 days. After 60 days of fortified diet, the fish underwent intraperitoneal injection with bacteria (*Aeromonas hydrophila)*, and subsequent relative percentage survival (RPS) was observed. The growth performance, including “weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR) and feed efficiency ratio (FER)”, were notably higher in the T3 group (5%) than in others. The hematological values of White blood cell, hematocrit, and hemoglobin revealed higher levels with a fortified diet. The dietary supplementation of nettle reduced serum cholesterol and glucose concentration, whereas it increased albumin, globulin, and total protein in the fish blood. Enhancements in lysozyme and myeloperoxidase activity were observed in the intervention groups with feed containing nettle supplementation. The nettle diet at a 5% concentration demonstrated a higher RPS than the others following injection with *A. hydrophila*. The findings indicate the potential of nettle as a valuable nutritional supplement for increasing fish immunological reaction and bolstering pathogen resistance.

Keywords *Urtica dioica* · *Labeo rohita* · Immune response · Growth performance · Lysozyme activity

Introduction

In light of the current expansion of the global population, it is imperative to prioritize the production of additional animal protein. The increasing need for fish consumption and the constraints posed by limited natural resources have spurred the worldwide advancement of aquaculture (Naylor et al. 2021). This development can be ascribed to the exceptional quality of aquatic animal protein, lipid level, and beneficial amino acids (Jolly et al. 2023). Among the

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freshwater-farmed fish species, rohu (*Labeo rohita*) is valued for its exceptional meat quality and market demand (Nissa et al. 2022). For the successful and sustainable growth of fish farming, it is imperative to prioritize nutrition principles and implement effective health management practices. This includes a particular emphasis on controlling feeding costs within fish farms (Engle et al. 2020), and considering the main goals of aquaculture, which aim for optimal development and survival rates, investigating the appropriate nutritional requirements for farmed fish becomes notably more vital. Therefore, to attain these objectives, using nutritional supplements becomes imperative. Beyond meeting the nutritional needs of farmed fish, these supplements enhance their immunity, growth and health within the cultivation setting (Gatlin and Yamamoto 2022). The historical utilization of medicinal plants has been intricately woven into the tapestry of human existence. Indeed, during that era, plants constituted the sole therapeutic choices available to humans for managing diseases (Rigos et al. 2024). In the current timeframe, research on using plants in aquaculture has been conducted in different areas of the world. Most of these studies examine the impact of these plants on enhancing growth performance, stimulating appetite, exerting anxiolytic effects, influencing haematology and serum biochemical status, promoting health and immune function improvements, and augmenting resistance against pathogens (Hu et al. 2022; Fazio et al. 2022; Wu et al. 2024; Xu et al. 2024). The rohu, is a commercially significant fish species in Asia, contributing substantially to aquaculture production (Majumder and Saikia 2020). *Aeromonas hydrophila* stands out as one of the highly pathogenic bacteria associated with diseases in freshwater fishes, particularly affecting carp species (Dien et al. 2023). Additionally, rohu is known to be susceptible to infections caused by *A. hydrophila*, a prevalent pathogen in aquaculture settings (Gupta et al. 2021). Under stressful conditions, when the immune system of the fish is already weakening due to poor water quality, overcrowding, temperature fluctuations, handling, or inadequate nutrition, this opportunistic bacterium undergoes a transformation into a pathogenic state (Ofek et al. 2023), resulting in symptoms such as widespread hemorrhages, caudal fin erosion, ulceration, and a pallid gill color. These manifestations contribute to elevated mortality rates, leading to significant economic losses in global aquaculture (Semwal et al. 2023). The studies related to nettle in aquaculture practices, particularly for carp species, are limited. Hence, examining the impact of nettle supplementation on the growth, hematology, immunity, and resistance to bacteria (*A. hydrophila*) in fingerlings of rohu holds great potential for advancing sustainable aquaculture practices.

Studies on the nutritional and therapeutic attributes of nettle reveal its richness in bioactive compounds, including polyphenols, iron, flavonoids, acetylcholine, vitamins, salicylic acid, thymol, histamine, carotenoids, serotonin, sterols, lecithin and formic acid (Bhusal et al. 2022; Devkota et al. 2022). Moreover, it possesses antioxidant, immune-stimulating, antifungal, anti-tumor, anti-irritant and antiviral properties (Awad et al. 2013; Koraqi et al. 2023).

The aim of the research was to evaluate the influence of three different concentrations of nettle leaves powder on growth efficiency, blood profile, and immunity parameters in rohu fingerlings within an experimental setting. Additionally, the research explores the special effects of incorporating dietary nettle supplements on the infection resistance of rohu against *A. hydrophila*.

Materials and methods

Plant preparation

Nettle (*Urtica dioica*) was gathered near Tarki Khel Village, Karak, Khyber Pakhtunkhwa, Pakistan. The plant was authenticated and identified by a botanist from the Department of Botany, University of Sargodha, Pakistan. After collection, the samples underwent washing with distilled water, with subsequent separation of leaves from stems. The separated components were then air-dried in the shade. After this, the plant was milled into a fine particles and kept at temperature (4 °C) prior to use.

Experimental diet preparation

The experimental diet was formulated using locally sourced ingredients, which included nettle powder at concentrations of 1 g kg^{-1}, 3 g kg^{-1}, and 5 g kg^{-1} (Table 1). Initially, all the ingredients were carefully blended together with water, forming a homogenous mixture. This mixture was then shaped into pellets using a manual pelletizer and subsequently dried at room temperature of 25 °C for a duration of 24 h. Once dried, the pellets were placed in an airtight container and stored in a cool, dry environment at 4 °C until needed (Zare et al. 2021). The proximate composition of the examined feed was assessed using the standard AOAC (2000) protocol.

Fish and experimental design

Healthy rohu were sourced from a reputable aquaculture facility in Southern Punjab, Pakistan. They were transported to the aquarium facility at the Research Centre of Gomal University (31.8188° N, 70.8971° E) using newly installed polyethylene tanks equipped with aeration systems. The fish underwent a two-week adaptation period in the new

Table 1 Experimental diet formulation

Basic Ingredients (%)	Control group	Experimental feed		
	Basal	1%	3%	5%
Soybean meal	36.0	36.0	36.0	36.0
Wheat offal	5	5	5	5
Common salt (NaCl)	0.2	0.2	0.2	0.2
Fish meal	24	24	24	24
Vitamin and mineral premix[1,2]	1.5	1.5	1.5	1.5
Palm oil	0.5	0.5	0.5	0.5
$Ca_3(PO_4)_2$	0.3	0.3	0.3	0.3
Maize powder	32.5	31.5	29.5	27.5
Nettle leaves	00	1	3	5
Total	100	100	100	100
Proximate composition				
Crude protein %	30.8	30.43	30.35	30.37
Moisture %	2.2	2.3	2.4	2.4
Crude fiber %	10.1	10.6	10.8	10.4
Crude lipid %	7.74	7.63	7.76	7.66
Ash %	7.73	7.34	7.46	7.54
Gross energy (kj.g^{-1} DM)	19.32	19.37	19.35	19.38

Vitamin premix[1](Per kilogram): Vitamin A (16,000 IU); Vitamin D3 (2200 U); Vitamin E (200 mg); Vitamin K3 (10 mg); Vitamin B1 (20 mg); Vitamin B2 (50 mg); Vitamin B3 (250 mg); Vitamin B6 (15 mg); Vitamin B12 (0.05 mg); Vitamin C (150 mg); Choline chloride (2000 mg); Folic acid (10 mg); Calcium d–pantothenate (40 mg); Biotin (1 mg); Inositol (100 mg)

Mineral prmix[2]: Iron (70.2 mg); Copper (12.7 mg); Manganese (200 mg); Zinc (80 mg); Iodine (2.5 mg); Selenium (0.312 mg); Cobalt (1 mg)

environment while provided with a basal diet. After acclimation period the fish average weight (5.23 ± 0.34 g) was measured. The experiment was conducted in triplicate, distributing a total of 180 fish in 12 aquariums (40 × 28 × 45 cm; $n = 15$ per aquarium) containing dechlorinated static water provided with air stones. The feed was provided at various levels to different treatment groups (control: 0 g kg^{-1}, T1: 1 g kg^{-1}, T2: 3 g kg^{-1}, T3: 5 g kg^{-1}) (Table 1). The fish were maintained in ambient lighting conditions, following a day-night schedule “12-h light and 12-h dark”. Fish were fed to the point of satiety, where they ceased eating, three times a day, specifically at 9am, 12pm, and 3pm. Additionally, 10% of the total water volume was replaced daily. Throughout the trial, the key water parameters (APHA 2005) were assessed with average temperature (25.12 ± 0.3 °C), dissolved oxygen, DO (5.78 ± 0.43 mg L^{-1}), pH (7.7 ± 0.4), ammonia (0.02 ± 0.001mg L^{-1}), and nitrite (0.017 ± 0.001mg L^{-1}).

Growth performance

After 60 days of the experimental period, the weight of every fish in the aquariums was measured separately. Growth performance was evaluated using the formulae given below.

$$\begin{gathered}\text{Weight gain } (\%)\\ = (\text{FW}) - (\text{IW}) \times 100/\,(\text{IW})\\ \text{SGR (Specific growth rate } (\%)\\ = [\ln\ (\text{FW}) - \ln\ (\text{IW})\ /\text{N}] \times 100)\\ \text{FCR (Feed conversion ratio)}\\ = \text{Feed given (DW)}/\text{body weight gain (WW)}\\ \text{FER (Feed efficiency ratio)}\\ = 1/\text{FCR},\ \text{SGR } (\%)\\ = [\ln\ (\text{FW}) - \ln\ (\text{IW})/\text{N}] \times 100\end{gathered}$$

Here,

FW	represents the final weight
IW	represents the initial weight
DW	represents the dry weight
WW	represents the wet weight
In	stands for the natural log
N	denotes the number of culture days

Blood samples collection

Healthy fish (in triplicate), showing no signs of infection, were selected from each aquarium, totaling 9 per treatment group, and anesthetized with clove oil at a concentration of 25 mgL^{-1}. Blood samples (0.6 mL) were then collected from the caudal vein using a syringe, following a 60-day feeding trial. Afterwards, half of blood samples were placed into plastic tubes containing heparin and sealed with lids, while the remaining portion was stored in aseptic tubes without anticoagulant agent (Habib et al. 2024). The blood-filled tubes without anticoagulant were allowed to stand at normal room temperature for 2 h. Following blood coagulation, the tubes underwent centrifugation at 5000 rpm for 12 min. Subsequently, the serum samples were transferred into labeled tubes based on their respective treatments and stored in a refrigerator at -20 °C for further experimental use (Duman et al. 2019). All chemicals and reagents used in this research were of analytical grade and were purchased from Sigma-Aldrich.

Analysis of hematological and serum biochemical parameters

Blood samples (heparinized) were utilized to ascertain white blood cell (WBC) and red blood cell (RBC) counts employing a counting chamber (Neubauer hemocytometer). Hematocrit (Ht) levels were assessed by subjecting microhematocrit tubes to centrifugation at 3000 rpm for 5 min. Hemoglobin (Hb) concentration was assessed using the cyanmethemoglobin method, involving the measurement of optical density at 540 nm using spectrophotometry (Goldenfarb et al. 1971). The spectrophotometric method, using kits from Bioanalytic Diagnostic Industry, İstanbul, Turkey, was used to analyze total protein, plasma glucose, albumin, globulin, and cholesterol levels.

Lysozyme activity

Serum lysozyme levels were evaluated by means of a turbidometric assay (Caruso et al. 2002). A suspension (*Micrococcus lysodeikticus*), comprising 875µL of "Sigma, ATCC 4698" at amount (0.2 mg/mL Phosphate buffer saline (PBS), was mixed with serum samples (25µL). At 530 nm spectrophotometric readings were taken after specific time intervals of 0.5 and 4.5 min at temperature (25 °C) through a spectrophotometer. The activity of lysozyme was measured by determining the quantity of serum that caused a decrease in absorbance by 0.001 min^{-1}.

Myeloperoxidase (MPO) activity

In rohu blood the MPO content was evaluated using the procedure outlined by Quade and Roth (1997), with some adjustments. Serum sample of 30 µL were diluted in Eppendorf tubes with (HBSS 370mL) without Mg^{2+} or Ca^{2+}. About 100 µL of 0.1 mg/mL (w/v) "3,3', 5,5'-tetramethylbenzidine dihydrochloride" and freshly prepared of H_2O_2 (0.06%) were added. The reaction kinetics were observed by monitoring the increase in absorption. Reaction rate were quantified in International Units (IU), with one IU defined as the enzyme quantity needed to cause a 0.001 absorbance rise per minute for every 0.5 ml of the reaction mixture "ΔA 450/min/mL".

Table 2 Growth performance of rohu fingerlings provided with fortified feed at different concentration

Parameters	Control	T1 (1%)	T2 (3%)	T3 (5%)
Weight gain, WG (%)	31.21 ± 1.37^{d}	37.41 ± 1.28^{c}	44.63 ± 1.57^{b}	54.28 ± 1.47^{a}
Specific growth rate, SGR	1.27 ± 0.04^{c}	1.32 ± 0.03^{c}	1.38 ± 0.06^{b}	1.55 ± 0.04^{a}
Feed conversion ratio, FCR	1.86 ± 0.03^{c}	1.77 ± 0.06^{bc}	1.62 ± 0.03^{b}	1.45 ± 0.04^{a}
Feed efficiency ratio, FER	0.57 ± 0.03^{d}	0.59 ± 0.05^{c}	0.65 ± 0.04^{b}	0.73 ± 0.05^{a}

In the row, the presence of distinct superscripts in the mean indicates significant differences

Challenge test

The *A. hydrophila* strain was provided on request by the Microbiology Department at the University of Punjab in Pakistan. For cultivation, the bacterium was grown in Tryptic Soy Broth (TSB; Merck, Germany) at temperature of 31 °C for a duration of 24 h. After the cultivation period, the bacterial suspension was subjected to centrifugation at 5000×*g* for 5 min at 25 °C. The supernatant was discarded, and the bacterial pellet underwent three washes with sterile physiological saline buffer (PBS). The pellet was then resuspended in the same PBS solution, and the concentration was adjusted to achieve an optical density of 0.5 at 456 nm (Gobi et al. 2016), corresponding to 1×10^{7} cells mL^{-1} After completing the feeding experiment, fish (15 per treatment group, with 5 fish per aquarium) were administered an intraperitoneal injection of 0.1mL of the *A. hydrophila* suspension (containing 10^{7} cells mL^{-1}). The survival rates were monitored across all groups for a period of 14 days following the infection. Throughout the post-challenge phase, the fish were provided with the experimental diets and maintained under the identical rearing conditions implemented during the feeding period.

Relative percentage survival

Based on the formula shown below, the relative percentage survival (RPS) at 14 days post-infection was calculated (Amend 1981).

$$\mathrm{RPS}\ (\%) = \frac{\text{Numbe of live fish after challange test}}{\text{No. of fish injected with bacteria}} \times 100$$

Statistical analysis

The data were analyzed using SPSS-22, applying one-way analysis of variance (ANOVA), followed by the Duncan multiple range test. The presentation of findings included arithmetic means accompanied by standard error (SE). Statistical significance was assessed using a significance level ($p < 0.05$).

Results

The results illustrating the growth response of rohu in control and treated groups are given in Table 2. The fish (T3 group) with a 5% experimental diet demonstrated a notably higher ($p < 0.05$) weight gain percentage than control and other treatment groups. The SGR of the T3 group revealed a substantially greater ($p < 0.05$) result. The best FCR (1.45 ± 0.04) was observed in the T3 group with a 5% nettle plant-based diet, followed by a T2 (3%) group (1.62 ± 0.03), while in the control group, a higher FCR (1.86 ± 0.03) was found. FER was markedly greater ($p < 0.05$) in the T3 group than others.

Table 3 presents the hematological parameter findings for various treatment groups. Fish nourished with 5% nettle (T3 group) showed a notable increase ($p < 0.05$) in WBC count among the other treatments and control. No significant difference ($p > 0.05$) was recorded in the RBC count among all the groups. The higher ($p < 0.05$) level of hematocrit (HCT) and hemoglobin (HB) was observed in T3 with 5% nettle addition.

The findings of serum biochemical parameters of rohu fingerlings in control and treated groups are depicted in Table 4. The total protein value in the T3 group of rohu, reared on 5% nettle diet, tended to increase ($p < 0.05$). Glucose levels decreased significantly in the T3 group, whereas no significant differences ($p > 0.05$) were observed among the control, T1, and T2 groups. The T3 group with 5% nettle markedly increase ($p < 0.05$) the albumin and globulin concentration levels than other groups. However, the cholesterol results showed no significant difference ($p > 0.05$) among the treatments and control group.

Figure 1(A) illustrates a notable rise ($p < 0.05$) in serum lysozyme activity in the fortified diet with nettle powder at all concentrations in rohu fingerlings. Considerable difference ($p < 0.05$) persisted consistently throughout the trial. Fish provided a diet supplemented with nettle powder exhibited consistently higher lysozyme activity in the serum across all concentrations, than the control group. Within the different nettle levels, the treatment receiving nettle powder of 5% demonstrated the utmost lysozyme activity.

Figure 1(B) shows the impact of a diet supplemented with nettle powder on the MPO activity of leukocytes in the serum of fish. The fingerlings nourished with experimental feed of 3% and 5% recorded the maximum and statistically significant ($p < 0.05$) MPO values. The MPO amount was markedly higher in all treatments than control group.

After a 60-day trial, the fish underwent a challenge with bacteria (*A. hydrophila*), and the RPS was determined over a 14-day period. Figure 1(C) illustrates the RPS of rohu fingerlings. The RPS showed a significant increase across all treated groups compared to the control. However, fish provided with 5% fortified feed showed an RPS of 73.81%, which was higher than those fed with 3%, 1%, and the control diet.

Table 3 Analysis of haematological parameters of rohu fingerlings provided with fortified feed at different concentration

Parameters	Control	T1 (1%)	T2 (3%)	T3 (5%)
WBCs (10^3 / µl)	27.42 ± 1.15^d	33.28 ± 1.32^c	39.34 ± 1.47^b	44.94 ± 1.61^a
RBCs (10^6 / µl)	1.32 ± 0.12^a	1.33 ± 0.14^a	1.33 ± 0.19^a	1.35 ± 1.19^a
Hematocrit HCT (%)	25.62 ± 0.27^c	26.69 ± 0.24^c	32.41 ± 0.42^b	36.55 ± 1.19^a
Haemoglobin HB (%)	6.96 ± 0.19^c	7.86 ± 0.24^{bc}	8.88 ± 0.26^b	11.06 ± 0.22^a

In the row, the presence of distinct superscripts in the mean indicates significant differences

Discussion

The use of chemicals in fish farming to enhance growth and prevent bacterial diseases poses various challenges, including the emergence of issues such as antibiotic resistance. Moreover, these chemicals can harm the well-being of fish, consumer health, and the ecosystem (Santos and Ramos 2018). As a result, significant focus has been directed toward exploring natural products as alternatives to antibiotics in aquaculture. Among the potential applications of natural products, the utilization of herbs is particularly significant. Some herbs like *Cymbopogon citratus*, *Mentha* spp, *Ocimum basilicum* and *Anethum graveolens* can benefit aquatic species in terms of growth and overall well-being (Filep et al. 2016; Adebayo et al. 2020; Knaus et al. 2020; Kesbiç et al. 2022). Keeping in view previous studies, current study examined how nettle leaf powder influences the growth efficiency, blood profile, immunological response, and resistance of rohu fingerlings when exposed to *A. hydrophila*.

Enhanced growth and improved fish health offer advantages in aquaculture by reducing production periods, lowering FCR, and increasing overall productivity. In our investigation, adding powdered nettle to the diet led to significant enhancements in WG, SGR, FCR, and FER than the values observed in the control group. Comparable findings

Table 4 Analysis of serum biochemical parameters of rohu fingerlings provided with fortified feed at different concentration

Parameters	Control	T1 (1%)	T2 (3%)	T3 (5%)
Total protein (g/dl)	1.94 ± 0.15^d	2.05 ± 0.25^c	2.22 ± 0.27^b	2.94 ± 0.37^a
Glucose (mg/dl)	58.32 ± 6.72^a	57.18 ± 2.54^a	55.27 ± 3.16^a	50.75 ± 7.24^b
Albumin (g/dl)	1.38 ± 0.18^c	1.44 ± 0.32^{bc}	1.47 ± 0.33^b	1.95 ± 0.36^a
Globulin (d/dl)	0.62 ± 0.16^c	0.76 ± 0.37^b	0.82 ± 0.25^b	0.98 ± 0.27^a
Cholesterol (mg/dl)	66.56 ± 12.42^a	63.86 ± 14.06^a	64.36 ± 9.74^a	63.48 ± 11.52^a

In the row, the presence of distinct superscripts in the mean indicates significant differences

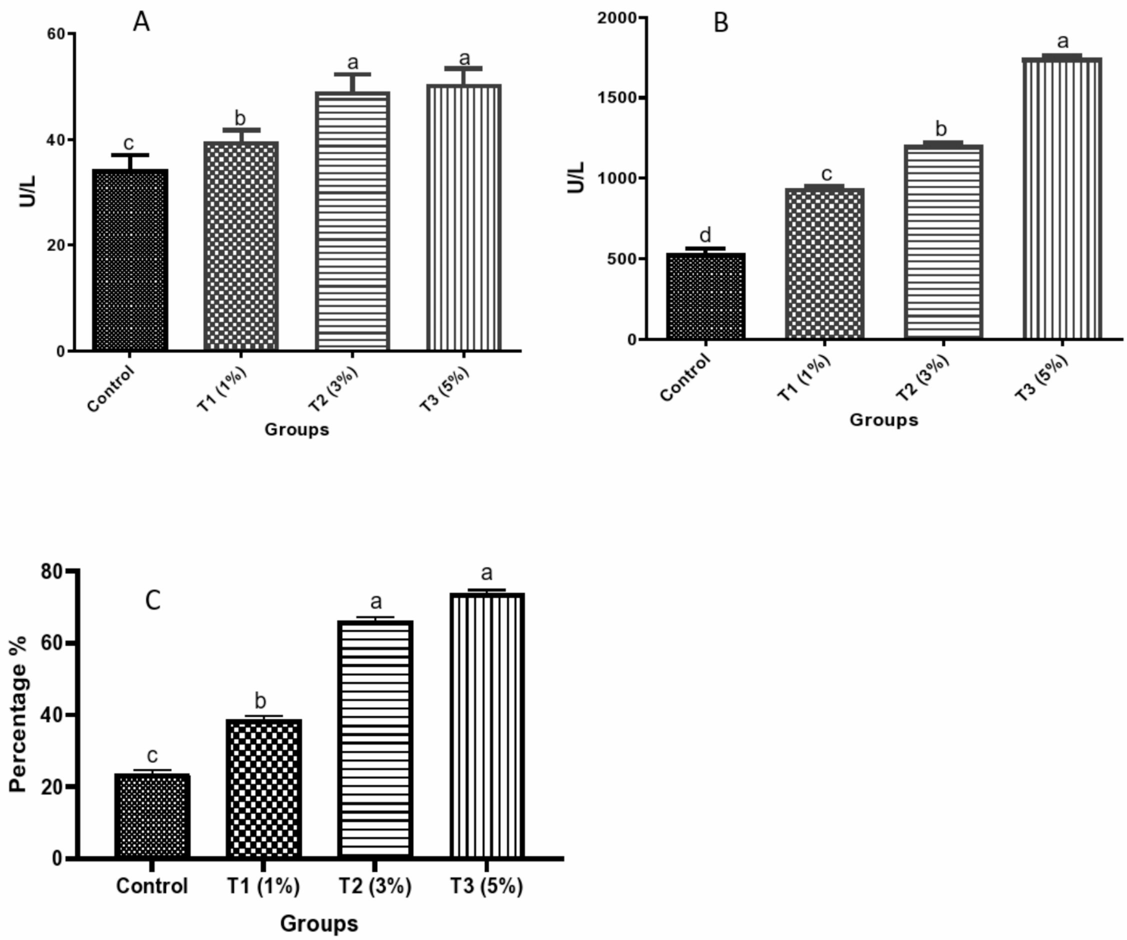

Fig. 1 Effect of fortified feed at different concentration on rohu fingerlings (**A**) Serum lysozyme activity (**B**) Myeloperoxidase activity (MPO) and (**C**) Relative percentage survival (RPS)

regarding the improvement of fish growth and dietary efficiency were reported in several studies, such as Mehrabi and Firouzbakhsh (2020) studied the immediate impact of incorporating powdered nettle (*U. dioica*) and Aloe vera (*Aloe barbadensis*) into the diet on the growth parameters of *Oncorhynchus mykiss*; their findings shows that the Aloe vera (1.5%) and nettle powder (0.5%) with basal diet exhibited a significant increase in feed efficiency and growth performance of fish than a control for 30 days. In our study, 5% nettle powder shows a substantial increase. Fazio et al. (2023) reported that 1.5% and 2% fruit extract of *Withania coagulans* supplemented diet significantly increased the SGR and FCR of rohu than the control. Ngugi et al. (2015) stated that increasing the nettle concentration (1%, 2% and 5%) shows higher growth performance (weight gain, FCR, SGR and FER) of *Labeo victorianus* than control. Similar observations were noted in the context of our investigation. Jafari et al. (2022) observed higher *Amatitlania nigrofasciata* final weight (FW), WG and SGR nourished on a fortified diet with 0.1% nettle for 56 days. The favorable growth response observed in rohu may be ascribed due to the growth-promoting components found in nettle. It is commonly known that nettle contains carvacrol, capsaicin, and cinnamaldehyde, which have demonstrated the ability to enhance growth in aquatic species (Taştan and Salem 2021). Herbs and other plant derived products have the potential to regulate and restrict the proliferation and presence of various disease-causing and harmful bacterial species in the digestive tracts of fish. This can contribute to increased efficiency in food utilization, ultimately leading to improved growth and enhanced feed efficiency (Syahidah et al. 2015; Zhang et al. 2021). Herbs, spices and diverse plant extracts possess properties that stimulate appetite and digestion in certain animals, potentially enhancing food utilization and conversion efficiency and consequently leading to enhanced growth prospects (Kuralkar and Kuralkar 2021).

The analysis of hematological parameters provides insights into the fish's health (Fazio 2019; Habib et al. 2021). The findings of this investigation demonstrated elevated levels of WBCs, HCT, and HB in response to the influence of nettle powder. However, no noticeable difference was found in fish RBC count among the treated and control groups. The RBC count in current research are in line with the investigation reported by Nobahar et al. (2015), who found no significant change in RBCs of *Huso huso* fed on 1% of the nettle diet for 60 days. On the other hand, HCT and HB values significantly increased in fish fed a diet containing 5% nettle. According to Mehrabi and Firouzbakhsh (2020), higher HCT and HB values were observed in *Oncorhynchus mykiss* fed nettle powder (0.5%) for 30 days. Interestingly, RBC counts did not change significantly with increasing nettle concentration, though HCT and HB did. This may be due to variations in RBC size (mean corpuscular volume - MCV), as HCT measures the percentage of blood volume occupied by RBCs. Larger RBCs lead to higher HCT, while smaller RBCs result in lower HCT, independent of RBC count (Ho et al. 2018; Mondal and Budh 2019). Another contributing factor is the variation in HB content per RBC (mean corpuscular hemoglobin - MCH). Differences in the amount of HB in each RBC can alter HB concentration without affecting RBC count. Additionally, changes in the concentration of HB within RBCs (mean corpuscular hemoglobin concentration – MCHC) can influence overall HB levels (Rudrappan et al. 2021). Hydration status and plasma volume also play a role; for instance, dehydration decreases plasma volume and increases RBC and HB concentrations, resulting in higher HCT and HB values without altering RBC count (Ho et al. 2018; Pałka et al. 2023). The current research illustrates a rise in the WBC count in fish that were provided with nettle supplements, attributed to the immune-stimulating properties of this plant. Our results are according to Ngugi et al. (2015), who found that 5% nettle leaf powder enhances the *Labeo victorianus* WBCs. Our study also aligns with the study of Fazio et al. (2022), who stated that the extract of olive leaf (1%) considerably improves the WBC count of *Oreochromis niloticus*. Significant increases in WBC count were observed in *Huso huso* that were fed with nettle (6% and 12%) over 8 weeks (Binaii et al. 2014). The elevated WBC levels in fish fed with nettle-fortified feed indicate an enhanced immune response rather than stress or infection because the well-established role of WBCs as a primary defense mechanism protects the body from infections caused by chemical and biological agents (Srivastava and Pandey 2015; Hu and Feng 2017). Nettle contains bioactive compounds like vitamins, minerals, and antioxidants that stimulate WBC production and activity, strengthening the fish immune system (Jan et al. 2017). The absence of signs of infection or stress confirms that this increase is a positive immunomodulatory effect. This result supports the research objective, demonstrating that nettle fortification can enhance fish health and disease resistance, making it a valuable addition to aquaculture diets. This is further confirmed by the elevated levels of serum proteins, globulins, and albumin, which are associated with an enhanced innate immune reaction (Alfonso et al. 2024).

Assessing the serum characteristics of fish is vital for comprehending both normal physiological processes and the impacts of pathological and toxicological factors (Esmaeili 2021). Furthermore, it was found that the group fed with 5% nettle had a considerably increased total protein value compared to the other tested groups. This is according to the other studies using different plant extracts with supplemented diets, such as *Avena sativa* extract for *Cyprinus carpio* (Baba et al. 2016), dandelion extracts for *Trachinotus ovatus* (Tan et al. 2017), and dietary Aloe vera for tilapia (Gabriel et al. 2015).

Globulin serves as a primary contributor to the production of immunoglobulins, and when its levels rise in the serum, it promotes increased activation of the immune system (Kumar et al. 2022). Besides this increased level of WBC, serum protein levels, globulins, and albumin, the enhanced immunity of the fish is further supported by the decreased level of glucose, as a rise in glucose levels is identified as an indicator of stress in fish (Odhiambo et al. 2020). The decrease in glucose levels in fish supplemented with nettle can be attributed to several scientific mechanisms. Nettle possesses hypoglycemic properties due to compounds like flavonoids, sterols, and polysaccharides, which enhance insulin secretion and improve insulin sensitivity, facilitating better glucose uptake by cells (Samakar et al. 2022). Additionally, the rich antioxidant content of nettle, including vitamins C and E, carotenoids, and phenolic compounds, reduces oxidative stress and improves pancreatic beta-cell function, aiding in glucose regulation. Nettle anti-inflammatory effects, resulting from bioactive compounds such as flavonoids and tannins, also contribute to improved insulin sensitivity. Furthermore, nettle can inhibit digestive enzymes like alpha-glucosidase and alpha-amylase, slowing carbohydrate digestion and glucose absorption, thus lowering postprandial blood glucose levels. The high dietary fiber content in nettle slows down carbohydrate digestion, preventing rapid spikes in blood glucose. Moreover, nettle rich mineral content, including magnesium and zinc, plays a crucial role in enhancing insulin function and glucose metabolism (Jan et al. 2017; Grauso et al. 2020). This aligns with the findings reported by Paray et al. (2020), who used leaf extract of *Quercus castaneifolia* for *C. carpio*, and Kavitha et al. (2012), who used seed extract of *Moringa oleifera* for *C. carpio*. The current findings suggest that the utilization of nettle with fortified diets led to an increase in albumin and globulin levels in fish. This agrees with other studies; for example, Baba et al. (2016) used supplemented diet with oat extract for *C. carpio*, and Mehrabi and Firouzbakhsh (2020) used Aloe vera and nettle supplementation for rainbow trout. Globulin serves as a primary contributor to the production of immunoglobulins, and when its levels rise in the serum, it promotes increased activation of the immune system (Sahu et al. 2007). In this study, the cholesterol level shows no significant difference, which is consistent with other author studies that used plant extracts (Metwally 2009; Immanuel et al. 2009). Lysozyme activity constitutes a crucial element at the forefront of the innate immune system barrier (Ferraboschi et al. 2021). Biological and synthetically manufactured immunity booster products are recognized for potentially promoting serum lysozyme activity (Wang et al. 2017). In this current investigation, fish that were provided diets enriched with varying levels of nettle leaf powder exhibited notably enhance the lysozyme actions than the control. Comparable findings have been documented in rohu, which were fed diets incorporating herbal immunostimulants (Das et al. 2015; Rao et al. 2006). The higher lysozyme level noted in this research demonstrates an enhanced non-specific immune response in rohu, provided with diets supplemented with nettle.

Additionally, nettle antioxidant properties play a crucial role in reducing oxidative stress, thereby improving the overall function of immune cells. The antioxidants in nettle protect these cells from oxidative damage, helping to maintain or enhance MPO production and activity, thus boosting the immune response. Furthermore, the anti-inflammatory effects of nettle, attributed to compounds such as flavonoids and tannins, help regulate chronic inflammation, which can otherwise impair immune function. By modulating inflammation, nettle allows immune cells to function more effectively and produce higher levels of MPO (Grauso et al. 2020). Nettle is also a rich source of essential nutrients, including iron, which is vital for the proper functioning of neutrophils and macrophages. Improved nutrient status supports the metabolic needs of these cells, enhancing their ability to produce MPO and other antimicrobial agents. Additionally, some components of nettle may have direct antimicrobial effects, which can synergize with the increased MPO activity to provide a more robust defense against pathogens (Harrison et al. 2022). Collectively, these mechanisms contribute to the significant increase in MPO activity observed in fish supplemented with nettle, indicating that nettle can enhance the innate immune response by stimulating immune cell activity, reducing oxidative stress, modulating inflammation, improving nutrient absorption, and potentially providing direct antimicrobial effects. This demonstrates the immunostimulatory potential of nettle in fish, contributing to a more effective eradication of microorganisms and overall improved immune function. Several researchers have noted an increase in MPO activity after the application of immunostimulant compounds in fish (Mir et al. 2017; Zare et al. 2023). This study illustrated an extensive improvement in the survival rate of rohu against the pathogenic bacteria (*A. hydrophila*) when fed a fortified feed with nettle. In numerous studies, immunostimulants have been revealed to increase the fish resistivity against various bacterial pathogens, including *A. hydrophila* (Khanzadeh et al. 2024; Barui et al. 2024).

Conclusions

In conclusion, the research conducted on the utilization of nettle (*U. dioica*) in promoting the growth and immunity of rohu has provided valuable insights into the potential benefits of incorporating this natural supplement into aquaculture practices, specifically at a supplementation level of 5 g/kg, holds potential for enhancing growth, bolstering immune responses, and improving resistance to *A. hydrophila*. It is strongly recommended that additional studies be conducted that explore the potential application of nettle as an immunostimulant in aquaculture, examining its efficacy in different fishes and against different pathogens.

Acknowledgements The authors extend their appreciation to the Researchers Supporting Project (RSPD2024R758), King Saud University, Riyadh, Saudi Arabia.

Author contributions Conceptualization, J.A.U.; methodology and software, S.S.H.; validation, S.S.H. and R.A.; data curation, J.A.U.; investigation, S.F.H; formal analysis, R.A.; writing—original draft preparation, S.S.H.; writing—review and editing, F.F., O.S.K. and A.R.; resources, S.S.H. and A.I.B; funding acquisition, M.M. Every author has reviewed and approved the final form of the article for publication.

Funding This work was funded by the Researchers Supporting Project (RSPD2024R758), King Saud University, Riyadh, Saudi Arabia.

Data availability No datasets were generated or analysed during the current study.

Declarations

Informed consent Not applicable.

Institutional Review Board The research was reviewed and approved by the Advanced Research Board of the Department of Zoology, Khushal Khan Khattak University. All procedures conducted in the study were also approved by the Ethics Committee of Khushal Khan Khattak University, Karak, Pakistan (registration number: Zoo/KKKU/13).

Competing interests The authors declare no competing interests.

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