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RESEARCH PAPER

Assessment of the Haematological and Biochemical Effects of Himalayan Herb *Urtica dioica* Leaves Diets Fed Amur Carp, *Cyprinus carpio haematopterus*

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Abstract

The various properties of *Urtica dioica* (Stinging nettle) supplemented feeds were evaluated in amur carp (*Cyprinus carpio haematopterus*). Four iso-protein and iso-energy diets were prepared by using different dietary levels of *U. dioica* i.e., 0.5 g (T_1), 0.75 g (T_2), and 1.0 g/kg (T_3) respectively, and the treatment T_C (control) consist of the basal diet with 0% of *U. dioica.* The phytochemical analyses of *U. dioica* revealed the presence of flavonoids (25.39 µg/mL), alkaloids (30.21 µg/mL), tannin (2.97 µg/mL), terpenoid (6.15 µg/mL), glycosides (10.12 µg/mL), phenolic (25.16 µg/mL) and saponin was absent. The best result of growth parameters such as net weight gain (415±0.55 g), net length gain (4.20±0.04 cm), weight gain percent (85.65±0.40%), specific growth rate (0.30±0.01%), gross conversion efficiency (0.229±0.01%), feed conversion ratio (4.37±0.13) and condition factor (1.53±0.01) were recorded in treatment T_3 in comparison with the other treatments. No mortality was observed in all the treatments resulting in a 100% survival rate. The haematological parameters (like haemoglobin level, total erythrocyte count, total leukocyte count, etc.) and serum biochemical parameters (including total serum protein, albumin, and globulin) showed better results in treatment T_3.

Introduction

Fisheries is a vital area of food production that ensures the nation's nutritional security while also providing a source of income for a substantial portion of the population, particularly the fishermen's community. India provides about 7.7% of global fish production and is the fourth largest exporter of fish products in the world. The total fish production during 2019-20 was 141.64 lakh tonnes with a contribution of 37.27 lakh tones from the marine sector and 104.37 lakh tonnes from the inland sector (Handbook on Fisheries Statistics 2020). Various growth promoters are currently used in various culturable fishes like carp fishes, catfishes, tilapia, African catfish etc. to enhance fat and protein distribution and boost the feed-to-muscle conversion rate (Nowosad *et al*., 2023). Growth promoters have been proven to improve feed efficiency, production, immunological function, and fish health in farmed aquatic species, which is important given current aquaculture goals of significant growth rate and disease resistance (Mehrabi *et al.,* 2020; Mehrabi and Firouzbakhsh, 2020). Phytobiotics are plant-derived compounds that are added to feed to improve aquatic species' performance and are mostly leaves, roots, tubers, or fruits of herbs, spices, and also other plants. Commonly used in shrimp and fish culture to improve growth performance. Herbs provide a more cost-effective means of advancing aquaculture. Herbs are employed instead of costly pharmaceuticals and growth stimulants (Ghafoor, 2020). Medicinal plants are profoundly ingrained in the cultural histories of many people from diverse nations and hence are inextricably linked to good health (Galina *et al.,* 2009). Medicinal plants have been shown to offer growth promotion, antistress, and anti-microbial effects (Pandey *et al.,* 2012; Semwal *et al*., 2023) due to the existence of many active principal components such as alkaloids, flavonoids, phenolics, essential oils, and so on (Ravikumar *et al.,* 2010; Dawood *et al.,* 2018), which are employed as immunostimulants (Van Hai, 2015). Plants are far safer to utilize, and phytochemicals are more readily available. Most of the phytochemicals are redox-active substances with antioxidant capabilities, which may aid the general physiological status of fish (Chakraborty and Hancz, 2011). Various parts of medicinal plants and their extracts added to fish diets have recently been studied in aquaculture with positive outcomes for fish growth or disease resistance (Dada, 2015a; Dada 2015b; Giri *et al*., 2015; Kumar *et al*., 2021; Abdel-Latif *et al*., 2022a; Abdel-Latif *et al*., 2022b; Abdel-Latif *et al*., 2022c; Kumar 2022; Kumar *et al*., 2022a; Abd El-Naby *et al*., 2023; Semwal et al., 2023; Singh et al., 2023). Most of the herbs/plants/spices are used as Phytobiotics (mostly used leaves, roots, tubers, or fruits) in the diets of the fish cultured either in the laboratory or in the field/pond conditions. As they have many benefits such as eco-friendly (Citarasu, 2010), easy access, cheapness, less toxicity (Madhuri *et al.,* 2012; Olusola *et al.,* 2013), and food security as natural additives (Jian and Wu, 2004).

Himalayan herbs are generally aromatic and medicinal plants having properties in curing diseases and improving growth and immunity. *Urtica dioica* (Stinging nettle), is a perennial plant belonging to the genus *Urtica* of the family Urticaceae (Ahmed and Parasuraman 2014). This plant has become both medicine and food in many nations due to its ease of harvesting and extensive distribution, as well as its extraordinary biological properties (Asgarpanah and Mohajerani, 2012). The presence of many chemical components in the plant, such as flavonoids, tannins, phytosterols, and phenolic acids suggested that the plant may be cheaply cultivated and used for naturopathy. Antioxidants, anti-inflammatory, analgesics, antiviral, immunomodulatory, and anticancer properties have all been identified for the plant. According to a detailed proximate study on a wet basis, proteins (3.7%), fat (0.6%), ash (2.1%), dietary fiber (6.4%), total carbohydrate (7.1%), and total calories (45.7 kcal/100 g) are present in Stinging nettle. Stinging nettle is also high in vitamin A, vitamin C, calcium, iron, salt, and a diverse fatty acid profile (Rutto *et al.,* 2013). In all culture systems, the performance of Amur carp (*Cyprinus carpio haematopterus*) was consistently superior to that of other stocks for critical qualities. It has an excellent food conversion rate, exceptional ability to accept artificial feed, and minimal fat level, and its viability is excellent. Because of its superior growth, the Amur strain of common carp has higher promise in low-input aquaculture systems (Basavaraju and Reddy, 2013). The present investigation aims to study the effect of feeding of Himalayan herb *Urtica dioica* leaves enriched diets in strengthening the hematological and biochemical systems in *Cyprinus carpio haematopterus*.

Materials and Methods

Collection of Experimental Fishes

The experimental fishes were obtained from the Instructional Fish Farm of the College of Fisheries, Govind Ballabh Pant University of Agriculture and Technology located at Pantnagar, Udham Singh Nagar, Uttarakhand, India. The stock of fish was acclimatized for 15 days and fed with a basal diet (rice bran, mustard oil cake). The protein ingredient in the diet was the same (mustard oil cake) as that of Kaur *et al*. (2022) used in her experiment. Aerators were used to provide aeration in all the FRP tanks.

Collection of *Urtica dioica*

The leaves of *U. dioica* were collected from the Bhimtal, Nainital, Uttarakhand. Gloves were used on both hands while the leaves were carefully removed with a sharp instrument since they irritate the skin when they came into touch. The freshly collected green leaves of *U. dioica* were put outside for sun drying to complete the removal of moisture from the leaves. The dried leaves were finely grounded in the grinder and powder was made. After that *U. dioica* leaves powder was kept in an airtight plastic jar for future use.

Preparation of Experimental Diet

Three different experimental diets were made by combining a weighted amount of different feed ingredients (like rice bran, mustard oil cake, and maize) with three different quantities of *U. dioica* leaves powder (Kaur *et al*., 2022). To produce a satisfactory dough consistency, the materials were uniformly mixed with an acceptable amount of water. A hand-operated pelletizer was used to pelletize the prepared dough separately. The pellets were well-dried in the sun and stored in airtight containers with proper labeling. Each experimental treatment had three replicates. The final diet had 24.50% (crude protein), 7.10% (crude fat), and 46.10% (carbohydrate) (Table 1 and Table 2).

Experimental Design

In this experiment, 12 (4 treatments and 3 replicates, 4 x 3 = 12) circular FRP tanks with a capacity of 600 L each were placed indoors at the wet lab of the College of Fisheries. The stocking density was 10 fish/tank with an average body weight of 48.70±4.51 g.

All the tanks' aerators were appropriately fitted. Feed was given to the fish at a rate of 3% of their total body weight twice a day @ 9.00 am (IST) and 5.00 pm (IST). The fish group weight from all tanks was measured at intervals of 15 days. The total experimental duration was 90 days.

Phytochemical Analysis of *U. dioica* and Preparation of Extract

Plant powder was properly combined with methanol in a 1:10 ratio in a conical flask. The mixture is then put in the shaker at room temperature. After 48 h, filter the supernatant using sterilized Whatman no. 1 filter paper. To obtain the final concentrated mixture, the leaves residue was re-extracted for 24 h with the addition of solvent and then kept in a water bath at 40°C for complete evaporation of methanol. The dried extract was then kept at 4°C for study use.

Qualitative phytochemical analysis for the herb was done for alkaloid (Mayer's test), flavonoid (H_2SO_4 test), terpenoid *(Salkowski test,* phenol *(Lead acetate test),* saponin (Frothing test), tannin (Ferric chloride test) and glycoside (Salkowski test). Quantification of phytochemicals was done by spectrophotometric method (Table 3).

Water Quality Analysis

Standard procedures (APHA, 2017) were used to analyze the physico-chemical parameters of water in all the experimental tanks for the 90-day experiment (Table 4).

Growth Parameters of Experimental Fish

Growth performances were calculated by using the electric balance and mathematical scale analyzed.

Net Length Gain (cm) = Final length at the end of the experiment - Initial length at the start of the experiment.

Net Weight Gain (g) = Final weight at the end of the experiment - initial weight at the start of the experiment.

Table 1. Formulation of basal diet composition (%).

Ingredients	Treatments*			
	T_1	T_2	T_3	T_C
Mustard oil cake	55	55	55	55
Rice bran	36	36	36	36
Maize	8	8	8	8
Vitamin and mineral	0.95	0.93	0.9	1
Nettle (*Urtica dioica*)	0.05	0.08	0.1	0

*T_1 = Nettle leaves powder 0.5 g/kg feed + Basal diet; T_2 = Nettle leaves powder 0.75 g/kg feed + Basal diet; T_3 = Nettle leaves powder 1 g/kg feed + Basal diet; T_C (control) = Without nettle leaves powder + Basal diet.

Table 2. Proximate nutrient composition of experimental diets.

Contents	Composition (%)
Crude protein	24.50
Crude fat	7.10
Carbohydrate	46.10
Crude fiber	6.30
Ash content	7.0
Moisture	9.00
Calory value (kcal/kg)	3.46

Table 3. Qualitative and quantitative phytochemical estimation of in *Urtica dioica* leaves.

Phytochemical parameters	Observations	Present/ absent	Method	Concentration (µg/mL)
Alkaloid	Cream color reddish brown solution/ precipitate	+	Mayer's test	30.21
Flavonoid	Yellow-orange reddish brown/orange color precipitate	+	H_2SO_4 test	25.39
Terpenoid	Reddish brown precipitate	+	Salkowski test	6.15
Phenol	Deep blue to black color formation white precipitate	+	Lead acetate test	25.16
Saponin	Stable persistent	-	Frothing test	Absent
Tannin	Brownish green/blue black	+	$FeCl_3$ test	2.97
Glycoside	A brown ring at the interface and green color in the acetic layer was observed	+	Salkowski test	10.12

Table 4. Growth parameters (Net weight gain, Net length, Weight gain, SGR, FCR, GCE and Condition factor) of amur carp. Means with different superscripts in the same row are significantly different.

Treatments	Growth parameters						
	Net weight gain (g)	Net length gain (cm)	Weight gain (%)	SGR gain (%)	FCR	GCE	Condition factor (K)
T_1	$319±0.35^a$	$2.30±0.02^b$	$65.15±0.07^a$	$0.24±0.01^a$	$5.24±0.11^c$	$0.19±0.01^a$	$1.49±0.01^a$
T_2	$356±0.75^b$	$3.20±0.01^c$	$72.64±0.53^b$	$0.26±0.01^b$	$4.87±0.11^b$	$0.21±0.01^b$	$1.51±0.01^b$
T_3	$415±0.55^c$	$4.20±0.04^d$	$85.65±0.40^c$	$0.30±0.01^c$	$4.37±0.13^a$	$0.23±0.02^c$	$1.53±0.01^b$
T_C	$305±0.89^a$	$1.70±0.10^a$	$63.26±0.54^a$	$0.24±0.01^a$	$5.41±0.07^c$	$0.19±0.01^a$	$1.48±0.01^a$

Data expressed as mean ± SE (n=3), the mean value in the same column sharing different superscripts are significantly different (P<0.05).
*T_1 = Nettle leaves powder 0.5 g/kg feed + Basal diet; T_2 = Nettle leaves powder 0.75 g/kg feed + Basal diet; T_3 = Nettle leaves powder 1 g/kg feed + Basal diet; T_C (control) = Without nettle leaves powder + Basal diet.

$$\text{Weight Gain (\%)} = \frac{\text{Final weight (g)} - \text{initial weight (g)}}{\text{Initial weight (g)}} x100$$

$$\text{Specific Growth Rate (SGR)} = \frac{\text{Log final weight} - \text{Log initial weight}}{\text{Duration of experiment}} x100$$

$$\text{Feed Conversion Ratio (FCR)} = \frac{\text{Feed consumed (g)}}{\text{Weight gain (g)}}$$

$$\text{Gross Conversion Efficiency (GCE)} = \frac{\text{Weight gain (g)}}{\text{Feed consumed (g)}} x100$$

$$\text{Condition Factor (K)} = \frac{\text{Weight of fish (g)}}{\text{Length3 of fish (cm)}} x100$$

$$\text{Survival Rate (\%)} = \frac{\text{Final number of fishes}}{\text{Initial number of fishes}} x100$$

Blood Sampling and Biochemical Analysis

Changes in haematology and serum biochemical parameters were studied using blood samples taken at the initial and final stages of the experiment. The blood sample was collected from the caudal vein of the fish taken in the 4°C conditions using ice flakes and ice packs.

Two fish from each replication were collected using a disposable syringe of (5 mL) for blood collection. After that blood was transferred to ethylene diamine tetra-acetic acid (EDTA) vials. Then the vials were gently moved for proper mixing. The sample was kept at 4°C overnight for clotting, then the sample was centrifuged at 400×g (4°C for 10 min) and serum was stored at -20°C for various assays. For biochemical parameters such as; total serum protein was calculated using Autospan Liquid Gold Protein bromocresol green, endpoint assay at 546nm, and serum albumin Autospan Liquid Gold Albumin Bromocresol green (BCG) endpoint assay at 630 nm wavelength was used for calculation (Kaur *et al*., 2022).

Statistical Analysis

Using IBM SPSS software, the research sample data were processed for One-Way ANOVA (analysis of variance) to obtain the means and standard error readings. Duncan's multiple range tests were applied for analyzing post-hoc comparison of means to observe significant differences at a 5% probability level (P<0.05).

Results

Phytochemical Analysis

Qualitative analysis results of *U. dioica* leaves showed the presence of alkaloids (30.21 ug/mL), flavonoids (25.39 ug/mL), terpenoids (6.15 ug/mL), phenol (25.16 ug/mL), tannin (2.97 ug/mL), and glycoside (10.12 ug/ml). Alkaloids (30.21 ug/mL) are the highest and tannin (2.97 ug/mL) were lowest observed in the leaves of the plant, while saponin was absent (Table 3).

Growth Parameters

The maximum net weight gain (g) was recorded in treatment T_3 (415±0.55 g) diet-fed fish which was provided with 1 g/kg of *U. dioica* based diet fed daily and the lowest growth was observed in the treatment Tc (305±0.89 g) diet-fed fish which was fed with the control diet. Comparatively higher net length gain was recorded in the treatment T_3 (4.20±0.03 cm) diet-fed fish which was supplemented with 1 g/kg of *U. dioica* based diet fed daily and the lowest was observed in treatment T_C (1.70±0.11 cm) diet-fed fish which was provided with the control diet. The maximum percent weight gain was observed in the treatment T_3 (85.65±0.40 %) diet-fed fish which was supplemented with 1 g/kg of *U. dioica* based diet and the lowest was in treatment T_C (63.26±0.54 %) diet-fed fish which was provided with the control diet. There were no significant (P>0.05) differences in percent weight gain of fish fed with T_C and T_1.

Among all the treatments, the higher specific growth rate (SGR %) gain was recorded in treatment T_3 (0.30±0.01 %) diet-fed fish which was supplemented with 1 g/kg of *U. dioica* diet followed by treatment T_2 (0.26±0.01) diet supplemented with 0.75 g/kg and T_1 (0.24±0.01 %) diet supplemented with 0.5 g/kg of *U. dioica* diet-fed fish. The lowest SGR gain was observed in the treatment T_C (0.24±0.01%) diet-fed fish which was a control diet without any supplementation with *U. dioica*. The findings revealed that increasing *U. dioica* in the diet increased SGR. The higher gross conversion efficiency (GCE %) was recorded in the treatments which were supplemented with *U. dioica* in comparison to the control diets fed fishes. The herb supplemented with 1 g/kg for treatment T_3 diet-fed fishes showed the maximum GCE of (0.30±0.01). The T_3 diet-fed fishes showed the best feed conversion ratio (FCR) of (4.37±0.13%) which was supplemented with 1 g/kg of *U. dioica* in comparison with the treatment T_C (5.41±0.07%) which was not supplemented with *U. dioica*. The highest condition factor (K) was observed in the treatment T_3 (1.53±0.01) and lowest in the treatment T_C (1.48±0.01) incondite-fed diet-fed fish. The treatment T_3 showed a higher condition factor which was supplemented with 1 g/kg of *U. dioica* in comparison to the treatment Tc which was given in the control diet diet-fed fish. There were no significant differences (P>0.05) in condition factors in fish fed with T_2 and T_3. The T_3 treatment diet-fed fishes showed a significant difference with Tc treatment (Table 4).

Haematological Parameters

During this study, the levels of haemoglobin (8.70±0.06 g/dl), TEC (2.07±0.01 10^6/mm^3), TLC (44.70±0.06 10^3/mm^3) and PCV (32.23±0.09 %) were increased from the initial haemoglobin (7.60±0.12 g/dl), TEC (1.01±0.01 10^6/mm^3), TLC (40.40±0.06 10^3/mm^3) and PCV (25.03±0.15 %) levels in the blood showed the highest results in the treatment T_3 diet-fed fish which was supplemented with 1 g/kg of *U. dioica* in comparison to Tc (control) treatment diet-fed fish. There was negative growth of MCV, HCH, and MCHC found in T_3 diet-fed fish (Table 5).

Serum Biochemical Parameters

The maximum level of total serum protein was observed in treatment T_3 (6.96±0.39 g/dl) followed by T_2 and T_1 diet-fed fish. Similarly, the maximum level of serum albumin was observed in treatment T_3 (3.56±0.12 g/dl) followed by treatment T_2 (3.03±0.09 g/dl) and T_1 (2.63±0.12 g/dl) diet-fed fishes. Serum globulin was observed maximum in treatment T_3 (3.40±0.29 g/dl) and treatment T_C showed (1.86±0.03 g/dl) as diet-fed fish the lowest amount of serum globulin was recorded, although there was no significant difference (P>0.05) in serum globulin content of fish fed with diet T_C and T_1 (Table 6).

Table 5. Haematological parameters of amur carp in different experimental treatments. Means with different superscripts in the same row are significantly different.

Parameters		Treatments*			
		T_1	T_2	T_3	T_C
Haemoglobin (g/dl)	Initial	7.43±0.03^b	7.63±0.09^b	7.60±0.12^b	7.10±0.06^a
	Final	8.13±0.09^b	8.40±0.06^c	8.70±0.06^d	7.63±0.09^a
TEC (10^6/mm^3)	Initial	1.03±0.01^b	0.97±0.01^a	1.01±0.01^b	0.95±0.06^a
	Final	1.86±0.01^b	1.93±0.01^c	2.07±0.01^d	1.63±0.09^a
TLC (10^3/mm^3)	Initial	40.60±0.06^c	40.30±0.06^{ab}	40.40±0.06^b	40.20±0.06^a
	Final	42.30±0.12^b	43.40±0.12^c	44.70±0.06^d	41.33±0.09^a
PCV (%)	Initial	25.30±0.06^a	25.13±0.03^a	25.03±0.15^a	24.83±0.26^a
	Final	30.33±0.09^b	31.30±0.06^c	32.23±0.09^d	29.70±0.06^a
MCV (fl)	Initial	244.86±2.62^a	259.10±1.86^b	247.00±0.76^a	261.40±4.34^b
	Final	162.76±2.62^b	161.86±1.86^b	155.66±0.77^a	181.83±4.34^c
MCH (pg)	Initial	71.93±0.32^a	78.68±0.46^b	75.00±1.36^a	74.74±1.06^a
	Final	43.65±0.80^a	43.45±0.49^a	42.02±0.04^a	46.73±0.78^b
MCHC (%)	Initial	29.37±0.20^{ab}	30.36±0.38^b	30.36±0.64^b	28.58±0.07^a
	Final	26.81±0.37^b	26.83±0.23^b	26.98±0.11^b	25.69±0.35^a

Data expressed as mean ± SE (n=3), the mean value in the same column sharing different superscripts are significantly different (P<0.05).
*T_1 = Nettle leaves powder 0.5 g/kg feed + Basal diet; T_2 = Nettle leaves powder 0.75 g/kg feed + Basal diet; T_3 = Nettle leaves powder 1 g/kg feed + Basal diet; T_C (control) = Without nettle leaves powder + Basal diet.

Table 6. Biochemical parameters of amur carp in different experimental treatments. Means with different superscripts in the same row are significantly different.

Parameters		Treatments*			
		T_1	T_2	T_3	Tc
Total serum protein (g/dl)	Initial	3.53±0.03^a	3.90±0.06^b	4.03±0.03^b	3.40±0.10^a
	Final	4.83±0.23^a	5.63±0.09^b	6.96±0.38^c	4.23±0.03^a
Serum albumin (g/dl)	Initial	2.30±0.12^a	2.10±0.06^a	2.33±0.15^a	2.03±0.03^a
	Final	2.63±0.12^a	3.03±0.09^b	3.56±0.12^c	2.36±0.07^a
Serum globulin (g/dl)	Initial	1.23±0.12^a	1.80±0.12^c	1.70±0.12^{bc}	1.36±0.09^{ab}
	Final	2.20±0.15^{ab}	2.60±0.15^b	3.40±0.29^c	1.86±0.03^a

Data expressed as mean ± SE (n=3), the mean value in the same column sharing different superscripts are significantly different (P<0.05).
*T_1 = Nettle leaves powder 0.5 g/kg feed + Basal diet; T_2 = Nettle leaves powder 0.75 g/kg feed + Basal diet; T_3 = Nettle leaves powder 1 g/kg feed + Basal diet; T_C (control) = Without nettle leaves powder + Basal diet.

Water Quality Parameters

There was no significant difference in water quality parameters in all the different units during the experimental period. All the major water quality parameters were observed and were within the ideal range. As a result, adding *U. dioica* to the fish diet had no negative impact on the water quality of the different units (Table 7).

Discussion

Modern aquaculture places a high priority on improving and optimizing fish rearing because doing so will have numerous quantifiable economic benefits. It is necessary to create and produce efficient, secure, and non-polluting herbal compounds in order to boost and accelerate the aquaculture industry's growth. Affordable and producing top-notch outcomes are herbal medications (Rashidian *et al*., 2021). Pharmacology of numerous herbal medicines and compound preparations lack functions that treat aquatic animals, prevent disease, promote growth and improve the quality of aquatic products. They can be utilized in whole or in part with the aid of a mixture of extract compounds or former immunostimulants. The use of plants as a natural, harm-free composition has maybe replaced antibiotics in aquaculture (Chaolan *et al*., 2014; Van Hai, 2015). The aquaculture industry depends on phytotherapy because it has been shown to offer advantages such as enhancing the delivery system, bioavailability, and sustained release of bioactive substances (Jeyavani *et al*., 2022). One strategy to improve contemporary aquaculture practice is to reduce the number of pathogenic bacteria that are present in the intestines. This may favor the development of probiotic microflora (Nowosad *et al*., 2022). In order to increase the survival, growth rate, or disease resistance of farmed fish, various research projects have focused on these issues. It's interesting to note that adding functional bioactive ingredients to fish meals, such as vitamins, prebiotics, and herbal additions, has been shown to improve several aspects of fish culture (Abdel-Latif *et al*., 2022a; Abdel-Latif *et al*., 2022b; Abdel-Latif *et al*., 2022c; Abd El-Naby *et al*., 2023). Additionally, research shows that bioflavonoids, which are plant compounds with estrogenic activity, stimulate common carp growth (Dada *et el*., 2015b).

The present study results concluded that the supplementation of Himalayan herb *U. dioica* at a rate of 1 g/kg in the feed of Amur carp (*Cyprinus carpio haematopterus*) showed increased growth performance, haematological and serum biochemical parameters. The study also revealed that there was no negative impact of herb *U. dioica* supplemented diets on the fish's health and resulted in no mortality. The survival rate of fish was not affected after fed with the Himalayan herb *U. dioica* to Amur carp (*Cyprinus carpio haematopterus*), all fish survived. Awad and Austin (2010) investigated that, feeding rainbow trout a diet containing 1 % stinging nettle considerably reduced mortality after being challenged with *Aeromonas hydrophila*. They also saw a rise in haematocrit, haemoglobin, total protein in the *U. dioica* fed group by bacterial infection. The mixture of herbs (*Achyranthes aspera*, *Lupinus perennis, Mangifera indica, and U. dioica*) with various doses for two months with various fishes (rainbow trout, rohu and magur) resulted in considerably higher weight increase, stress control, low mortality, increase disease resistant capacity, fish length, and growth rate in treated fish than in control fish (Citarasu, 2010; Awad *et al.,* 2012; Kumar *et al.,* 2019; Sharma *et al.,* 2019; Kumar *et al.,* 2021; Kumar *et al.,* 2022b; Sharma *et al.,* 2022). Mehrabi and Firouzbakhsh (2020), reported the impacts of nettle (*U. dioica*) on rainbow trout growth, immunology, and gene expression after an 8-week feeding trial. The fish fed with 0.5% nettle powder had the maximum body WG value (28.98±0.76 g), which did not significantly differ from the other treated groups ($P>0.05$). The control fish had the lowest WG (18.07±1.51 g), which was significantly different from the other supplemented groups ($P<0.05$). The SGR and FCR values of all nettle-fed treatments were nearly identical ($P>0.05$), but the two indices showed statistical differences concerning the control ($P<0.05$). Throughout the 8-week feeding period, it was observed that all of the treatments had a 100% survival rate.

Conclusion

An experimental trial was conducted to evaluate the dietary effect of *Urtica dioica* on the growth performance, haematological and serum biochemical parameters of Amur carp (*Cyprinus carpio haematopterus*). The treatment T_3 showed the best

Table 7. List of water quality parameters analyzed during the experiment.

Parameters	T_1	T_2	T_3	T_C
Temperature (°C)	28.71±0.92	28.7±0.72	29.32±0.69	29.01±1.01
pH	7.29±0.039	7.26±0.21	7.43±0.25	8.06±0.11
Dissolved oxygen (mg/L)	5.33±0.12	6.02±0.31	5.63±0.51	5.98±0.31
Ammonia (mg/L)	0.01±0.00	0.01±0.00	0.02±0.00	0.01±0.00
Total alkalinity (mg/L)	155.43±5.12	159.34±6.23	158.78±5.93	157.61±4.93

T_1 = Nettle leaves powder 0.5 g/kg feed + Basal diet; T_2 = Nettle leaves powder 0.75 g/kg feed + Basal diet; T_3 = Nettle leaves powder 1 g/kg feed + Basal diet; T_C (control) = Without nettle leaves powder + Basal diet.

result in all parameters like growth parameters, survival rate, haematological parameters, and biochemical parameters. The survival rate of fish was not affected after being fed with the Himalayan herb *U. dioica* to Amur carp (*Cyprinus carpio haematopterus*), all fish survived. All parameters have significant relationships (P<0.05) between treatments and the control group. The survival rate was observed 100% in all the treatments. The herb, *U. dioica* (1 g/kg) is beneficial for enhancing the growth parameters as well the immunity of experimental fish, amur carp.

Ethical Statement

The authors followed all applicable international, national, and institutional guidelines for the care and use of fish.

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Author Contribution

KJ, RSC, AK, R, NK: designed the experiment; KJ, AK, NK; conducted the experiment and analyzed the samples; KJ, RSC, AK, R, NK; prepared the manuscript; KJ, AK, NK; prepared the tables; AK, NK; evaluate the final design of the manuscript.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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