



Potential Role of Purslane (*Portulaca oleracea*) Extract Supplemented Sunflower Meal-Based Diets on The Performance of Commercially Important Fish, *Labeo rohita*

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ABSTRACT

This study investigates the potential of incorporating purslane (*Portulaca oleracea*) extract into sunflower meal-based diets on *Labeo rohita* fingerlings (average weight: 6.29±0.02 g), evaluating its effects on growth performance, hematology, whole-body composition, and mineral content. Each of the seven test diets-T₀ (no extract), T₁ (0.5%), T₂ (1%), T₃ (1.5%), T₄ (2%), T₅ (2.5%), and T₆ (3%), were administered twice per day at a rate of five percent of their body mass. The fish were maintained in V-shaped tanks for a duration of 90 days. Each group included fifteen fingerlings, and each diet was administered in triplicate. The present study indicates that growth was considerably ($p<0.05$) improved in groups fed purslane extract-supplemented sunflower meal-based diets compared to T₀ and T₆, respectively. In comparison to T₀ and T₆, all groups showed a substantial decrease in fat content and a significant increase in protein content. Ash and mineral contents differed non-significantly across treatments. The inclusion of purslane extract in sunflower meal-based diets resulted in a significant increase in blood factors such as white blood cells, red blood cells, hemoglobin and platelets. Nonetheless, there was a decline in mean corpuscular Hb and volume. The results also showed that mineral content in the body of fingerlings enhanced markedly ($p<0.05$) in all studied diets when compared to T₀ and T₆. According to this study, a diet containing 1.5% purslane extract is the best option for fish performance.

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Authors' Contribution

MF: Writing-original draft; SMH: Conceptualization, data curation, supervision. TS, AR: Investigation, formal analysis. SA: Writing-review and editing. All authors read and approved the final manuscript.

Key words

Purslane, Sunflower, *Labeo rohita*, Growth performance, Hematology, Body composition

INTRODUCTION

Globally, aquatic products represent a major source of both animal and human protein and food (Golden et al., 2021). Aquaculture has emerged as the most rapidly expanding food industry globally over the course of many decades (FAO, 2020). Global fish production increased by

60% from the average of 1960 to 2020, reaching 214 million tons. Aquaculture production is expected to reach a record-breaking level of 122.6 mt in 2020, according to the report of the FAO (2022). The majority of the protein consumed by indigenous communities and animals worldwide comes from fish and other aquatic sources. Fish comprises 17% of the animal protein in the diet, but only 7% of the overall protein. From 1973 to 2021, Pakistan's fish production surged from 214,231 metric tons to 733,025 metric tons, a rise of 4.9% per year (Khan et al., 2023).

In the field of aquaculture, fishmeal (FM) is often considered to be the most exceptional protein source. The increasing worldwide demand and limited accessibility of FM impose limitations on its use as the only protein source in aquafeed, hence compelling fish nutritionists to explore alternate protein-based aquafeeds (Hossain et al., 2024). There are many advantages of using functional feed additives in aquafeed (Abdel-Latif et al., 2020). For fish to

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flourish, feed additives are an integral part of the feeding process. For cultured fish to reach their full potential, feed additives must be included in fish feed (Syanya *et al.*, 2023). Herbs and their active extracts are frequently used in fish farming as effective feed additives (Magouz *et al.*, 2021).

Portulaca oleracea commonly called as purslane, is a member of the portulacaceae family and possesses nutritional, phytoremedial, ornamental, and medicinal properties (Ocampo and Columbus, 2012; Kumar *et al.*, 2022). It has been shown that this plant has over 30 unique biological activities and over 60 medicinal uses (Sangeetha *et al.*, 2020). As one of the most frequently utilized plant species, the World Health Organization (WHO) has recognized it as a “Global Panacea” (Miao *et al.*, 2019). Phytochemical study suggests that this plant contains a variety of bioactive chemicals, such as organic acids, fatty acids, flavonoids, alkaloids, terpenoids, vitamins, and minerals (Zhou *et al.*, 2015). According to Hanan *et al.* (2014), purslane extract contains ash (3.8%), fiber (0.82%), protein (4.9%) and total carotenoids (40.40 mg per 100 g). Incorporating this herb, *P. oleracea*, as fish feed is a sustainable and possibly cost-effective approach (Sourani *et al.*, 2023). This invention promotes circular economy principles by converting trash into profitable feedstock (Amalia *et al.*, 2024).

Oilseed meals are plant-based protein additives made by extracting oil from oilseeds such as soybeans, cottonseed, canola, peanuts, and sunflower seeds (Bernard, 2016). The study discovered that sunflower meal (SFM) is one of the plant protein sources with promising applications (Rodrigues *et al.*, 2012). The product that is extracted from sunflower seeds through direct leaching or pre-pressing is called SFM. SFM is less expensive than soybean meal and has a lower sulfur-containing amino acid content than soybean meal. Other amino acids, such as glutathione and aspartic acid, were more evenly distributed (Köprücü and Sertel, 2012; Shchekoldina and Aider, 2014). Its high protein content, excellent nutritional value, and low level of anti-nutritional factors, make it a suitable component for fish feed (Shchekoldina and Aider, 2014; Saleh *et al.*, 2021).

The primary aquaculture species in the Indian Subcontinent is the freshwater Indian major carp, *Labeo rohita* (Rohu) (Mridul *et al.*, 2024). Rohu is a mid-layer water column resident, and its growth performance in culture implies that they prefer relatively constant water temperatures ranging from 28 to 30°C (Rahi and Shah, 2012; Roychowdhury *et al.*, 2020). The current study sought to evaluate the potential of incorporating purslane (*P. oleracea*) extract into SFM-based diets on parameters of *L. rohita* fingerlings.

MATERIALS AND METHODS

Sample extract

The dried, firmly powdered herb was suspended in an ethanol solution comprising 60% volume/volume served as the extraction solvent. Initially, 2000 g of powdered purslane were introduced into a Soxhlet apparatus for extraction using ethanol as the solvent. After extraction, the resulting product underwent filtration and was concentrated to dryness through a rotary evaporator. Finally, the concentrated product was stored at -20°C for future applications.

Experimental fish

Fingerlings of *L. rohita* (average weight: 6.29±0.02 g) were procured from a local market and transported to the designated experimental site. They were allowed to acclimate under controlled laboratory conditions for a period of 15 days. The fingerlings were treated with five grams per liter solution of NaCl to mitigate parasites and minimize microbial infections (Rowland and Ingram, 1991). Prior to the experimental trial, the fingerlings were provided with a basal diet until they exhibited signs of satiation, as recommended by Allen and Rowland (1992). A capillary aeration system was employed to ensure continuous oxygen supply to the experimental tanks, while daily monitoring and maintenance of water quality parameters such as dissolved oxygen (7.5 mg/L), temperature (26-29 °C) and pH (7.7) were diligently performed.

Experimental design

P. oleracea was utilized as a test component to formulate the test diet, resulting in the preparation of seven diets for this study project. One group served as a control and received a diet without extract (the basal diet), while the other groups received purslane extract at various concentrations. Each experimental group was assigned to triplicate tanks, each containing fifteen fingerlings. SFM-based diets supplemented with purslane extract were administered to the fingerlings twice daily, equivalent to 5% of the fish's body weight. After each two-h feeding session, any residual feed in each tank was removed by opening the tank valves. Following a thorough cleaning process to eliminate any remaining feed particles, the tanks were refilled with water. Valves I and II were opened to collect feces from each tank using a fecal collecting tube. Precautions were taken to prevent the breakage of small fecal strands to minimize nutrient loss. A 90-day feeding study was conducted.

Test diet

Initially, the diet ingredients (Table I) were grinded and crushed to achieve a uniform consistency. Following

this, the crushed materials, along with purslane extract, mixed for five minutes to ensure even distribution. Subsequently, fish oil gradually incorporated into the mixture, providing essential nutrients. To attain the ideal dough-like texture for pelleting, 10-15% water introduced. Finally, the dough passed through specialized pelleting equipment to form uniform pellets, facilitating ease of handling and distribution as animal feed (Lovell, 1989). Seven test diets were formulated using purslane extract at levels of 0%, 0.5%, 1%, 1.5%, 2%, 2.5%, and 3%.

Table I. Test diet composition (%).

Ingredients	T ₀ (Control)	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆
<i>P. oleracea</i> extract (%)	0	0.5	1	1.5	2	2.5	3
Sunflower meal	54	54	54	54	54	54	54
Fish meal	17	17	17	17	17	17	17
Wheat flour*	11	10.5	10	9.5	9	8.5	8
Rice polish	8	8	8	8	8	8	8
Fish oil	6	6	6	6	6	6	6
Vitamin premix**	1	1	1	1	1	1	1
Mineral mixture***	1	1	1	1	1	1	1
Ascorbic acid	1	1	1	1	1	1	1
Chromic oxide	1	1	1	1	1	1	1

* Purslane extract supplemented at the cost of wheat flour. **Mineral premix kg-1: Fe: 1000 mg, Ca: 155 g, Co: 40 mg, P: 135 g, Se: 3 mg, Na: 45 g, Zn:3000mg, Cu: 600 mg, Mg: 55 g, Mn: 2000 mg, I: 40 mg. ***Vitamin (Vit.) premix kg-1: Vit. B12: 40 mg, Vit. D3: 3,000,000 IU, Vit. A: 15,000,000 IU, Vit. C: 15,000 mg, B2: 7000 mg, Vit. K3: 8000 mg, Vit. B6: 4000 mg, Vit. Ca pantothenate: 12,000 mg, Folic acid: 1500 mg, Nicotinic acid: 60,000 mg.

Proximate analysis

Carcass composition of fingerlings was determined by using standard protocols of AOAC (2016). Moisture content was determined by subjecting the samples to a 12-h oven-drying process at 105 °C. The evaluation of crude protein (CP) content (N×6.25) was conducted using a micro Kjeldahl apparatus. Ether extract (EE) was obtained via the petroleum ether extraction method, employing the Soxhlet HT2 1045 system. Ash content was determined through a 12-h combustion process in an electric furnace at 650°C (Eyela-TMF 3100) until reaching a constant weight. The overall energy content of both feces and diet samples was ascertained using an adiabatic oxygen bomb calorimeter.

Growth assessment

Growth of fish in means of weight gain (WG), weight gain percentage (WG%), feed conversion ratio (FCR), specific growth rate (SGR) and protein efficiency ratio

(PER) was measured using following standard equations:

$$WG = \text{final body weight} - \text{initial body weight}$$

$$\% WG = \frac{\text{Final weight (g)} - \text{Initial weight(g)}}{\text{Initial Weight}} \times 100$$

$$FCR = \frac{\text{Total dry feed intake (g)}}{\text{Wet weight gain (g)}}$$

Specific growth rate

$$SGR = \frac{[\ln(\text{final weight}) - \ln(\text{initial weight})]}{\text{Duration of trial in day}} \times 100$$

$$PER = \text{Gain in weight (g)} / \text{protein intake in feed (g)}$$

$$\text{Fish survival (\%)} = 100 (\text{final fish number}/\text{initial fish number})$$

Hematological studies

Blood samples were collected from the caudal vein of anesthetized fish using an anticoagulated syringe and transported to the Department of Zoology at GCUF for hematological analysis. By using clove oil (60 mg/L) for at least 05 min, fish were immobilized (Javahery *et al.*, 2012). Hematocrit levels were determined using the micro-hematocrit method with capillary tubes, following the protocol outlined by Brown (1988). White blood cells (WBCs) and red blood cells (RBCs) were enumerated using a hemocytometer equipped with a certified Neubauer counting chamber, as described by Blaxhall and Daisley (1973). Hemoglobin (Hb) concentration was measured based on the protocols established by Wedemeyer and Yastuk (1977). Mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV), and mean corpuscular hemoglobin (MCH) were calculated by using standard equations described by Hussain *et al.* (2024).

Mineral analysis

By using an atomic absorption spectrophotometer, the mineral content of fingerlings was determined (AOAC, 2005).

Statistical analysis

A microcomputer was applied to statistically analyze data on growth, body composition, hematology, and body-mineralization. The data were determined using one-way analysis of variance (ANOVA) (Steel *et al.*, 1996). Tukey's honest significant difference test was used to compare means, with $p < 0.05$ indicating significance (Snedecor and Cochran, 1991). The statistical analysis was carried out using the Co-Stat software (Version 6.303, PMB 320, Monterey, CA, 93940 USA).

RESULTS

Growth and survival rate of fish

Current findings indicated that when concentrations of purslane extract increased from 0.5% to 1.5%, there was

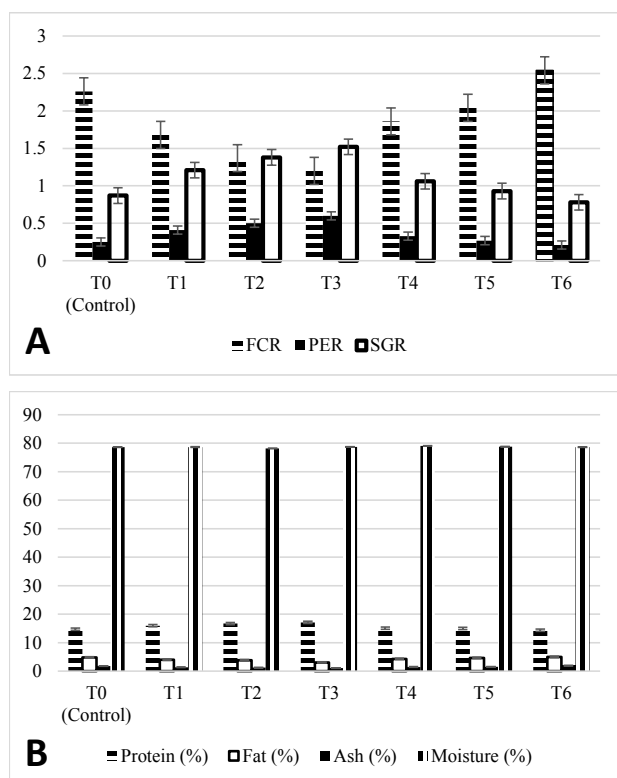


Fig. 1. Effect of different concentrations of purslane extract supplemented sunflower meal-based diets on growth performance (A) and body composition (B) of *L. rohita*. FCR, food conversion ratio; SGR, specific growth rate; PER, protein efficiency ratio. T₀, T₁-T₆, purslane extract at 0%, 0.5%, 1%, 1.5%, 2%, 2.5% and 3%, respectively.

an improvement in growth indices such as FW, WG, WG%, SGR, and survival rate. However, at higher concentrations (2% and 2.5%), there was a slight decrease in growth performance compared to the optimal concentrations (0.5%, 1% and 1.5%). Furthermore, increasing purslane extract

concentrations (0.5%, 1% and 1.5%) improved the FCR and PER, indicating that feed was used more efficiently for growth. These results demonstrated that the inclusion of purslane extract up to 1.5% in aquafeed formulations is the optimal level for the growth performance of fish (Table II and Fig. 1A).

Proximate composition of fish

Figure 1B shows the carcass composition of *L. rohita* fed meals containing varying percentages of purslane extract supplemented with SFM. As the concentrations of purslane extract increased from T₁ to T₃ (0.5%-1.5%), protein content increased significantly. Conversely, as the concentrations of purslane extract increased, fat content decreased. Ash content slightly varied across all treatments and moisture content did not vary significantly among treatments. Overall, the results suggest that adding purslane extract (up to 1.5%) into the diet, positively altered the protein and fat composition of fish, demonstrating its potential as a dietary supplement for improving nutritional quality.

Hematological indices

This study found that, as the concentrations of purslane extract increased from T₁ (0.5%) to T₃ (1.5%), various parameters showed notable changes. RBCs increased from T₁ to T₃, maximum at 1.5% purslane extract concentration, then dropping at control (0%) and higher concentration (3%), respectively. WBCs rise steadily with increasing purslane extract content, climaxing at T₃ and platelet count followed a similar trend as well. Hb levels increased up to T₃ and then decreased somewhat. PCV progressively rose up to T₃ before slightly dropping. The observed changes in MCHC, MCH, and MCV indicate potential alterations in the size and hemoglobin content of RBCs, reflecting possible improvements in erythrocyte function and morphology (Table III).

Table II. Growth of *L. rohita* fed with different concentrations of purslane extract supplemented sunflower meal-based diets.

Test diets/ Purslane extract	T ₀ (Control) (0%)	T ₁ (0.5%)	T ₂ (1%)	T ₃ (1.5%)	T ₄ (2%)	T ₅ (2.5%)	T ₆ (3%)
Initial weight (g)	6.29±0.02 ^a	6.26±0.03 ^a	6.16±0.02 ^c	6.15±0.03 ^c	6.19±0.02 ^{bc}	6.23±0.02 ^{ab}	6.24±0.03 ^{ab}
Final weight (g)	13.75±0.59 ^{ef}	18.54±0.59 ^c	21.28±0.73 ^b	24.17±0.75 ^a	16.04±0.35 ^d	14.34±0.47 ^c	12.61±0.64 ^f
Weight gain (g)	7.46±0.61 ^{ef}	12.28±0.57 ^c	15.12±0.74 ^b	18.02±0.73 ^a	9.84±0.34 ^d	8.11±0.46 ^e	6.36±0.65 ^f
Weight gain (%)	118.73±9.90 ^{ef}	195.99±8.32 ^c	245.40±12.07 ^b	293.19±10.65 ^a	158.93±5.29 ^d	130.10±7.24 ^c	101.93±10.53 ^f
Feed intake	0.19±0.01 ^{bc}	0.23±0.001 ^a	0.23±0.01 ^a	0.24±0.001 ^a	0.20±0.01 ^b	0.18±0.01 ^c	0.18±0.001 ^c
Survival rate (%)	96	100	100	100	99	98	95

Rows with various superscripts indicate significant differences at a significance level of $p < 0.05$. The values represent the mean of three replicates.

Table III. Effect of different concentrations of purslane extract supplemented sunflower meal-based diets on hematology of *L. rohita*.

Test diets/ Purslane extract	T ₀ (Control) (0%)	T ₁ (0.5%)	T ₂ (1%)	T ₃ (1.5%)	T ₄ (2%)	T ₅ (2.5%)	T ₆ (3%)
RBCs (10 ⁻⁶ mm ⁻³)	1.96±0.05 ^c	2.63±0.11 ^{bc}	2.79±0.03 ^b	3.01±0.10 ^a	2.44±0.07 ^c	2.17±0.09 ^d	1.84±0.04 ^c
WBCs (10 ⁻⁶ mm ⁻³)	6.57±0.16 ^d	7.02±0.13 ^{bc}	7.23±0.09 ^{ab}	7.49±0.23 ^a	6.87±0.12 ^{bcd}	6.71±0.22 ^{cd}	6.43±0.09 ^d
PLT	57.16±0.17 ^f	62.09±0.14 ^c	63.38±0.25 ^b	67.55±0.24 ^a	60.59±0.32 ^d	58.34±0.29 ^c	54.44±0.21 ^g
Hb (g/100 mL)	7.34±0.11 ^d	8.32±0.16 ^{bc}	8.67±0.12 ^{ab}	9.00±0.20 ^a	7.95±0.15 ^c	7.51±0.16 ^d	7.26±0.13 ^d
PCV (%)	22.07±0.13 ^{ef}	22.95±0.13 ^{bc}	23.28±0.11 ^b	24.05±0.13 ^a	22.74±0.14 ^{cd}	22.38±0.16 ^{de}	21.98±0.17 ^f
MCHC (%)	33.25±0.37 ^c	35.83±1.47 ^{ab}	37.22±0.67 ^{ab}	37.41±0.83 ^a	34.95±0.85 ^{bc}	33.53±0.60 ^c	33.03±0.46 ^c
MCH (pg)	40.35±1.05 ^b	31.67±1.87 ^{cd}	31.02±0.38 ^{cd}	29.86±0.42 ^d	32.56±1.56 ^{cd}	34.66±0.84 ^c	44.08±2.02 ^a
MCV (fl)	112.86±3.15 ^a	87.34±3.26 ^{cd}	83.35±0.82 ^d	79.85±2.49 ^d	93.12±2.26 ^c	103.39±3.70 ^b	119.49±2.96 ^a

WBC, white blood cell; RBC, red blood cell; PLT, platelet; PCV, packed cell volume; Hb, hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin. Rows with various superscripts indicate significant differences at a significance level of $p < 0.05$. The values represent the mean of three replicates.

Table IV. Effect of different levels of purslane extract supplemented sunflower meal-based diets on whole-body mineralization of *L. rohita*.

Test diets/ Purslane extract	T ₀ (Control) (0%)	T ₁ (0.5%)	T ₂ (1%)	T ₃ (1.5%)	T ₄ (2%)	T ₅ (2.5%)	T ₆ (3%)
Ca (%)	0.78±0.16	0.96±0.11	1.03±0.10	1.11±0.19	0.89±0.17	0.83±0.15	0.72±0.17
Na (mg/g)	5.50±0.10 ^{cd}	5.85±0.10 ^{abc}	5.94±0.10 ^{ab}	6.04±0.14 ^a	5.74±0.19 ^{abcd}	5.61±0.18 ^{bcd}	5.41±0.10 ^d
P (%)	0.91±0.14 ^b	1.10±0.13 ^{ab}	1.19±0.15 ^{ab}	1.32±0.14 ^a	1.03±0.15 ^{ab}	0.97±0.13 ^{ab}	0.86±0.17 ^b
Mg (%)	2.94±0.12 ^{bc}	3.17±0.16 ^{abc}	3.29±0.15 ^{ab}	3.41±0.16 ^a	3.06±0.16 ^{abc}	3.01±0.09 ^{abc}	2.80±0.27 ^c
Fe (μg/g)	48.09±0.32 ^f	57.91±0.17 ^c	60.97±0.11 ^b	64.09±0.14 ^a	55.10±0.23 ^d	51.32±0.20 ^e	45.83±0.36 ^g
Mn (μg/g)	8.04±0.11 ^{ef}	10.35±0.21 ^c	11.39±0.34 ^b	12.75±0.23 ^a	9.10±0.34 ^d	8.67±0.20 ^{de}	7.74±0.23 ^f
Zn (μg/g)	3.21±0.20 ^{de}	3.84±0.18 ^{abc}	4.05±0.17 ^{ab}	4.33±0.18 ^a	3.62±0.31 ^{bcd}	3.38±0.21 ^{cde}	2.97±0.29 ^e
K (%)	7.29±0.15 ^{cd}	8.36±0.07 ^{ab}	8.54±0.38 ^{ab}	8.96±0.19 ^a	7.98±0.14 ^{bc}	7.54±0.46 ^{cd}	7.02±0.15 ^d
Cu (μg/g)	3.04±0.14 ^{cd}	3.92±0.10 ^{bc}	4.12±0.14 ^{ab}	4.36±0.19 ^a	3.60±0.16 ^{bc}	3.37±0.20 ^{cd}	2.85±0.19 ^d

Rows with various superscripts indicate significant differences at a significance level of $p < 0.05$. The values represent the mean of three replicates.

Fish body-mineralization

This study suggested that addition of 1.5% of purslane extract resulted in an significant increase in Ca, P, Mg, Fe, Mn, Zn, K, and Cu, compared to the control group (0%) and T₆ (3%). However, at the control group (T₀) and maximum concentration of purslane extract (T₆), mineral levels decreased as compared to T₃. Furthermore, Na levels varied widely across diets, indicating a less consistent association with purslane extract content. This suggested that purslane extract could enhance mineralization in *L. rohita* at moderate levels (T₁, T₂ and T₃) but excessive supplementation could lead to diminishing uptake and deposition of minerals (Table IV).

DISCUSSION

Feed additives offer nutritional security to fish by

improving their growth, health, diet stability, flavor, and palatability. The goal of feed additives is to improve the texture and stability of fish diets by using their significance in fish feed (Yadav *et al.*, 2021). Incorporating the herb *P. oleracea* into fish feed, is a sustainable and potentially cost-effective solution (Sourani *et al.*, 2023). This invention advances circular economy principles by transforming waste into valuable feedstock (Amalia *et al.*, 2024). Purslane has been widely used in animal diets in recent years to improve growth and health because of its high concentration of bioactive compounds (Wang *et al.*, 2021). This is the first study to look at how purslane extract supplementation affects the overall performance of rohu fish.

Several studies have reported influence of purslane added into fish feed on growth performance. Mohammadalikhani *et al.* (2020) have shown that adding

varying quantities of purslane extract to rainbow trout diets boosted fish growth efficiency. A similar type of study with goldfish done by [Sahin et al. \(2021\)](#) found that administering purslane extract increased fish growth and survival. A further study indicated that adding purslane to broiler chicken's diets greatly improved the growth performance ([Wang et al., 2021](#)). The present investigation supported [Ahmadifar et al. \(2020\)](#), who found that adding 0.5% purslane to the fish feed improved the growth performance of grass carp. [Zenhom et al. \(2014\)](#) utilized purslane seeds in the feed of Nile tilapia and found that growth performance improved dramatically when compared to the control group. Furthermore, increasing the amount of purslane seeds in fish diets greatly boosted their survival rates. [Okafor et al. \(2014\)](#) claimed that adding purslane in the diet of broiler chickens improved the growth performance due to high antioxidant capacity of purslane. Purslane's nutritional profile, notably its omega-3 fatty acid concentration, is important for improving growth parameters. Besides, for aquaculture animals, it has been found that bioactive substances in weeds can act as tasty appetizer for animals ([Ahmadifar et al., 2020](#)). [Zhao et al. \(2013\)](#) have reported that purslane extract enhances the flora of beneficial bacteria, as lactic acid bacteria, in broiler chickens. Contradictory results were found at the end of the feeding experiment, and noticed that growth performance dramatically decreased when increasing the amounts of purslane in the diet of Nile tilapia, relative to the control group ([Abdel-Razek et al., 2019](#)). According to [Zemheri-Navruz et al. \(2019\)](#), variations in feeding periods, feed additive dosages, and fish species themselves could lead to inconsistent growth results amongst fish species. It has also been found that anti-nutritional substances such as oxalate and phytate in purslane, could adversely affect the growth of fish when purslane added at high concentrations ([Ahmadifar et al., 2020](#)).

Purslane has the potential to improve body composition in aquaculture due to its high nutritional value, which includes omega-3 fatty acids, proteins, vitamins, minerals, and antioxidants. The present study found that purslane extract supplemented SFM-based diets considerably improved the body composition of fish compared to the control group. [Zenhom et al. \(2014\)](#) discovered that supplementing Nile tilapia with purslane seeds greatly improved their body composition. Authors indicated that increasing the different percentages of purslane in fish feed resulted in higher protein content and reduced lipids in the fish body compared to the control group.

The results of the current study revealed that purslane extract supplemented SFM-based diets substantially enhanced the hematological parameters of fish fingerlings

compared to the control group. [Muhammad et al. \(2020\)](#) employed purslane in rainbow trout diets and found that hematological parameters such as RBC, WBC, and HB were significantly improved compared to control groups. [Habibian et al. \(2017\)](#) reported the inclusion of purslane in broiler chicken diets enhanced Hb concentration and RBC counts. These positive effects are linked to phenolic acids, flavonoids, vitamin E, and vitamin C ([Varmaghany et al., 2015](#)), as well as due to high concentration of n-3 fatty acids in purslane ([Okafor et al., 2014](#)).

Our findings suggested that supplementing *L. rohita* diets with different levels of purslane extract enhanced the fish's overall mineral composition. It could be because purslane leaves contain minerals including Mg, P, Na, K, and Ca ([Srivastava et al., 2023](#)). [Shalaei et al. \(2014\)](#) fed purslane seed to laying hens and found that the concentration of mineral contents in the blood plasma, including calcium, phosphorus, iron, and magnesium, was not significantly altered by the purslane-based diet.

CONCLUSION

In conclusion, the incorporation of purslane (*P. oleracea*) extract into SFM-based diets has shown promising potential in improving the growth, body composition, hematology and body mineralization of *L. rohita* fingerlings. This study suggested that inclusion of 1.5% purslane extract in the diet is the optimal level for performance of rohu fish. Future studies needed to explore the underlying mechanisms (effects on gene expression, hormone regulation and metabolic pathways) through which purslane extract exerts its effects on performance of rohu fish.

DECLARATIONS

Acknowledgments

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IRB approval

All the procedures and methods used in this study followed the ethical guidelines provided by Government College University Faisalabad (Ref No. GCUF/ERC/436).

Ethical statement

All applicable institutional, national and international guidelines for the care and use of animals were followed.

Statement of conflict of interest

The authors have declared no conflict of interest.

REFERENCES

- Abdel-Latif, H.M., Abdel-Tawwab, M., Dawood, M.A., Menanteau-Ledouble, S. and El-Matbouli, M., 2020. Benefits of dietary butyric acid, sodium butyrate, and their protected forms in aquafeeds: A review. *Rev. Fish. Sci. Aquac.*, **28**: 421-448. <https://doi.org/10.1080/23308249.2020.1758899>
- Abdel-Razek, N., Awad, S.M., and Abdel-Tawwab, M., 2019. Effect of dietary purslane (*Portulaca oleracea* L.) leaves powder on growth, immunostimulation, and protection of Nile tilapia, *Oreochromis niloticus* against *Aeromonas hydrophila* infection. *Fish Physiol. Biochem.*, **45**: 1907-1917. <https://doi.org/10.1007/s10695-019-00685-8>
- Ahmadifar, E., Hoseinifar, S.H., Adineh, H., Moghadam, M.S. and Dawood, M.A., 2020. Assessing the impact of purslane (L.) on growth performance, anti-oxidative, and immune activities in grass carp. *Annls Anim. Sci.*, **20**: 1427-1440. <https://doi.org/10.2478/aoas-2020-0042>
- Allen, G. and Rowland, S., 1992. Development of an experimental diet for silver perch (*Bidanus bidanus*). *Austas. Aquacult.*, **6**: 39-40.
- Amalia, R.L.R., Suryaningrum, L.H., Sumitro, S., Budiyaniti, B., Rohmy, S., Nur, B. and Mulyasari, M., 2024. Valorization of weed *Portulaca oleracea* L. as an alternative to fish feed ingredient. In: *BIO Web Conf.*, EDP Sciences. **87**: 03029. <https://doi.org/10.1051/bioconf/20248703029>
- AOAC, 2005. *Official methods of analysis*. 15th Ed. Association of Official Analytical Chemists. Washington, D.C. USA., pp. 1094. <https://doi.org/10.1002/0471740039.vec0284>
- AOAC, 2016. *Official methods of analysis of AOAC International (20th ed)*. AOAC International.
- Bernard, J.K., 2016. *Feedingredients: Feed concentrates-oil seed and oilseed meals*. *Encyclopedia of dairy sciences* (2nd ed.). Academic Press, New York. pp. 349-355. <https://doi.org/10.1016/B978-0-12-374407-4.00165-5>
- Blaxhall, P.C. and Daisley, K.W., 1973. Routine hematological methods for use with fish blood. *J. Fish Biol.*, **5**: 771-781. <https://doi.org/10.1111/j.1095-8649.1973.tb04510.x>
- Brown, B.A., 1988. *Hematology: Principle and Procedures*. pp. 7-122.
- FAO, 2022. *The state of world fisheries and aquaculture 2022*. Towards blue transformation. Rome, FAO. <https://doi.org/10.4060/cc0461en>
- Golden, C.D., Koehn, J.Z., Shepon, A., Passarelli, S., Free, C.M., Viana, D.F. and Thilsted, S.H., 2021. Aquatic foods to nourish nations. *Nature*, **598**: 315-320. <https://doi.org/10.1038/s41586-021-03917-1>
- Habibian, M., Sadeghi, G. and Karimi, A., 2017. Effects of purslane (*Portulaca oleracea* L.) powder on growth performance, blood indices, and antioxidant status in broiler chickens with triiodothyronine-induced ascites. *Arch. Anim. Breed.*, **60**: 315-325. <https://doi.org/10.5194/aab-60-315-2017>
- Hanan, A.A., Sobhy, M.H., Kawkab, A.A., Azza, K.A., Zeinab, A.R. and Wedad, A.H., 2014. Chemical and remedial effects of purslane (*Portulaca oleracea*) plant. *Life Sci. J.*, **11**: 31-42.
- Hossain, M.S., Small, B.C., Kumar, V. and Hardy, R., 2024. Utilization of functional feed additives to produce cost-effective, ecofriendly aquafeeds high in plant-based ingredients. *Rev. Aquac.*, **16**: 121-153. <https://doi.org/10.1111/raq.12824>
- Hussain, S.M., Naeem, E., Ali, S., Adrees, M., Riaz, D., Paray, B.A. and Naeem, A., 2024. Evaluation of growth, nutrient absorption, body composition and blood indices under dietary exposure of iron oxide nanoparticles in Common carp (*Cyprinus carpio*). *J. Anim. Physiol. Anim Nutr.*, **108**: 366-373. <https://doi.org/10.1111/jpn.13898>
- Javahery, S., Nekoubin, H. and Moradlu, A.H., 2012. Effect of anaesthesia with clove oil in fish. *Fish Physiol. Biochem.*, **38**: 1545-1552. <https://doi.org/10.1007/s10695-012-9682-5>
- Khan, M.W., and Abbas, G., 2023. Studies on the use of aquatic food in Pakistan. *J. Zool. Syst.*, **1**: 40-57. <https://doi.org/10.56946/jzs.v1i2.246>
- Köprücü, K. and Sertel, E., 2012. The effects of less-expensive plant protein sources replaced with soybean meal in the juvenile diet of grass carp (*Ctenopharyngodon idella*): Growth, nutrient utilization and body composition. *Aquacult. Int.*, **20**: 399-412. <https://doi.org/10.1007/s10499-011-9471-7>
- Kumar, A., Sreedharan, S., Kashyap, A.K., Singh, P. and Ramchiary, N., 2022. A review on bioactive phytochemicals and ethnopharmacological potential of purslane (*Portulaca oleracea* L.). *Heliyon*, **8**. <https://doi.org/10.1016/j.heliyon.2021.e08669>
- Lovell, T., 1989. *Nutrition and feeding of fish*. Van Nostrand Reinhold, New York. **260**. <https://doi.org/10.1007/978-1-4757-1174-5>
- Magouz, F.I., Mahmoud, S.A., El-Morsy, R.A., Paray, B.A., Soliman, A.A., Zaineldin, A.I. and Dawood,

- M.A., 2021. Dietary menthol essential oil enhanced the growth performance, digestive enzyme activity, immune-related genes, and resistance against acute ammonia exposure in Nile tilapia (*Oreochromis niloticus*). *Aquaculture*, **530**: 735944. <https://doi.org/10.1016/j.aquaculture.2020.735944>
- Miao, L., Tao, H., Peng, Y., Wang, S., Zhong, Z., El-Seedi, H. and Xiao, J., 2019. The anti-inflammatory potential of *Portulaca oleracea* L. (purslane) extract by partial suppression on NF- κ B and MAPK activation. *Fd. Chem.*, **290**: 239-245. <https://doi.org/10.1016/j.foodchem.2019.04.005>
- Mohammadalikahni, M., Shamsaie, M.M., Haghghi, M., Soltani, M. and Kamali, A., 2020. Effects of oral administration of dried purslane (*Portulaca oleracea*) extract on some growth indices, carcass quality and intestinal microbial flora of rainbow trout (*Oncorhynchus mykiss*) fry. *J. Anim. Environ.*, **12**: 229-236.
- Mridul, M.M.I., Zeehad, M.S.K., Aziz, D., Salin, K.R., Hurwood, D.A. and Rahi, M.L., 2024. Temperature induced biological alterations in the major carp, Rohu (*Labeo rohita*): Assessing potential effects of climate change on aquaculture production. *Aquacult. Rep.*, **35**: 101954. <https://doi.org/10.1016/j.aqrep.2024.101954>
- Ocampo, G. and Columbus, J.T., 2012. Molecular phylogenetics, historical biogeography, and chromosome number evolution of *Portulaca* (Portulacaceae). *Mol. Phylogen. Evol.*, **63**: 97-112. <https://doi.org/10.1016/j.ympev.2011.12.017>
- Okafor, I.A., Ayalokunrin, M.B. and Orachu, L.A., 2014. A review on *Portulaca oleracea* (purslane) plant its nature and biomedical benefits. *Int. J. Biomed. Res.*, **5**: 75-80. <https://doi.org/10.7439/ijbr.v5i2.462>
- Rahi, M.L. and Shah, M.S., 2012. Triploidization in rohu \times mrigal hybrid and comparison of growth performance of triploid hybrid. *Aquacult. Res.*, **43**: 1867-1879. <https://doi.org/10.1111/j.1365-2109.2011.02996.x>
- Rodrigues, I.M., Coelho, J.F. and Carvalho, M.G.V., 2012. Isolation and valorisation of vegetable proteins from oilseed plants: Methods, limitations and potential. *J. Fd. Engin.*, **109**: 337-346. <https://doi.org/10.1016/j.jfoodeng.2011.10.027>
- Rowland, S.J. and Ingram, B.A., 1991. Diseases of Australian native fishes. *Fish Bull.*, **4**: 21-23.
- Roychowdhury, P., Aftabuddin, M. and Pati, M.K., 2020. Thermal stress altered growth performance and metabolism and induced anaemia and liver disorder in *Labeo rohita*. *Aquacult. Res.*, **51**: 1406-1414. <https://doi.org/10.1111/are.14486>
- Şahin, D., Meryem, Ö.Z., Aral, O., Bahtiyar, M. and Taşçi, S., 2021. Growth and pigmentation of goldfish (*Carassius auratus* L, 1758) fed on a diet supplemented with purslane (*Portulaca* sp.) extract. *Magnesium (Mg, ppm)*, **1727**: 1725. <https://doi.org/10.17582/journal.pjz/20210318090307>
- Saleh, A.A., El-Awady, A., Amber, K., Eid, Y.Z., Alzawqari, M.H., Selim, S. and Shukry, M., 2021. Effects of sunflower meal supplementation as a complementary protein source in the laying hen's diet on productive performance, egg quality, and nutrient digestibility. *Sustainability*, **13**: 3557. <https://doi.org/10.3390/su13063557>
- Sangeetha, S., Kiran, R.S., Abbulu, K. and Battu, S., 2020. A review on traditional herb *Portulaca oleracea*. *World J. Pharm. Res.*, **9**: 578-601.
- Shalaei, M. and Hosseini, S.M., 2014. *Effect of different levels of purslane seed on blood parameters, plasma minerals, liver enzymes and some egg characteristics in laying hens*. http://ar.guilan.ac.ir/article_90_2c7efd3d99b0ff9650f5b81f323dc777.pdf
- Shchekoldina, T. and Aider, M., 2014. Production of low chlorogenic and caffeic acid containing sunflower meal protein isolate and its use in functional wheat bread making. *J. Fd. Sci. Technol.*, **51**: 2331-2343. <https://doi.org/10.1007/s13197-012-0780-2>
- Snedecor, G.W. and Cochran, W.G., 1991. *Statistical methods*. 8th Ed. Iowa State University, Press, Americans. USA, pp. 503.
- Sourani, Z., Shirian, S., Shafiei, S., Mosayebi, N. and Nematollahi, A., 2023. Modulation of immune-related gene expressions in zebrafish (*Danio rerio*) by dietary purslane (*Portulaca oleracea*) extract. *Mar. Biotechnol.*, **25**: 214-221. <https://doi.org/10.1007/s10126-022-10195-z>
- Srivastava, R., Srivastava, V. and Singh, A., 2023. Multipurpose benefits of an underexplored species purslane (*Portulaca oleracea* L.): A critical review. *Environ. Manage.*, **72**: 309-320. <https://doi.org/10.1007/s00267-021-01456-z>
- Steel, R.G.D., Torrie, J.H. and Dickey, D.A., 1996. *Principles and procedures of statistics* (3rd ed.). McGraw Hill international Book Co. Inc., New York. USA, pp. 336-352.
- Syanya, F.J., Mathia, W.M. and Harikrishnan, M., 2023. Current status and trend on the adoption of fish feed additives for sustainable tilapia aquaculture production: A review. *Asian J. Fish. aquat. Res.*, **22**: 10-25. <https://doi.org/10.9734/ajfar/2023/v22i3571>
- Varmaghany, S., Torshizi, M.A.K., Rahimi, S.,

- Lotfollahian, H. and Hassanzadeh, M., 2015. The effects of increasing levels of dietary garlic bulb on growth performance, systolic blood pressure, hematology, and ascites syndrome in broiler chickens. *Poult. Sci.*, **94**: 1812-1820. <https://doi.org/10.3382/ps/pev148>
- Wang, C., Liu, Q., Ye, F., Tang, H., Xiong, Y., Wu, Y. and Huang, J., 2021. Dietary purslane (*Portulaca oleracea* L.) promotes the growth performance of broilers by modulation of gut microbiota. *AMB Express*, **11**: 1-11. <https://doi.org/10.1186/s13568-021-01190-z>
- Wedemeyer, G.A. and Yasutake, W.T., 1977. Clinical methods for the assessment of the effects of environmental stress on fish health. *Dept. Interior; Fish Wildl. Ser.*, **89**.
- Yadav, M., Khati, A., Chauhan, R., Arya, P. and Semwal, A., 2021. A review on feed additives used in fish diet. *Int. J. Environ. agric. Biotechnol.*, **6**. <https://doi.org/10.22161/ijeab.62.21>
- Zenhom, M.M. and Khames, M.K., 2014. Effect of purslane seeds (*Portulaca oleraceae*) on growth performance, feed utilization and body composition of Nile-tilapia (*Oreochromis niloticus*). *Abbassa Int. J. Aquacult.*, **7**: 210-224.
- Zhao, X.H., He, X., Yang, X.F. and Zhong, X.H., 2013. Effect of *Portulaca oleracea* extracts on growth performance and microbial populations in ceca of broilers. *Poult. Sci.*, **92**: 1343-1347. <https://doi.org/10.3382/ps.2012-02434>
- Zhou, Y.X., Xin, H.L., Rahman, K., Wang, S.J., Peng, C. and Zhang, H., 2015. *Portulaca oleracea* L.: A review of phytochemistry and pharmacological effects. *BioMed Res.*, **2015**: 925631. <https://doi.org/10.1155/2015/925631>

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