

Research Article

Partial Replacement of Fish Meal with an Aquatic Macrophyte, *Ceratophyllum demersum* in the Diet of Common Carp, *Cyprinus carpio* var. *communis* Fingerlings

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A 12-week feeding trial was conducted to investigate the nutrient utilization, growth performance, and hematological indices of *Cyprinus carpio* var. *communis* fingerlings fed diets based on aquatic macrophyte, *Ceratophyllum demersum* as a replacement of fish meal (FM). Six isonitrogenous and isocaloric diets containing graded levels of *C. demersum* 0%, 5%, 10%, 15%, 20%, and 25%, respectively, as replacer of FM were formulated. Total of 360 fingerlings with an initial weight of 3.65 ± 0.98 g were randomly stocked in 70 L plastic tanks water volume 60 L connected with a continuous flow-through system (1–1.5 L/min) for each treatment and were run in triplicate having 20 fish in each tank. At the end of the feeding trial, it was observed that the *C. carpio* var. *communis* fingerlings receiving 5% and 10% *C. demersum* in the diet showed improvements in live weight gain (LWG), specific growth rate (SGR), feed conversion ratio (FCR), and protein efficiency rate (PER). While further addition of *C. demersum* as a replacement of FM from 15% to 25% led to the progressive decline in the values of LWG, SGR, FCR, and PER. Hematological data also exhibited a linear declining trend beyond the 10% *C. demersum* replacement level. The fish fed with higher inclusions of *C. demersum* in each diet significantly ($P < 0.05$) affected whole body composition with the lowest protein and fat amounts recorded at higher replacement levels significantly ($P < 0.05$). The highest protein and fat contents were observed at 5% and 10% diets. Except serum glucose, cholesterol, alanine aminotransferase (ALT), and aspartate aminotransferase (AST), the other serum indices exhibited a decreasing trend with increasing inclusion of *C. demersum* in the diet. The results of the present study clearly demonstrated that the inclusion of 10% *C. demersum* did not affect the growth and other parameters of *C. carpio* var. *communis* fingerlings compared to the 50% FM diet. However, it is recommended that 10% FM can be substituted without compromising the growth and nutritional quality of fish.

1. Introduction

The contribution of worldwide aquaculture to the total production of fish got increased from 25.7% to 46% between 2000 and 2018, and it is anticipated that it will reach up to 50% by 2030 [1, 2]. Aquaculture sector plays an important role in reducing hunger and enhancing food security in accordance with the sustainable development goals of the United Nations. One of the main sources of protein in fish diet is fish meal, due to its high protein content, balanced amino acids, and good digestibility. However, due to its enormous demand, severe decline in supply, and rising market prices [3], the fish meal inclusion level in fish feed has been

significantly declined [4]. Moreover, because of the high cost of fish meal, a sizable amount of expenditures associated with producing farmed fish are involved in the formulation of feed [5, 6].

In order to reduce the cost of fish feed in aquaculture and to ensure a sufficient supply of nutrients for optimum growth, less expensive protein source from both plant and animal origin have been studied as prospective substitutes for fish meal [7]. The sustainability of aquaculture could be increased by substituting a more sustainable protein source that does not compete with human food for fish meal in the diets of fish fries and fingerlings [8]. Scientists must therefore devise new methods to supply the necessary quantities of

high-quality protein in order to satisfy the expanding demand [9]. Although, many authors have suggested plant-based protein components as a possible alternative to fish meals, particularly in the light of their perceived lower cost compared to fish meals [10]. In this scenario, the replacement of fish meal with aquatic macrophytes has a great potential to revolutionize aquaculture and helps the world's population to satisfy its protein needs.

Being the principal producers in aquatic ecosystems, aquatic macrophytes have a considerable impact on biodiversity at the ecosystem level; therefore, turning these weeds into fecund plants would be expected if it could at least partially offset the costs of mechanical removal [11]. Macrophytes are enriched with protein (11%–32%), lipids (3%–16.8%) in case of naturally grown [12], and up to 37% protein and 21% ash in case of cultivated ones [13]. These have been acknowledged as a natural resource for nutrient contents due to their possible biochemical profile [14]. In light of this, an aquatic macrophyte, *Ceratophyllum demersum*, has been used in the present study as an effective ingredient for the partial replacement of fish meal after being assessed for its near composition. *C. demersum*, sometimes referred to as coontail or hornwort, is a dicotyledonous aquatic angiosperm that is submerged and is a member of the Ceratophyllaceae family. It has a global range representing freshwater macrophytes. It grows as a nearly free-floating aquatic plant in still or slowly moving waters that are hard, calcareous, nutrient-rich, or eutrophic in streams, ditches, canals, ponds, and lakes where it may form large masses [15]. Due to its endurance in a variety of aquatic circumstances and the ability to supply fish and other aquatic animals with a great living habitat, it is one of the most well-known plants in the aquatic industry [16].

The common carp, *Cyprinus carpio* is one of the most widely grown and economically considerable freshwater fish species in the world [17]. It is an omnivorous fish that can consume a variety of simulated protein-rich foods, such as minced fish, frog and snake flesh, dried insects, fish meal, carcass meal, and blood meal, including aquatic insects, macroinvertebrates, zooplanktons, macrophytes, and phytoplanktons [18, 19]. It is essential to assemble materials that are both reasonably priced and widely available while cultivating common carp. However, the majority of research indicated that using fish meal as a source of protein in addition to other animal and plant sources is significantly more effective than using a single source of protein [20]. For the same reason, various experiments have been done on substituting less expensive plant protein sources, such as aquatic macrophytes for more expensive proteins of animal origin. In order to reduce the cost of feed, the current study's objective was to quantitatively compare the growth of *Cyprinus carpio* var. *communis* fingerlings fed with various levels of formulated diets that contained varying percentages of the aquatic macrophyte, *C. demersum*. The successful substitution of less expensive yet nutritious alternatives for fish meals in the diet of *C. carpio* var. *communis* can increase its output and will enhance aquaculture's contribution to global food and nutritional security.

2. Materials and Methods

2.1. Sample Collection. For the current study, fresh *C. demersum* was brought to the wet laboratory, Department of Zoology, University of Kashmir, in clean plastic bags from different selected sites of Dal lake (Figure 1). The weed was properly cleaned with tap water in order to remove any remaining dirt and debris and was then dried in the sun (30–35°C) beneath the shade. After that, it was uniformly crushed into fine flour (200 µm), which was then stored in airtight plastic polythene bags and preserved in a refrigerator at 4°C until used. The analyzed proximate composition of *C. demersum* meal comprised of 9.68% moisture, 15.45% protein, 0.43% fat, 18.85% ash, and 2,726.89 Cal/g energy, which was evaluated as per AOAC [21].

2.2. Diet Preparation. *C. demersum* meal was substituted for fish meal in six dry diets at amounts of 0%, 5%, 10%, 15%, 20%, and 25% (Table 1). The diets were supplemented with vitamins and mineral mixtures [22]. For *C. carpio* var. *communis* fingerlings, the amount of dietary protein in the tested diet was fixed at 42% based on the earlier studies and past research conducted in our lab and the gross energy content of the diet was fixed at 362 kcal/100 g dry food. Corn and cod liver oils were used as a lipid source. The prior process was used to prepare the experimental diets [23]. After the gelatin was separately dissolved in a known volume of water with steady heating and consistent stirring, the other ingredients added included poultry intestine (56%) and soyabean meal (42%) at 80°C. When the mixer bowl was attached to a Hobart electric mixer (Hobart Corp., Troy, Ohio, USA.) and the heat was turned off, the dextrin was then added. The lukewarm (40°C) dish was gradually topped off with other components, such as vitamin and oil premixes while being vigorously combined. Carboxymethyl cellulose was added to the mixture and the speed of the blender was then gradually raised as the diet began to solidify. A teflon-coated pan was used to hold the final diet while it was air dried and was then maintained at 4°C until further utilized.

2.3. Experimental Fish. For this experiment, the common carp, *C. carpio* var. *communis* fingerlings were used. Its selection was supported by factors including great growth rate, commercial importance, extensive distribution, accessibility, etc. It can live in a range of aquatic settings because of its tolerant and hardy nature. Its seed is in high demand among aqua-farmers for both monoculture and polyculture applications. Healthy *C. carpio* var. *communis* fingerlings were obtained from the fish hatchery (Manasbal), Union Territory, Government Fishery Department. These fingerlings were brought to the Fish Feeding Experiment Laboratory at the Department of Zoology, University of Kashmir, in oxygen-filled polythene bags. The collected fingerlings were first prophylactically dipped in KMnO₄ (1 : 3,000) for around 30 s to rule out any potential illnesses and were then stocked for acclimatization in circular plastic tanks equipped with a continuous flow-through system for a period of 2 weeks. During the period of acclimatization, they were fed with a

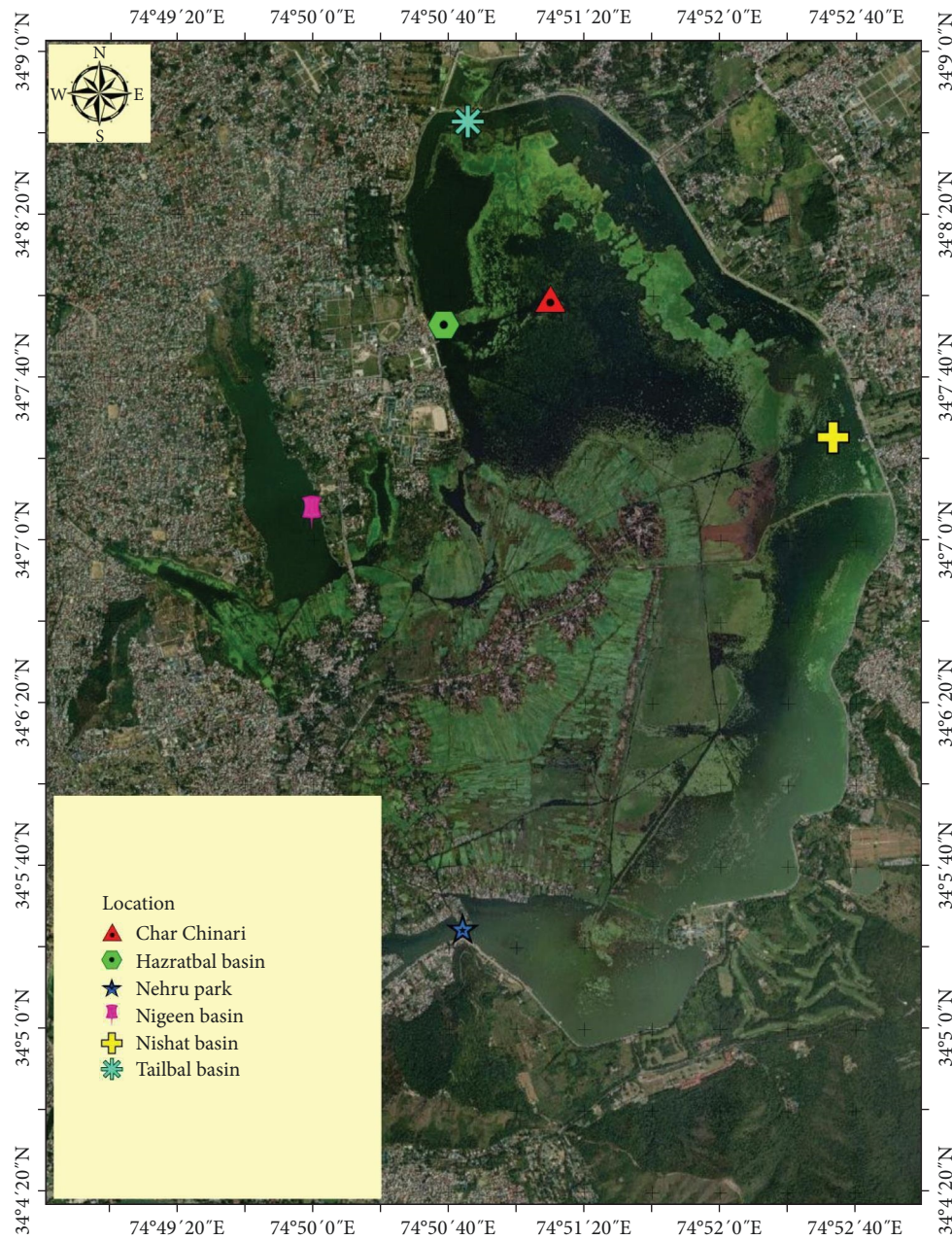


FIGURE 1: Map giving the representation of various selected sites of Dal Lake, Srinagar.

realistic diet that included fish meal, mustard oil cake, soya-bean meal, and rice bran. The fish were afterward acclimatized further for 2 more weeks and were given a synthetic diet called H-440 [22].

2.4. Feeding Trial. From the acclimatized lot, around 360 *C. carpio* var. *communis* fingerlings were sorted out and distributed in triplicate with 20 fish in each trough ($n = 3$) in 70-L circular polyvinyl plastic tanks (water volume 60 L) connected to a running water with a flow rate of 1–1.5 L/min. The experimental trial lasted for 84 days in order to achieve clear results. Feeding was carried out twice in a day at 08:00 and 18:00 and test diets were given until it seemed that satiation has been attained. Before and after feeding, dietary

intake was thoroughly monitored and feces were routinely emptied. On the day of the weekly measurements, fish were not fed and a top-loading balance (Sartorius CPA-224S, 0.1 mg sensitivity, Goettingen, Germany) was used to gauge their mass weight in order to ascertain other growth characteristics.

2.5. Physicochemical Parameter Evaluation. Physicochemical analysis of the experimental samples was carried out on a daily basis. pH, temperature, free carbon dioxide, dissolved oxygen, and total alkalinity were analyzed using standard techniques [24] and the data are presented in Table 2.

2.6. Proximate Analysis. The proximate evaluation of each constituent and experimental diet was done using standard

TABLE 1: Composition and proximate analysis of experimental diets used for replacement of fish meal with *C. demersum*.

Ingredients (g/100g, dry diet)	Diet 1 0.0	Diet 2 5.0	Diet 3 10.0	Diet 4 15.0	Diet 5 20.0	Diet 6 25.0
Fish meal ¹	25.81	24.51	23.23	21.94	20.65	19.35
<i>C. demersum</i> ²	—	5.34	10.67	16	21.34	26.67
Poultry intestine ³	12.5	12.5	12.5	12.5	12.5	12.5
Soyabean meal (SOM) ⁴	16.66	16.66	16.66	16.66	16.66	16.66
Gelatin ⁵	12.90	12.90	12.90	12.90	12.90	12.90
Corn oil	1.0	1.0	1.0	1.0	1.0	1.0
Cod liver oil	1.0	1.0	1.0	1.0	1.0	1.0
Carboxy methyl cellulose (CMC)	2.0	2.0	2.0	2.0	2.0	2.0
Mineral mix	2.0	2.0	2.0	2.0	2.0	2.0
Vitamins mix ⁶	3.0	3.0	3.0	3.0	3.0	3.0
Dextrin white	14.84	12.43	10.02	7.62	5.21	2.81
Alpha cellulose	8.29	6.65	5.02	3.38	1.74	0.10
Total	100	100	100	100	100	100
Calculated crude protein	42.0	42.0	42.0	42.0	42.0	42.0
Analyzed crude protein	41.23	41.46	40.87	41.11	41.35	41.17
Gross energy (kcal/100 g, dry diet) ⁷	400.0	400.0	400.0	400.0	400.0	400.0
Analyzed energy (kcal/100 g, dry diet) ⁸	399.26	402.23	398.75	397.60	398.42	397.85

¹Fish meal 62%; ²*C. demersum* 15%; ³Poultry intestine 56%; ⁴Soyabean meal 42%; ⁵Gelatin 93% CP; ⁶1 g Vitamin mix + 2g α -cellulose; ⁷Calculated on the basis of fuel values 4.1, 2.72, 5.50, 5.30, 4.83, 3.83, and 9 k cal/100 g for fish meal, *C. demersum*, intestine, soyabean, gelatin, dextrin and oils, respectively; ⁸Estimated on Bomb calorimeter (Model 6400; Parr, Moline, Illinois, USA).

TABLE 2: Physicochemical properties of water tank in the wet laboratory during the period of acclimation.

Parameters	Mean \pm SEM
pH	7.35 \pm 0.71
Temperature ($^{\circ}$ C)	14.80 \pm 1.26
Free carbon dioxide (mg/L)	9.83 \pm 1.54
Dissolved oxygen (mg/L)	7.02 \pm 1.18
Total alkalinity (mg/L)	124.52 \pm 3.89

methods [21]. At the start of the feeding trial, 40 samples of *C. carpio* var. *communis* fingerling samples were pooled for biochemical analysis after being euthanized with tricaine methanesulfonate (MS-222) at 0.1 mg/L concentration [25]. At the culmination of the feeding trial, 45 fish samples (15 per replicate per group) were taken from each diet, pooled and subjected to proximate analyses. The overall moisture content was calculated using a digital hot air oven for around 24 hr at 105 $^{\circ}$ C (Bells, India). The amount of crude protein ($N \times 6.25$) was calculated using Kjeltac 8,400 (FOSS Denmark) analyzer. Soxtec automated analyzer (Foss Avanti automatic 2050, Sweden) utilizing solvent extraction technique with petroleum ether (BP = 40–60 $^{\circ}$ C) was used to measure the crude fat content of the samples. The amount of ash in the samples was measured by following the oven incineration method using a muffle furnace (Bells-India) at 650 $^{\circ}$ C for 4–6 hr. The energy content of the experimental feed and fish samples was measured by using a bomb calorimeter (6400 Parr USA).

2.7. Blood Collection and Hemato-Biochemical Evaluation. On the completion of the feeding experiment, 15 anesthetized

fish specimens (5 fish per replicate per group) from each diet treatments were used for blood collection for analyzing different hematological and serum biochemical indices. A disposable heparinized syringe of 2 mL equipped with 24-gauge needle was used to collect the blood from the haemal arch after severing the caudal peduncle of fishes [26–28]. Approximately, a total of 1 mL of blood was obtained from 15 pooled fish samples of each diet treatment. Out of 1 mL of blood sample, 0.5 mL of blood was placed in lithium heparinized vials for hematological analysis such as hemoglobin (Hb) content, total erythrocyte (RBC) count, and hematocrit (Hct) percentage and rest 0.5 mL of blood was placed in Eppendorf tube for serum collection. The acquired blood placed in lithium heparinized vials was immediately put on ice for future evaluation [29]. Hb content analysis was performed using the cyanmethemoglobin technique [29]. According to Parida et al. [30], RBC count was determined using an improved Neubauer hemocytometer and the Natt-Herrick's diluent method [31]. Utilizing microhematocrit capillaries, the hematocrit content was calculated and the results were reported in terms of percentages [32]. The blood placed in Eppendorf tubes was centrifuged in mini-serum centrifuge (Tarsons, Spinwin, MC-02) and the supernatant in the form of serum was collected with the help of pipette for the estimation of different serum biochemical indices such as glucose, cholesterol, total protein, albumin, globulin, calcium, sodium, potassium, phosphorus, alanine aminotransaminase (ALT), and aspartate aminotransaminase (AST) using a Vet scan biochemistry analyzer (VS2 Abaxis, USA).

2.8. Statistical Analysis. After the experiment was over, the growth data were collected and SPSS was used to perform

TABLE 3: Growth and conversion efficiencies of *C. carpio* var. *communis* fingerlings fed fish meal replaced diets^{1,2}.

	Varying levels of <i>C. demersum</i> (g/100 g, dry diet)					
	0.0	5.0	10.0	15.0	20.0	25.0
Average initial weight (g)	3.42 ± 0.03	3.45 ± 0.02	3.43 ± 0.03	3.41 ± 0.04	3.46 ± 0.05	3.43 ± 0.04
Average final weight (g)	13.61 ± 1.08 ^a	13.31 ± 1.09 ^a	12.96 ± 1.07 ^a	11.18 ± 1.04 ^b	10.03 ± 1.06 ^c	8.57 ± 1.05 ^d
Live weight gain (%) ³	298.85 ± 4.15 ^a	286.32 ± 4.09 ^b	278.75 ± 4.19 ^c	228.43 ± 4.07 ^d	190.76 ± 4.09 ^e	150.28 ± 4.04 ^f
Specific growth rate ⁴	1.64 ± 0.07 ^a	1.61 ± 0.05 ^a	1.58 ± 0.04 ^a	1.41 ± 0.05 ^b	1.27 ± 0.04 ^c	1.09 ± 0.06 ^d
Feed conversion ratio ⁵	1.65 ± 0.45 ^f	1.74 ± 0.48 ^e	1.89 ± 0.39 ^d	2.38 ± 0.45 ^c	2.62 ± 0.47 ^b	2.86 ± 0.49 ^a
Protein efficiency ratio ⁶	1.70 ± 0.09 ^a	1.64 ± 0.07 ^a	1.51 ± 0.04 ^b	1.20 ± 0.05 ^c	1.09 ± 0.03 ^d	0.99 ± 0.07 ^e
Protein gain ⁷	1.75 ± 0.05 ^a	1.65 ± 0.04 ^b	1.50 ± 0.06 ^c	1.10 ± 0.07 ^d	0.78 ± 0.07 ^e	0.48 ± 0.05 ^e
Percentage survival	100	100	100	98	95	92

¹Mean values of three replicates ± SEM. ²Mean values sharing the same superscripts in the same row are insignificantly different ($P > 0.05$). ³Live weight gain (%) = final body weight (g) – initial body weight (g) / initial body weight × 100. ⁴Specific growth rate = $100 \times \ln(\text{mean final weight}) - \ln(\text{mean initial weight}) / \text{no. of days}$. ⁵Feed conversion ratio (FCR) = feed given (dry weight basis) / body weight gain (wet weight basis). ⁶Protein efficiency ratio (PER) = wet weight gain (g) / protein fed (g). ⁷Protein gain = (final body protein × final body weight) – (initial body protein × initial body weight).

TABLE 4: Carcass composition and hematological indices of fingerling *C. carpio* var. *communis* fingerlings fed fish meal replaced diets^{1,2}.

	Varying levels of <i>C. demersum</i> (g/100 g, dry diet)						
	Initial	0.0	5.0	10.0	15.0	20.0	25.0
Moisture (%)	78.63 ± 3.14	76.12 ± 3.09 ^c	76.19 ± 3.18 ^c	76.28 ± 3.43 ^c	76.47 ± 3.45 ^c	77.64 ± 3.31 ^b	78.51 ± 3.36 ^a
Crude protein (%)	14.82 ± 1.05	16.45 ± 2.68 ^a	16.09 ± 2.43 ^a	15.23 ± 2.39 ^b	14.04 ± 2.72 ^c	12.58 ± 2.28 ^d	11.09 ± 2.37 ^e
Crude fat (%)	4.65 ± 0.09	4.88 ± 1.09 ^a	4.73 ± 1.38 ^b	4.51 ± 1.64 ^c	4.38 ± 1.53 ^d	4.12 ± 1.48 ^e	3.98 ± 1.25 ^e
Crude ash (%)	2.58 ± 0.09	2.32 ± 0.07 ^a	2.46 ± 0.05 ^a	2.23 ± 0.08 ^a	2.52 ± 0.07 ^a	2.62 ± 0.09 ^a	2.42 ± 0.04 ^a
Hemoglobin (g/dL)	—	8.78 ± 1.51 ^a	8.32 ± 1.67 ^a	7.98 ± 1.43 ^{ab}	5.85 ± 1.38 ^c	5.35 ± 1.74 ^d	5.12 ± 1.69 ^e
Hematocrit value (%)	—	32.81 ± 3.21 ^a	31.94 ± 3.18 ^a	30.68 ± 3.27 ^{ab}	26.42 ± 3.31 ^c	23.71 ± 3.15 ^d	19.93 ± 3.27 ^e
RBC ($\times 10^6/\text{mm}^3$)	—	3.45 ± 1.09 ^a	3.12 ± 1.05 ^a	3.04 ± 1.12 ^{ab}	2.26 ± 1.15 ^c	1.97 ± 1.07 ^d	1.63 ± 1.08 ^e

¹Mean values of three replicates ± SEM. ²Mean values sharing the same superscripts in the same row are insignificantly different ($P > 0.05$).

statistical analysis (version 23 for Windows 2010). All growth data obtained throughout the survey were subjected to one-way analysis of variance (ANOVA) [33, 34]. The significant differences between the treatment means were determined using the Tukey's significant difference test [35] at $P < 0.05$ level of significance.

3. Results

In the present study, the efficacy of the aquatic macrophyte, *C. demersum* has been tested as an alternative replacer of the fish meal in *C. carpio* var. *communis* fingerling which shows that the former has a significant potential in the replacement of FM with *C. demersum*. The results are shown in the following subsections.

3.1. Growth Performance and Survival. The effects of *C. demersum* as an alternative replacement of fish meal in *C. carpio* var. *communis* fingerling were investigated by analyzing live weight gain (LWG %), specific growth rate (SGR), feed conversion ratio (FCR), protein gain (PG), protein efficiency rate (PER), and percentage survival during 12-week feeding experiment (Table 3). No morphological deformities or evident deficiency indications were observed in any of the treatment groups, however fish fed higher levels of *C. demersum* showed a small amount of mortality (Table 3). Fish fed diets containing 5% and 10% *C. demersum* levels produced significantly ($P < 0.05$) the highest growth rate, FCR, SGR, PER, and PG,

respectively. Overall, the fish fed D1 (0%) as a basal diet grew faster in terms of LWG, SGR, PER, and PG, and its growth rate was equivalent to that of fish fed diets containing 5% (D2) and 10% (D3) *C. demersum*. However, greater doses of *C. demersum* meal substituted for fish meal starting at D4 (15%), D5 (20%), and D6 (25%), respectively, revealed a substantial ($P < 0.05$) reduction in the growth parameters. The best-FCR was observed at 0% (D0) and 5% (D2) inclusion of *C. demersum*, while poor FCR values were observed at increasing inclusion of *C. demersum*, i.e., Diet 5 and Diet 6, respectively.

3.2. Carcass Constituents and Hematological Characteristics. *C. carpio* var. *communis* carcass composition and hematological traits indicated a significant ($P < 0.05$) difference when fed a diet with graded levels of *C. demersum* (Table 4). For the groups receiving diets containing lower dosages of fish meal, with high inclusion level of *C. demersum* (D4–D6) exhibited a significant ($P < 0.05$) drop in body protein content which indicated that the protein synthesis rate may continue to be at its optimum only at the inclusion of 5% and 10% *C. demersum* in the diets. When fed aquatic macrophytes up to 10% (D3), the whole body protein level of *C. carpio* var. *communis* fingerlings showed no significant difference ($P > 0.05$) from that of the group receiving a diet low in *C. demersum*. Whole body moisture content of the fish significantly ($P < 0.05$) increased when the highest amounts of fish meal were

TABLE 5: Serum biochemical indices of *C. carpio* var. *communis* fingerlings fed fish meal replaced diets^{1,2}.

	Varying levels of <i>C. demersum</i> (g/100 g, dry diet)					
	0.0	5.0	10.0	15.0	20.0	25.0
Glucose (mmol/L)	5.28 ± 0.04 ^{cd}	5.89 ± 0.05 ^c	6.07 ± 0.07 ^c	7.12 ± 0.09 ^b	8.23 ± 0.07 ^a	8.76 ± 0.05 ^a
Albumin (g/L)	13.76 ± 0.52 ^a	13.29 ± 0.41 ^a	12.87 ± 0.56 ^{ab}	10.43 ± 0.48 ^c	9.12 ± 0.63 ^d	8.31 ± 0.47 ^e
Globulin (g/L)	12.73 ± 1.15 ^a	12.41 ± 1.67 ^a	11.98 ± 1.15 ^{ab}	10.56 ± 1.27 ^c	9.32 ± 1.15 ^d	8.82 ± 1.24 ^e
Total protein (g/L)	24.94 ± 1.46 ^a	24.26 ± 1.51 ^a	23.91 ± 1.32 ^{ab}	21.12 ± 1.41 ^c	19.76 ± 1.28 ^d	18.06 ± 1.37 ^e
Alanine aminotransferase (U/L)	0.62 ± 0.07 ^{de}	0.71 ± 0.05 ^d	0.79 ± 0.03 ^d	1.19 ± 0.04 ^c	1.57 ± 0.06 ^b	1.89 ± 0.07 ^a
Aspartate aminotransferase (U/L)	2.49 ± 0.40 ^{de}	2.63 ± 0.33 ^d	2.95 ± 0.27 ^d	3.85 ± 0.57 ^c	4.29 ± 0.42 ^b	4.93 ± 0.35 ^a
Calcium (mg/dL)	2.88 ± 0.57 ^a	2.56 ± 0.48 ^a	2.45 ± 0.39 ^a	1.91 ± 0.37 ^b	1.49 ± 0.29 ^c	1.12 ± 0.36 ^d
Cholesterol (mmol/L)	2.15 ± 0.08 ^d	2.28 ± 0.06 ^d	2.42 ± 0.07 ^d	2.98 ± 0.09 ^c	3.59 ± 0.05 ^b	4.03 ± 0.07 ^a
Phosphorus (mg/dL)	1.92 ± 0.08 ^a	1.78 ± 0.06 ^a	1.63 ± 0.07 ^{ab}	1.37 ± 0.09 ^c	1.14 ± 0.05 ^d	0.97 ± 0.07 ^{de}
Potassium (mmol/L)	1.88 ± 0.07 ^a	1.81 ± 0.05 ^a	1.73 ± 0.03 ^a	1.58 ± 0.05 ^b	1.32 ± 0.06 ^c	1.03 ± 0.04 ^d

¹Mean values of three replicates ± SEM. ²Mean values sharing the same superscripts in the same row are insignificantly different ($P > 0.05$).

replaced with aquatic macrophytes, *C. demersum* at D5 and D6. The finding suggested that the gradual addition of *C. demersum* to diet has a negative impact on the fish body moisture content. Fish fed a diet containing increasing levels of *C. demersum* showed a significant ($P < 0.05$) decline in body fat content, while as no appreciable change ($P > 0.05$) in body ash content was recorded with respect to increasing levels of *C. demersum* in the diets. Hematological analyses remained essentially insignificant ($P > 0.05$) when dietary fish meal replacement with macrophyte meal increased up to 10% (D2), demonstrating that replacing fish meal with aquatic weeds up to this percentage is feasible for this fish. The Hb concentration and RBC counts considerably ($P < 0.05$) decreased when *C. demersum* meal (D4–D6) was added to more than 10% of the dietary fish meal. For the groups fed diets D5–D6, where 20% and 25% of the dietary fish meal was replaced by *C. demersum* meal.

3.3. Serum Biochemical Characteristics. Due to the inclusion of *C. demersum* in the diet, substantial ($P < 0.05$) variations in the serum biochemical features of *C. carpio* var. *communis* were observed (Table 5). The serum biochemical indices, such as total protein, albumin, globulin, potassium, calcium, and phosphorus declined noticeably ($P < 0.05$) in groups fed diets with greater inclusion of fish meal, except for serum glucose, cholesterol, alanine aminotransferase (ALT), and aspartate aminotransferase (AST) content, which showed significant ($P < 0.05$) increase with sequential inclusion of *C. demersum* meal (D4–D6). Fish fed a basal diet (D0) had the highest concentrations of total protein, albumin, globulin, potassium, calcium, and phosphorus, followed by fish fed a diet containing 5% (D2) and 10% *C. demersum* (D3), respectively.

4. Discussion

The development of less expensive aqua feed will have a considerable positive influence on the spectacular expansion of aquaculture in the coming days. Feed accounts for about 60% of the total operating costs in this industry [10]. In many third world nations, the fish feed used in aquaculture is costly, inconsistent as well as available in very limited supply. Therefore, efforts are being made to lower down the cost of

feed by replacing expensive feed ingredients with less expensive and easily available ingredients. The key to the aquaculture industry's sustainable development is reducing its reliance on fish meal and other crucial ingredients for fish feed [36]. The availability, digestibility, palatability, acceptability of the fish, and cost of the ingredients are the main factors to be taken into account while choosing the right ingredients for fish feed [37]. Feed ingredients vary in terms of their nutritional quality, fish species, potential inclusion, and replacement amounts [38]. As a result, it is essential to make sure that the ingredients used for feed formulation possess the necessary nutrients for adequate growth and utilization by the fish species. This will depend on the protein quality and digestibility of the feed ingredients. Aquatic macrophytes, a feasible nontraditional source for fish farming, have been shown to partially replace fish meal in this context. Utilization benefits include affordability, accessibility, nutritional value, etc. [39]. Since the beginning of freshwater fish culture, aquatic macrophytes have been employed as supplemental feeds. They continue to represent a significant component of fish feed in vast culture systems [36]. These have been shown to include significant amounts of nutrients, protein, and minerals, pricey ingredients in fish feeds, and a crucial element impacting fish growth and feed costs. As a result, efforts have been made to partially substitute alternative protein sources for fish meal in various fish species diets in order to reduce the cost of fish feed, besides producing superior products [40, 41]. In the present study, *C. demersum*, a macrophyte, has been utilized as a good source for partial replacement of fish meal due to its capacity to produce significant amounts of biomass in a little period of time and its favorable biochemical profile. *C. demersum* can be utilized for culture in a cost-effective, safe, accessible, and ecologically friendly way [42]. Additionally, it is a fantastic phytoremediator for treating wastewater and eliminating contaminants like nitrate and phosphates [43].

The results of the present study are congruent with those of other researchers who have suggested that fish meal can partially be replaced with aquatic macrophytes [8, 44–49]. The results of the growth characteristics and feed consumption of *C. carpio* var. *communis* fingerlings showed that the

fish readily accepted the substitution of commercial feed with varying amounts of *C. demersum*, particularly at low levels of 5% (D2) and 10% (D3), respectively. *C. carpio* var. *communis* fingerlings can withstand up to 10% *C. demersum* inclusion when fed a control diet without suffering any deleterious effects on their growth. The live weight gain of *C. carpio* var. *communis* dropped as the amount of *C. demersum* in the diet increased and the fish fed the highest amount of *C. demersum* (D6) had recorded the lowest weight gain. However, weight gain for fish fed 5% and 10% *C. demersum* diets was found identical to the weight gain achieved at the 0% inclusion level, indicating that up to 10% replacement of fish meal did not alter the growth performance of the fish. More intriguingly, fish given 5% and 10% *C. demersum* showed the best-FCR results when compared to the control group, indicating that fish could utilize 10% *C. demersum* containing diets more effectively than other groups. The 5% and 10% *C. demersum* fed groups likewise had the best PER and PG values. This demonstrates how resources in *C. demersum*, particularly protein, are effectively converted into muscle weight gain while keeping the protein, fat, and energy that aid in growth. Similar conclusions about the favorable effects of dietary plant material at low levels on growth and feed efficiency metrics have previously been made in different fish species [50–53]. These favorable impacts on development and feed efficiency in several fish species may be caused by the highest concentrations of essential amino acids and crude proteins found in the aquatic weeds [54, 55]. Contrarily, it has been reported that using *C. demersum* weed meal up to 30% of the inclusion level in rabbit fish growth diets led to growth rates that were comparable to those of the control group [56]. Kiziloğlu et al. [57] also noted a tendency for fish growth performance and feed utilization to decline when macrophyte content in diets increased. This may have been caused by the comparatively high levels of fibers in *C. demersum*, which have been linked to fish growth problems due to their poor digestion. In support, Edwards [58] noted that it is well-known that consuming a diet high in fiber slows fish growth.

The type and composition of the feed used has an impact on the overall body composition of fish which is frequently utilized as a sign of the health of the fish and the quality of its meat [26]. Fish that were given different amounts of *C. demersum* in place of fish meal likewise showed variations in the whole body composition. Body moisture content increased throughout the time of raising *C. demersum* levels, reaching its maximum level at higher inclusion levels. The increase in plant-based inclusion that has led to deterioration in fish development and nutritional quality may be responsible for the increase in moisture content of fish fed different concentrations of *C. demersum* as a fish meal replacement for *C. carpio* var. *communis* fingerlings. Similar findings were observed for *Oreochromis niloticus* [59] and *Clarias gariepinus* [40]. In contrast to the moisture content, whole body protein and fat amounts of fish fed varying doses of *C. demersum* also showed differences. The highest value was found in the groups that were fed with 0%, 5%, and 10% of *C. demersum*. Additionally, diets with higher *C. demersum* inclusion

revealed a steady drop in protein and fat content. This drop could be attributed to the lower quality of plant protein than fish meal [10]. The enhanced plant protein produced from aquatic weeds in our investigation may be responsible for the high moisture and poor fat content in *C. carpio* var. *communis* fingerlings were given various integrated dietary treatments of *C. demersum*. Other researchers made similar observations reporting that the inverse relationship between body protein and fat was noted with the high inclusion of plant material in fish diets [53, 60–62]. Although, the increased inclusion of azolla in tilapia diets had no effect on the body proximate analysis [51]. The crude protein and ash content that may inhibit fish growth were successfully changed when the concentration of the plant was increased in various dietary inclusion treatments [63]. Madalla et al. [64] postulated that hunger brought on by insufficient feed intake at higher levels of plant meal inclusion may have decreased the nutrients in the fish's body. As a result, the fish ultimately had to use body fat reserves for vital bodily functions, which resulted in the lower values of fat content in various grades of dietary treatments, as was also observed in our investigation.

Since, hematological features are often employed as the main indicators for evaluating the well-being of different fish species, as they are directly related to how effectively different fish species respond to different biological and environmental stimuli [65, 66]. In the current study, the inferences depicted that the maximum values of Hb, RBC count, and Hct were displayed at 5% (D2) and 10% (D3) of dietary replacement levels, after which a significant decrease was noticed with an increase in plant meal concentration. An ideal Hb and RBC count could be seen as a sign of the fish's outstanding health. Our results showed that a drop in hematological markers is associated with poor development performance, especially in the diet D4 (15%)–D6 (25%) groups. It has been reported that feeding juvenile common carp moringa leaf meal (MOLM) at a 10% dietary inclusion level resulted in the best performance and highest levels of Hb and RBC count [67]. While *C. gariepinus* and *L. rohita* diets showed a similar trend of declining RBC count with an increase in MOLM levels [68, 69]. Similar observation has also been made with other fish species [70, 71].

In aquaculture, it is crucial to assess the serum biochemical parameters since they reveal the fish's physiological status [72]. Analyzing the biochemical composition of fish can be a useful and perceptive method for determining the physiological and pathological status of fish [73, 74]. Blood glucose, which is also regarded to be the parameter with the greatest range of change, serves as the primary energy source for fish. In the current study, it was found that increasing dietary *C. demersum* inclusion levels in different grades of dietary treatment led to higher serum glucose and cholesterol levels among all groups. The different ratios of *C. demersum* treated diets significantly affected fish glucose and cholesterol levels in a manner that was consistent with the findings of other researchers such as Kumar et al. [75], Jimoh et al. [76], Kari et al. [77], and Nandi et al. [78] who discovered that fish exposed to diets high in plant proteins had higher blood glucose and cholesterol levels compared to the lower

plant incorporated diets. The increase in catecholamine and corticosteroid hormones may be the cause of this increase [47]. This is because when fishes consume a diet with high levels of plant material, which might not be their ideal food source, it could stress their digestive and metabolic systems thereby leading to increased production of stress hormones. Higher doses of plant material in the diet may be less energy-dense and contain fewer carbohydrates and fats compared to typical fish diets. This dietary change can be perceived as a stressor by the fish, potentially leading to the release of catecholamine and corticosteroid hormones. During stressful situations, fishes may undergo gluconeogenesis, a process where the body synthesizes glucose from noncarbohydrate sources. This leads to an increase in blood glucose levels [79]. Cortisol can impact lipid metabolism, including cholesterol. It can increase the production of very-low-density lipoproteins (VLDLs) in the liver [80]. VLDLs transport lipids, including cholesterol, in the bloodstream. Elevated cortisol levels may lead to increased cholesterol levels as the body releases more VLDLs to transport lipids. In contrast, Xu et al. [81] reported that *C. carpio* var. *communis* fingerlings fed fish meal replacement diets had considerably lower blood cholesterol levels than the control group. Serum protein, which is made up of albumin, globulin, and fibrinogen, has a number of roles in fish physiology with their main function being to maintain the body's water balance [82, 83]. Albumin and globulin ensure that the system is healthy in addition to acting as plasma transporters [84]. Because fish fed higher doses of *C. demersum* meal had slower rates of protein synthesis, our study found that dietary *C. demersum* meal decreased the serum total protein of *C. carpio* var. *communis* fingerlings with an increase in their concentration in various dietary treatments. Abdali et al. [85] noticed that a decrease in protein is a symptom that toxins are having an adverse effect on the liver, spleen, and kidneys. On the other hand, several researchers reported that as the doses of plant material were increased, total protein, albumin, and globulin values increased [77, 78].

Blood electrolytes including calcium (Ca), phosphorous (P), and potassium (K) are among those that are frequently been tested in fish [86]. According to the various grades of diets used in our study, the levels of K, P, and Ca in fingerling *C. carpio* var. *communis* varied significantly among different dietary treatments (D2–D6) when compared to a group of fish fed a basic diet (D1). In addition to the control diet (D1), the maximum K, P, and Ca values were found at 5% (D2) and 10% (D3) of the dietary replacement levels. Thereafter, a significant decline was seen as the concentration of plant meal increased from D4 to D6. Fish, liver, kidney, and heart are just a few of the organs that contain inactive blood enzymes like ALT and AST [87]. These enzymes are released into the blood in cases of organ damage, providing information on the injury or dysfunction of the liver [88]. In our investigation, serum ALT and AST levels significantly increased when fish meal was replaced with *C. demersum* meal, indicating that the liver had been adversely affected by the fish meal replacement with *C. demersum* at levels of more than 10% inclusion level. Significant increase in ALT

and AST levels is in line with the other findings [77, 78]. On the other hand, Xu et al. [81] found that blood ALT levels in *C. carpio* var. *communis* fingerlings fed fish meal replacement diets were appreciably lower than those in the control group. However, *O. niloticus* did not show any variation in ALT and AST levels [89].

5. Conclusions

Proteins and minerals that are essential for fish growth, development, and health in the future of aquaculture are present in macrophytes in reasonable amounts. The aquatic plants used in aquadiets may be a useful, practical, and affordable substitute for fish meal to cut costs associated with the diet. In the present study, it was observed that among all dietary treatments, a replacement level of up to 10% (D3) *C. demersum* dietary inclusion or lower could favorably affect the growth, feed utilization, as well as improve the hemato-biochemical parameters of *C. carpio* var. *communis* fingerlings. This study sheds light on the use of new plant-based proteins as a cheap feed component for the development of nutritious fish feed.

Data Availability

The data will be available from Dr. Imtiaz Ahmed (email: imtiazamu1@yahoo.com) on reasonable request.

Ethical Approval

All appropriate international, national, and/or institutional standards for the handling and use of animals were adhered to during the current investigation. All protocols employed in this research as R. No. 801/Go/RE/S/2003/CPCSEA was approved by the Animal Ethical Committee.

Consent

All authors reviewed the manuscript and consented to its publication.

Conflicts of Interest

The authors declare that they have no potential conflict of interest.

Authors' Contributions

Imtiaz Ahmed offered professional assistance and scientific advice for designing and drafting the paper. Anzar Lateef and Younis Mohd Khan carried out the feeding trials and considerably contributed to the statistical analysis and interpretation of the data. Kousar Jan played a significant part in writing the manuscript. The final manuscript was read and approved by all writers.

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