Partial replacement of fishmeal by Azolla cristata in diets for fingerling common carp, Cyprinus carpio var. communis

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Research Article

Keywords: C. carpio. Azolla cristata, Growth, Replacement, Protein retention, Serum hematological parameters,

Posted Date: June 23rd, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1767137/v1

Abstract

A 12-week growth experiment was conducted to evaluate the suitability of *Azolla cristata* as fish meal substitute for fingerling *Cyprinus carpio var Communis*. Six iso-nitrogenous and isocaloric (16.17 kJ g^{-1} GE) diets were formulated to contain 42% crude protein and each treatment had three replicates with a mean initial weight of 3.4 ± 0.2 g. *A. cristata* in gradation of 0, 10, 20, 30, 40 and 50% were fed in order to check possible replacement of fish meal. No significant ($P > 0.05$) differences in growth performance of fish fed on diets containing up to 10% inclusion and the control were seen. However, further increase in *Azolla* meal resulted in progressively reduced growth performance of fish in terms of live weight gain (LWG), specific growth rate (SGR), feed conversion ratio (FCR) and protein retention efficiency (PRE). All hematological parameters had a linear declining trend as the proportion of *Azolla* meal in the diet was increased. Protein content was found significantly ($P < 0.05$) reduced in the fish fed higher inclusion of *Azolla* meal. Serum biochemical parameters were also found to reduce with increasing inclusion of *Azolla* meal, except for glucose, cholesterol, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) which showed higher concentration with increasing inclusion of *Azolla* meal. Based on the above results, it is suggested that 10% *Azolla* meal can be added as a replacement of fishmeal in practical diets for *C. carpio communis*, which would be helpful in reducing the cost of the feed as well as the appropriate use of aquatic macrophytes.

Introduction

In intensive aquaculture, feeding constitute more than 50% of operating cost with protein recognized as the most costly component of formulated feeds. Fish meal (FM) is conventionally used as the main ingredient in formulated feeds due to its known nutritional profile and high palatability, making it the most expensive protein source not only in aquaculture feeds but also in various animal feeds (Shpigel et al. 2017). However, due to upsurge of FM utilization in livestock and poultry, the global demand will soon exceed the production, thereby, further raising the price of FM (Hardy 2010). It is apparent that developing countries will be inept to depend on FM as a chief protein source in aquafeeds and the traditional ingredients used in formulated feeds like cottonseed meal, soybean meal groundnut oil and mustard oil cake are scanty, and thus not easily available to fish farmers and aquafeed producers. This has hampered the growth and cost of aquaculture industry in most of the developing countries (Ghosh et al. 2019). Therefore, research in this line is going on from past several decades to find out the possible replacement for FM with locally available and cheaper protein sources.

In the past, several attempts have been made to incorporate plant protein sources in fish feed and these sources have a great success in replacement of FM for the diets of fish up to some extent. The aquatic free-floating fern *Azolla cristata* often referred to as “super plant” due to its high productivity, has attracted attention as a nitrogen fertilizer and source of dietary nitrogen for herbivorous fish (Das et al. 2018). It is regarded as a superior ingredient in fish feed industry due to its excellent nutritional profile, growth promoter mediators and easy to cultivate (Sheeno and Sahu 2006; Das et al. 2018). The crude protein content of *Azolla* is found in the range from 20–30% (Basak et al. 2002; Das et al. 2018; Magouz et al. 2020) and found optimum for growth. Besides good protein content it is naturally rich in vitamins, minerals, biopolymers and often some probiotics (Pillai et al 2002). It is well documented that *Azolla* can be used as carp feed which convert its raw protein into best edible protein (Maity and Patra 2008; Datta 2011). It has been suggested that feeding dried and processed *Azolla* improved growth and feed utilization in rohu, common carp, silver carp, mrigal, *Tilapia mossambica*, and Nile tilapia (Fiogbe et al. 2004; Gangadhar et al. 2017). However, Sheeno and Sahu, (2006) reported that protein utilization ability of rohu fry was diminished after fed diet containing higher inclusion of *Azolla*. Moreover, it has also been suggested by some workers that *Azolla* meal should be used to replace sizeable quantity of FM from the diets of some cultured fish species such as 20% in *O. niloticus* (Abou, et al. 2007), 45% in *Cirrhinus mrigala* fry (Gangadhar et al. 2014) and 10–20% FM can be replaced in the diet of GIFT tilapia (Magouz et al. 2020), respectively.

Cyprinus carpio communis ranks third among thoroughly cultivated and economically significant fresh water fish species in the world (Odegard et al. 2010). It is commonly called as scale carp and is hardy in nature and can tolerate a wide variety of

conditions (Flajshans and Hulata 2006). *C. carpio communis* is the dominant pond culture fish with great economic value in domestic market in India. The fish is very nutritious, tasty and easily digested and more affordable and accessible, therefore highly preferred in locality (Ahmad et al. 2011). However, due to population expansion and increasing understanding that capture fisheries are depleting, therefore it is mandatory to find out possibilities offered by aquaculture through extensive/semi-intensive techniques for providing substitute to animal protein in the region. Being economically significant plus agreeable to aquaculture, the nutritional research on this valuable species is still going on. Since, the culture of scale carp mostly depends on FM as a feed input, there is currently no report available on the substitution of FM by *Azolla* meal for this species. Therefore, the present experiment was firstly designed to investigate whether dietary inclusion of *Azolla* meal can partially replace dietary fish meal and secondly to evaluate the effect of graded levels of *Azolla* meal on growth performance of fingerling *C. carpio*.

Materials And Methods

Sample collection

In this study, an attempt was made to utilize the aquatic macrophyte for feed preparation. *Azolla cristata* was freshly collected from the Dal Lake, Srinagar (Latitude 340 07' N and longitude 740 52' E, Altitude. 1580 m) and transported to Departmental wet laboratory in clean plastic bags. To eradicate residual soil and debris, the collected weed was thoroughly washed using tap water followed by sun-drying (32–35°C) and crushed homogenously into fine meal (200 µm) which was stored in air-tight plastic polythene bags and kept in refrigerator (4°C) until used for diet preparation.

Experimental Diets

Six dry diets were prepared in which fishmeal was replaced with *Azolla* meal at 0%, 10%, 20%, 30%, 40% and 50% levels. The diets were fortified with vitamins and mineral salts prepared as per Halver (2002). 42% dietary protein was fixed which is reported optimum for the growth of fingerling *C.carpio* with a gross energy fixed at 16.17 kJ g^{-1} for each diet. A mixture of corn and cod liver oil was used as the dietary lipid source to provide n-3 and n-6 fatty acids. The diets were prepared by our earlier adopted protocol (Ahmed 2007). Briefly, for preparing the test diets, gelatin was dissolved separately in a volume of water with constant heating and stirring followed by the addition of casein at 80°C. The mixer bowl was removed from heating and attached to a Hobart electric mixer (Hobart Corp., Troy, Ohio, U.S.A.) and dextrin was added. Other ingredients including vitamin and oil premixes were added to the lukewarm bowl (40°C) one by one with constant mixing. Lastly, carboxymethyl cellulose was added to the above mixture and the speed of the blender was gradually increased as the diet started to harden. The final diet obtained was poured into a Teflon-coated pan, air dried and stored at 4 ^{0}C until used.

Feeding trial

Fingerlings of *C. carpio* var. *communis* in healthy state were collected from the Union Teritory, Government Fishery, Department, fish hatchery (Manasbal). These fingerlings were transported to fish feeding trial laboratory at the Department of Zoology, University of Kashmir in polythene bags filled with oxygen. The obtained fingerlings were first up all offered a preventive dip in $KMnO_4$ (1:3000) for about thirty seconds to rule out any possible infection and afterwards they were transferred to aqua blue coloured indoor circular plastic fish tanks (water volume capacity = 600L), where they were acclimatized for two weeks. The fingerlings were sorted out from the acclimatized lot and were distributed in to triplicate groups in 70-liter circular polyvinyl tanks (water volume 60 liter) fitted with a continuous water flow-through (1-1.5 l min^{-1}) system with 20 fish each group (n = 3) having average body weight of 3.4 ± 0.2 g. The study was conducted for 84 days. Test diets in the form of semi-moist balls (5 mm in diameter) were fed to apparent satiation twice daily at 08:00 and 18:00 hours. Consumption of diet was carefully monitored and the faecal matter was siphoned daily before and after feeding. Fish were not given any feed on the day of weekly measurements and their mass weight was recorded on a top-loading balance

(Sartorus CPA- 224S 0.1 mg sensitivity, Goettingen, Germany) for calculating other growth parameters. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All the protocols used have been approved by Animal Ethical Committee registered under R. No. 801/Go/RE/S/2003/CPCSEA.

Water Quality Parameters

Standard methods of APHA (1992) were used to calculate water parameters and the average water temperature, dissolved oxygen, free carbon dioxide, pH and total alkalinity based on daily measurements were 22.8–24.5 °C, 5.9–6.7, 3.5–5.7, 7.1–7.4 and 93.3–114 mg L^{-1}, respectively.

Sample collection and biochemical analyses

For biochemical analysis 40 fishes at the start of the experiment were collected, sacrificed (MS-222) and pooled. Six subsamples were used from the pooled samples to analyze the initial whole body composition. At the end of the experiment, we took 10 fish from each replicate, sacrificed them, pooled and six subsamples of the pooled samples (n = 3×6) were analyzed for final carcass composition. Test diets, initial and final carcass were done using standard methods of AOAC (2005). Moisture content using thermostat (Yorko Instruments, New Delhi, India) at 105°C, crude protein by Kjeltec (8400, FOSS, Denmark), crude fat using soxlet extraction technique (FOSS Avanti automatic 2050, Sweden) gross energy content of test diets was determined using Parr calorimeter (Model 6400; USA) and ash was measured by combustion in a muffle furnace for 4–6 hrs (650°C).

Blood Collection and serum biochemical parameters

For hematological measurements, blood from six fish (n = 3×6) were collected from the caudal vein of anesthetized fish using heparinized syringe, pooled and three subsamples (n = 3×3) were used for analysis (hemoglobin; Hb g dL^{-1}, hematocrit; Hct% and red blood cells (RBCs×10^6 μL^{-1}). Erythrocyte count was determined by an improved Neubauer hematocytometer with Dacies fluid as the diluting medium. Blood haemoglobin was estimated following the cyanmethaemoglobin method using Drabkins fluid. Hematocrit levels were determined using a microhematocrit centrifuge (REMI RM-12C, India) (Del Rio-Zaragoza et al. 2008). Blood plasma were analyzed for blood enzymes such as alanine aminotransaminase (ALT), aspartate aminotransaminase (AST), glucose, total protein, albumin, globulin, cholesterol, calcium, sodium, potassium and phosphorus by using veterinary biochemistry analyzer (VS2 Abaxis, USA).

Statistical Analyses

All growth data were subjected to one-way analysis of variance (ANOVA; Snedecor and Cochran 1968; Sokal and Rohlf 1981). Differences among the treatment means were determined by Tukey's significant difference test (Tukey 1953) at a $P < 0.05$ level of significance. SPSS software was used for all statistical analysis.

Results

Growth response

Growth performance and percentage survival of fingerling *C. carpio communis* are shown in Table 2. No apparent deficiency symptoms or any morphological deformities have been observed in all the treatment groups. However, little mortality was recorded in fish fed higher inclusion levels of dietary *Azolla*. No significant ($P > 0.05$) differences were observed in growth performance among fish fed 0–10% *Azolla* incorporated diets. However, replacement of fishmeal with *Azolla* meal in diets D_3 (20%), D_4 (30%), D_5 (40%) and D_6 (50%) produced a significant ($P < 0.05$) difference with respect to growth rate, feed conversion, specific growth rate, protein gain and protein retention efficiency. Although maximum growth was achieved in fish fed the basal diet D_1 (0%), however, almost similar growth rate was also achieved with fish fed 10% *Azolla* diet (D_2). Significant ($P < 0.05$) decline in all growth parameters was recorded with fish fed diet having higher replacement of fishmeal

with *Azolla* meal (D_3 to D_6) indicating that a maximum of 10% fishmeal can be replaced by *Azolla* meal without affecting the growth and conversion efficiencies .

Table 1
Formulation and proximate composition of experimental diets

Diets						
Ingredients (g $100g^{-1}$, dry diet)	D_1(0)	D_2(10)	D_3(20)	D_4(30)	D_5(40)	D_6(50)
Fishmeal[1]	24.19	21.77	19.35	16.93	14.51	12.09
Azolla[2]	-	6.52	13.04	19.56	26.08	32.60
Casein[3]	22.5	18.75	18.75	18.75	18.75	18.75
Gelatin[4]	10	5.38	5.38	5.38	5.38	5.38
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
Mineral mix	2.0	2.0	2.0	2.0	2.0	2.0
Vitamin mix[5]	3.0	3.0	3.0	3.0	3.0	3.0
Dextrin white	27.6	24.0	20.3	16.6	12.9	9.2
Carboxy methyl cellolose	4.0	4.0	4.0	4.0	4.0	2.0
Cellulose	4.8	4.3	4.0	3.4	3.0	2.6
Total	100	100	100	100	100	100
Calculated Crude Protein	42.0	42.0	42.0	42.0	42.0	42.0
Analysed Crude Protein	41.75	41.12	40.95	41.38	41.56	41.24
Analysed lipid content	2.23	2.20	2.20	2.21	1.98	1.99
Analysed moisture content	10.25	10.78	11.21	12.63	10.09	9.98
Analysed ash content	20.95	21.23	23.56	23.12	24.31	24.08
Gross energy (kJ/g, dry diet)[6]	16.17	16.17	16.17	16.17	16.17	16.17

[1]Fishmeal 62%; [2]Azolla 23%, [3]Casein 80%; [4]Gelatin 93% crude protein; [5]1g Vitamin mix + 2g œ-cellulose; [6]Calculated on the basis of fuel values 16.8, 14.11, 21.84, 20.28, 15.96 and 37.8 kJ g^{-1} for fishmeal, azolla, casein, gelatin, dextrin and oils, respectively, as estimated on Gallenkamp ballistic bomb calorimeter.

Table 2

Growth and conversion efficiencies of fingerling *Cyprinus carpio communis* fed fishmeal replaced diets [a,b]

	Varying levels of *Azolla* (mg kg^{-1} dry diet)					
	D_1(0)	D_2 (10)	D_3 (20)	D_4 (30)	D_5 (40)	D_6 (50)
Average initial weight (g)	3.51±0.07	3.42±0.06	3.45±0.06	3.49±0.04	3.44±0.05	3.42±0.03
Average final weight (g)	12.98±0.52[a]	12.48±0.41[a]	11.55±0.56[b]	10.64±0.48[c]	9.63±0.63[d]	9.06±0.47[d]
Live weight gain (%)[c]	270±4.15[a]	265±3.67[a]	235±3.15[b]	205±2.27[c]	180±3.09[d]	165±3.41[e]
Specific growth rate[d]	1.16±0.07[a]	1.15±0.05[a]	1.07±0.03[b]	0.99±0.04[b]	0.91±0.06[b]	0.87±0.07[c]
Feed conversion ratio[e]	1.91±0.57[d]	1.75±0.48[e]	1.98±0.39[d]	2.4±0.37[c]	2.6±0.29[b]	2.7±0.36[a]
Protein efficiency ratio[f]	1.50±0.08[b]	1.63±0.06[a]	1.44±0.07[c]	1.19±0.09[d]	1.09±0.05[e]	1.05±0.07[e]
Protein gain[g]	1.58±0.07[a]	1.47±0.05[a]	1.28±0.03[b]	1.10±0.05[c]	0.90±0.06[d]	0.77±0.04[e]
Percentage survival	100	100	100	94	92	91

[a]Mean values of 3 replicates±SEM;

[b]Mean values sharing the same superscripts in the same row are insignificantly different (P > 0.05).

[c]Live weight gain (%) = Final body weight (g)-Initial body weight (g)/Initial body weight × 100.

[d]Specific growth rate = 100 × ln (mean final weight) − ln (mean initial weight)/No. of days.

[e]Feed conversion ratio (FCR) = Feed given (dry weight basis)/Body weight gain (wet weight basis)

[f]Protein efficiency ratio (PER) = Wet weight gain (g)/ Protein fed (g).

[g]Protein gain = (Final body protein × final body weight)−(Initial body protein × initial body weight).

Carcass composition and hematological parameters

Carcass composition and hematological parameters of *C. carpio communis* fed diets with different ratios of *Azolla* meal is depicted in Table 3. Body protein remained unaffected for the groups fed dietary aquatic macrophyte up to 10% (D_2), however, a significant decrease ($P < 0.05$) in the body protein was noted for the groups receiving diets with higher doses of fishmeal (D_3-D_6) indicating that rate of protein synthesis could remain maximum at the inclusion of 10% *Azolla* meal. Body moisture content remained almost unchanged with the increasing inclusion of aquatic weed up to 20% (D_3) and, thereafter at D_4-D_6 replacement of fishmeal with aquatic macrophyte, a significant increase ($P < 0.05$) in moisture content was noted. Body fat showed continued decline with the increasing ratio of dietary aquatic macrophyte. However, the body ash showed insignificant ($P > 0.05$) change in fish fed diets containing varying proportions of fishmeal with *Azolla* meal. The hematological parameters remained almost insignificant ($P > 0.05$) with the increase in the replacement of dietary fishmeal with *Azolla* meal up to 10% (D_2) indicating that replacement of fishmeal with aquatic weed up to this ratio is feasible for this fish. The RBC counts and Hb content decreased significantly ($P < 0.05$) when more than 10% fishmeal was replaced with *Azolla* meal (D_3-D_6) and such reduction in the hematological features was very high for the groups fed diet D_5-D_6 where 40 and 50% of the dietary fishmeal was replaced by *Azolla* meal indicating that these diets become deficient in some essential micronutrients when fishmeal was replaced beyond 40%.

Table 3

Carcass composition and hematological parameters of fingerling *Cyprinus carpio communis* fed fishmeal replaced diets [a,b]

Varying levels of *Azolla* (mg kg^{-1} dry diet)							
	Initial	D_1 (0)	D_2 (10)	D_3 (20)	D_4 (30)	D_5 (40)	D_6 (50)
Moisture (%)	78.61±5.21	75.47±3.4[e]	76.73±2.7[d]	76.15±2.4[d]	77.51±2.9[c]	78.68±2.6[b]	79.12±4.6[a]
Crude protein(%)	13.92±0.6	15.96±0.4[a]	15.64±0.1[a]	15.24±0.4[ab]	14.92±0.4[c]	14.38±0.2[d]	13.81±0.6[e]
Crude fat (%)	3.98± 0.14	4.91± 0.06[a]	4.47± 0.08[b]	3.85± 0.04[c]	3.38±0.09[d]	3.21±0.08[e]	2.97± 0.05[f]
Crude ash (%)	2.68± 0.09	2.41±0.02[a]	2.42±0.05[a]	2.36± 0.07[a]	2.38±0.03[a]	2.40±0.02[a]	2.37± 0.02[a]
Hemoglobin (g dL^{-1})		8.51±0.13[a]	7.64±0.16[b]	7.12±0.12[c]	6.52±0.25[d]	5.71±0.11[e]	5.17±0.17[f]
Hematocrit value (%)		34.81±0.23[a]	31.17±0.39[b]	27.66±0.17[c]	23.85±0.15[d]	19.23±0.19[e]	16.11±0.13[f]
RBC (× 10^6 mm^{-3})		3.68±0.13[a]	3.09±0.15[b]	2.81±0.17[c]	2.56±0.15[d]	2.13±0.18[e]	1.59±0.17[f]

[a]Mean values of 3 replicates±SEM.

[b]Mean values sharing the same superscripts in the same row are insignificantly different ($P > 0.05$).

Serum biochemical parameters

Serum biochemical parameters of fish fed with varying levels of aquatic macrophyte are presented in Table 4. Dietary fishmeal replacement with *Azolla* meal significantly impacted serum biochemical parameters which decreased significantly ($P < 0.05$) in groups fed diets with higher inclusion of fishmeal (D_3-D_6), except for AST, ALT, glucose and cholesterol content which showed a significant ($P < 0.05$) increase with incremental inclusion of *Azolla* meal. Maximum albumin, globulin, calcium, phosphorus, sodium, potassium and total protein content was obtained in fish fed basal diet (D_0) followed by D_2 (10%) inclusion of fishmeal indicating that replacement of fishmeal with *Azolla* meal up to this ratio could be possible without affecting the fish growth.

Table 4

Serum biochemical parameters of fingerling *Cyprinus carpio* fed fishmeal replaced diets [a,b]

	Varying levels of *Azolla* (mg kg^{-1} dry diet)					
	D_1(0)	D_2 (10)	D_3 (20)	D_4 (30)	D_5 (40)	D_6 (50)
Glucose (mmol L^{-1})	4.94±0.07^{d}	5.42±0.06^{c}	6.86±0.06^{b}	5.91±0.04^{c}	6.39±0.05^{b}	7.72±0.03^{a}
Albumin (g L^{-1})	13.98±0.52^{a}	12.78±0.41^{a}	11.14±0.56^{c}	10.64±0.48^{d}	9.63±0.63^{e}	9.06±0.47^{e}
Globulin (g L^{-1})	14.76±1.15^{e}	13.23±1.67^{b}	11.98±1.15^{c}	10.36±1.27^{d}	10.08±1.15^{d}	9.97±1.24^{d}
Total protein (g L^{-1})	26.89±1.46^{a}	24.63±1.51^{b}	21.91±1.32^{c}	20.06±1.41^{d}	18.71±1.28^{e}	17.64±1.37^{f}
Alanine aminotransferase (UL^{-1})	0.87±0.07^{d}	0.91±0.05^{bc}	0.99±0.03^{bc}	1.07±0.04^{b}	1.13±0.06^{b}	1.17±0.07^{a}
Aspartate aminotransferase (UL^{-1})	2.45 ± 0.40^{e}	3.15 ± 0.33^{d}	3.37 ± 0.27^{d}	3.81 ± 0.57^{c}	4.03 ± 0.42^{b}	4.57 ± 0.35^{a}
Calcium (mg dL^{-1})	1.91±0.57^{d}	1.75±0.48^{e}	1.98±0.39^{d}	2.4±0.37^{c}	2.6±0.29^{b}	2.7±0.36^{a}
Cholesterol (mmol L^{-1})	2.09±0.08^{a}	2.34±0.06^{b}	2.58±0.07^{ab}	2.72±0.09^{c}	2.79±0.05^{ab}	2.86±0.07^{b}
Phosphorus (mg dL^{-1})	1.63±0.08^{b}	1.50±0.06^{a}	1.44±0.07^{c}	1.19±0.09^{d}	1.09±0.05^{e}	1.05±0.07^{e}
Potassium (mmol L^{-1})	1.58±0.07^{a}	1.42±0.05^{b}	1.28±0.03^{c}	1.10±0.05^{d}	0.90±0.06^{d}	0.77±0.04^{e}

[a]Mean values of 3 replicates±SEM.

[b]Mean values sharing the same superscripts in the same row are insignificantly different (P > 0.05).

Discussion

For sustainable aquaculture practice, use of cost-effective and alternate plant protein sources in replacement studies is still on its infancy. The probable causes of considering non-conventional plant feedstuffs not suitable as feed ingredient for fishes include meager nutritive potential in terms of poor digestibility and presence of anti-nutritional factors (Li et al. 2021). Cruz-Velasquez (2014) pointed out that aquatic macrophytes are imperative nutritional sources for herbivorous-omnivorous fishes and could replace 25% of formulated diets and 50% of commercial feeds without any detrimental effects on growth and body composition of fishes. The aquatic fern *Azolla* proliferate at high rates in freshwater bodies have modest protein content (19–31%) depending on their sources, strain and growing media (Fasakin et al. 2001). Water fern has gained attention as natural sources of food for fish species, either directly in fresh forms or in combination with other feedstuffs. Our results reveal the inadequacy of total replacement of fish meal with water fern at high levels of incorporation in practical diets for *C. carpio communis* fingerlings. The results of the present study are in conformity with the findings of El-Sayed, (1992) and Almazan et al. (1986) who reported that growth of fish decreases with increasing levels of added aquatic plant ingredients. Fasakin et al. (1999) also reported similar growth retardation and poor FCR when increased levels of dried water fern and duckweed meals were incorporated in the Nile tilapia diets. In the present study, it was evident that *Azolla* could be a good substitute for fish meal, where the replacement up to 10% of fish meal has no effect on growth performance of *C. carpio communis* fingerlings. Earlier reports suggested that inclusion of 25% *Azolla microphylla* and *A. pinnata* mixture in the diet of *Labeo rohita* significantly improved the growth and SGR (Datta 2011). Similarly in our study, incorporation of fish meal along with *Azolla* meal satisfied the nutritional requirement of *C. carpio* fingerlings. However, reduced feed efficiency at higher inclusion of *Azolla* meal (D_5 (40) and D_6 (50)) may be because of presence of anti-nutritional factors (ANFs) and high dietary fiber content which in turn reduced the growth performance (Kamali-Sanzighi et al. 2019; Magouz et al. 2020). It has

been reported that higher dietary *Azolla* inclusion reduces the weight gain by increasing both the metabolic rate and energy expenditure, while decreasing the digestibility of ingredients, due to its ANF content (Ahmed et al. 2017; Mohammadi et al. 2018; Magouz et al. 2020).Nevertheless, the study of chemical and amino acid composition of water fern basically displayed the nutritional characteristics of these ingredients as fish feed (Chakrabarti et al. 2018).

Azolla can be used as a potential replacer of expensive FM in herbivorous species because of complementary digestive enzyme profile and the presence of ω-6 fatty acids from *Azolla* diet (Mosha 2018). In addition, fish fed *Azolla* supplemented diet resulted in improved protein conversion, mobilization and utilization of glycogenic amino acids. However, excess *Azolla* supplementation reduced fish growth and conversion efficiency, possibly due to the presence of high fiber content and low protein digestibility (Mosha 2018). *O. niloticus* and *T. mozambicuss* exhibited better growth performance in a range of 20–42% of dietary *Azolla* inclusion (Fiogbe et al. 2004, Ebrahim et al. 2007). Besides these, some reports propose positive growth even at 50% inclusion of *Azolla* meal (Almazan et al. 1986; El-Sayeed 1992). Nevertheless, despite being a microphagous omnivore fish, poor growth performance was reported in *Tilapia zillii* fed *Azolla* meal (Abdel-Halim et al. 1998). Similarly, *O. niloticus* and *T. rendalli* exhibited reduced growth pattern when fed *Azolla* incorporated diets (Micha et al. 1988). Literature suggests that various grades of *Azolla* levels have been incorporated in species belonging to family *Cyprinidae*. Improved feed utilization and growth rate was reported in rohu fed 10–50% *Azolla* meal in the diet (Tuladhar 2003; Datta 2011; Panigrahi 2014). While Orange fin labeo (Gangadhar et al. 2017), Catla (Umalatha 2018), silver carp and mrigal (Tuladhar 2003) and grass carp (Majhi et al. 2006) reported to have a range between 10–25% *Azolla* inclusion level in the diet (Kumari 2017). Our results from the current investigation are in line with the findings reported on above fish species with the inclusion levels of *Azolla* meal up to 10% that resulted in best growth performance of the candidate fish species.

Carcass composition is altered by numerous endogeneous and exogenous factors that often indicate quality of cultured fish species (Khan and Khan 2020a,b). Both protein and ash are controlled endogenously while as lipid values are altered by both of these factors. It is widely acknowledged that carcass protein and fat are the main attributes of interest and contribute to the suitability of fish meat for processing and storage. Further it has been suggested that feeding nutrient deficient diets results in impaired protein accretion and surplus fat deposition in liver, fillet or peritoneal cavity. In the present study, body protein decreased with higher inclusion of fishmeal with *Azolla* meal. This could be due to poor quality of plant protein compared to that of fish meal. However, the diets containing higher level of *Azolla* weed inclusion resulted in significantly high carcass moisture and lower lipid content. The high moisture and low lipid content of *C. carpio communis* fingerlings fed *Azolla* incorporated diets may be credited to the elevated plant protein derived from aquatic weed. Similarly, tilapia and common carp fed plant protein diets resulted in improved carcass moisture and reduced lipid content (Hassan and Edwards 1992; Hossain and Jauncey 1989). Datta (2011) reported that incorporation of *Azolla* resulted in reduction of body lipid content but their body protein content remained unaffected among all the treatments. Micha et al. (1988) reported the similar trend of body protein and lipid content in *O. niloticus* and *T. rendalli* fed *Azolla* supplemented diets in both the species.

One of the effective tools for understanding the physiological and pathological changes in fishes is ample knowledge of blood parameters. The blood parameters offer information on health status of a fish like metabolic disorders, deficiencies and chronic stress in response to changes related to diet, quality of water and ill health (Denson et al. 2003; Banaee et al. 2008; Khan and Khan 2021a,b,c,). Hematological parameters such as Hb, Hct%, RBCs showed an insignificant relationship with increase in dietary *Azolla* meal up to 10% (D_2) beyond which significant decline was apparent. The highest values of these data were observed in D_1 followed by D_2 group. It is well known fact that Hb, Hct and RBC count are related to the non-specific immune function, where high Hb and RBC count can be taken as an indication of good health. Earlier studies on fish fed diet rich in plant protein sources exhibited reduced blood Hct and Hb (Pham et al. 2008; Lim and Lee 2008). It is a well-known fact that hematological parameters are drastically affected by imbalanced diets, presence of anti-nutritional factors and environmental conditions (Garrido et al. 1990; Lim and Lee, 2009). The results of current research work determined that a reduction in haematological values corresponds with the poor growth performance, especially in the D_4 (30%) -D_6 (50%) group.

Serum biochemical parameters are one of the useful indices for monitoring the health and physiological condition of fishes and are widely been used to determine the effects of feed additives on fish health (Fanouraki et al. 2007; Shi et al. 2006; Hoseinifar et al. 2010; Parrino et al. 2018; Fazio et al. 2019). These serum parameters can prove a pivotal tool for detecting illness and response to therapy. In aquaculture it is necessary to evaluate the serum biochemical parameters which enable us to know the normal physiological condition of the fish under study (Patrichi et al. 2011). Proteins are among the leading sources of energy in fishes and play a significant role in the blood glucose level in fishes (Shweta et al. 2012). In our study dietary inclusion of *Azolla* meal decreased total serum protein of *C. carpio communis* fingerlings which may be due to reduced rate of protein synthesis in fish fed higher levels of dietary *Azolla* meal. Potassium, phosphorous, calcium etc, are some of the commonly analyzed blood electrolytes. Calcium is a component of bones and also regulates nerve and muscle functions in fish. Potassium ion is majorly found in intercellular fluid and possesses an important function of carbohydrate metabolism in nerve fibers of animals including fish. Alteration in potassium concentration affects heart function and causes neurotoxic damage to the central nervous system of the fish (Adediji 2010). Potassium level in our study shows significant variation. Similarly significant variation was also found in calcium and phosphorous activity of fingerling *C. carpio communis* when compared with the group of fish fed basal diet (D_0). On the other hand cholesterol shows the reverse trend means it starts to increase on higher inclusion of dietary *Azolla*. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are enzymes found in the different tissues of liver, kidneys, heart, skeletal muscle, pancreas, spleen, erythrocyte, brain and gills (Hadi et al. 2009). These blood plasma enzymes are calculated clinically as biomarkers for liver health. On injury these enzymes are being released directly into blood hence provide information about liver dysfunction. In our study serum ALT and AST increased significantly as FM was replaced with *Azolla* meal. It indicates that *Azolla* replacement of FM had negative impact on the liver. However, Xu et al. (2018) found that serum ALT and cholesterol levels were significantly lower than that of control group in *C. carpio* fed FM replaced diets. Serum glucose reflected the status of normal metabolism in the body and the healthy degree of liver function (Zhao 2006; Xu et al. 2018). Serum glucose levels were also found to increase with higher inclusion levels of dietary *Azolla* among all the groups. Our results suggested that fish was not able to maintain normal glucose metabolism at higher inclusion levels and causing burden to the liver metabolism which was also supported by the higher values of AST and ALT levels.

Conclusions

The present study demonstrated acceptable nutritional value of *Azolla* meal as an ingredient in the diets for *C. carpio communis* fingerling. The aquatic macrophyte seems to be a good replacer of fishmeal in practical diets for this fish at 10% inclusion level without any adverse effect on growth, conversion, hematological and serum biochemical parameters of *C. carpio communis*. *Azolla* meal could also be well incorporated to make eco-friendly, cost-effective practical feeds for mass production of fingerling *C. carpio communis* through its intensification.

Declarations

Acknowledgements

The authors are grateful to the Head, Department of Zoology,University of Kashmir, Hazratbal, Srinagar, India, for providing the laboratory facilities. We are also pleased to Department of Fisheries, Seed Hatchery, Manasbal, Ganderbal (Jammu & Kashmir) for providing carp fingerlings to carry out this experiment.

Author contribution

Imtiaz Ahmed: Provided expert assistance and is a scientific advisor for designing this study. He also contributed to the drafting of the paper; Younis Mohd Khan; The second author, Younis Mohd Khan conducted the feeding trial and substantially contributed to the writing of the manuscript, statistical analysis and interpretation of the data; Anzar Lateef: The third author, Anzar Lateef contributed to the writing of the manuscript and interpretation of the data; Aamir Majeed: The

fourth author, Aamir Majeed contributed to the writing of the manuscript and interpretation of the data; Manzoor A. Shah; The fifth author, Manzoor A. Shah contributed for the identification and collection of aquatic macrophytes.

Funding

This study was supported by Ministry of Environment, Forest & Climate Change (MoEF & CC) under its program National Mission on Himalayan Studies (NMHS), G.B. Pant National Institute of Himalayan Environment (NIHE), Kosi-Katarmal, Almora in its order No. GBPNI/NMHS-2020-21/MG/TSP/39

Data availability statement

The data used in this is including within the manuscript.

Ethical statement

During the present research work, all applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All the protocols used have been approved by Animal Ethical Committee registered under R. No. 801/Go/RE/S/2003/CPCSEA.

Competing interest

The authors declare that they have no competing of interests.

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