Indian Journal of Animal Sciences **94** (10): 908–913, October 2024/Article
https://doi.org/10.56093/ijans.v94i10.153662

Crassocephalum crepidioides as a sustainable protein supplement in Chocolate mahseer (*Neolissochilus hexagonolepis*) diets: Implications for growth, health, and economic viability in sustainable aquaculture

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Received: 10 July 2024; Accepted: 6 September 2024

ABSTRACT

This study investigated the potential of *Crassocephalum crepidioides*, an abundant weed as a protein supplement in chocolate mahseer (*Neolissochilus hexagonolepis*) diets. The plant replaced a combination of fish meal and mustard oil cake at 0%, 25%, 50%, 75%, and 100% in experimental diets. A 12-week feeding trial was conducted to assess growth performance, survival, biomass yield, digestive enzyme activity, blood parameters, and economic viability. Results indicated that *C. crepidioides* can effectively replace up to 61.3% of the combined fish meal and mustard oil cake protein without negatively impacting fish health or growth, while reducing feed costs. Fish fed diets with 25% replacement showed improved weight gain (15.5±0.7 g) and biomass yield (6.05±0.25 kg/m³) compared to the control (14.6±0.8 g and 5.75±0.28 kg/m³, respectively). Survival rates were high across all treatments (94.0-97.3%). Digestive enzyme activities and blood parameters remained within normal ranges for inclusion levels up to 75%. Economic analysis revealed potential cost savings of up to 15.5% when incorporating *C. crepidioides* into mahseer diets. This finding presents an opportunity to develop low-cost fish feed for resource-poor farmers in Meghalaya, effectively converting a local weed into a valuable aquaculture input, with broader implications for sustainable aquaculture practices globally.

Keywords: Alternative protein source, *Crassocephalum crepidioides,* Hill aquaculture, Low-cost aquafeed, *Neolissochilus hexagonolepis*, Sustainable aquaculture

Aquaculture, a key sector for global food security and economic growth, faces a significant challenge from the rising costs of fish feed, which account for 50-70% of production expenses, especially impacting small-scale farmers (FAO 2022). This issue is particularly pressing in developing regions like North-east India, where many fish farmers are marginalized with limited resources and purchasing power (Rahman 2019). The lack of affordable, locally sourced fish feed constrains aquaculture's potential to support rural livelihoods and food security (Das 2018).

A promising solution is the development of low-cost fish feeds using locally available plant materials. This approach could reduce feed costs, promote sustainable resource use, and enhance local aquaculture systems' resilience (Fregene *et al.* 2020). Plants abundant in the region, like *Crassocephalum crepidioides* (redflower ragleaf), could be utilized. This plant, prevalent in Meghalaya and other parts of North-east India, is noted for its high protein content exceeding 20% and its ease of cultivation (Dairo and Adanlawo 2007, Adjatin *et al.* 2013, Silalahi 2022).

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Transforming such locally available plants into fish feed aligns with sustainable agricultural practices, offering benefits like managing unwanted vegetation while producing valuable aquaculture inputs (Dorothy *et al.* 2018, Naseem *et al.* 2021). This is especially relevant in North-east India, where resource-poor farmers could greatly benefit from cost-effective, locally sourced feeds (Duarah and Mall 2020).

Although research on using *C. crepidioides* in fish feed is limited (Gangmei *et al.* 2018), studies on plant-based proteins in fish diets have been explored (Hardy 2010, Muziri *et al.* 2022, Hossain *et al.* 2023). This study focussed on *C. crepidioides* as a protein supplement for the chocolate mahseer (*Neolissochilus hexagonolepis*), a culturally significant fish in Meghalaya (Debnath *et al.* 2024). The aim was to establish a foundation for cost-effective, locally sourced fish feed, advancing sustainable aquaculture practices globally.

MATERIALS AND METHODS

Experimental design and fish: The study was conducted in North-east India (25.699'N latitude, 91.975'E longitude and altitude of 950 m above MSL). A total of 450 juvenile chocolate mahseer (locally sourced) with an initial average weight of 5.2±0.3 g were randomly distributed into

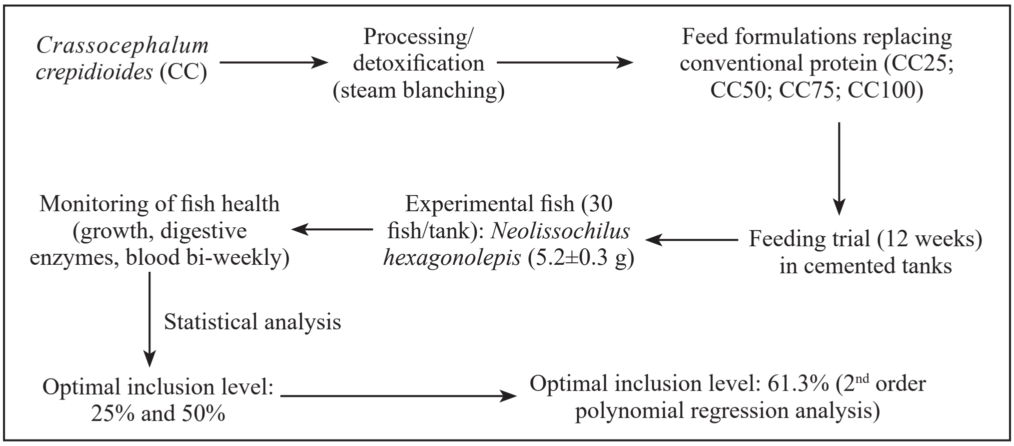

Fig. 1. Schematic diagram of experimenting *Crassocephalum crepidioides* as a feed ingredient for Chocolate mahseer.

15 cemented tanks (500 L capacity) at a stocking density of 30 fish per tank (Fig. 1). The experiment followed a completely randomized design with five treatments and three replicates per treatment.

Plant material and detoxification: Crassocephalum crepidioides was collected from the ICAR farm in Meghalaya. The plants were washed, dried at 60°C for 48 h, and ground into a fine powder. Detoxification was performed using the method described by Gangmei *et al*. (2018), involving steam blanching followed by squeezing and drying in the shade. The proximate composition of the processed *C. crepidioides* meal was tested following AOAC methods (2019).

Diet formulation and preparation: Five diets were formulated: Control (C): Basal diet (rice polish, mustard oil cake, fish meal), CC25: 25% replacement of combined fish meal and mustard oil cake protein with *C. crepidioides*, CC50: 50% replacement, CC75: 75% replacement and (5) CC100: 100% replacement (Table 1). The ingredients were mixed, pelletized, and dried at 60°C for 12 h. Proximate composition analysis of the diets was performed following AOAC methods (2019).

Feeding trial: The feeding trial lasted 12 weeks (May-July). Fish were fed at 3% body weight twice daily (0900 and 1600 h). Water quality parameters (temperature, dissolved oxygen, *p*H, ammonia) were monitored weekly and maintained within the optimal range for fish (Ayyappan *et al.* 2019).

Data collection and analysis: Fish were weighed biweekly to adjust feeding rates and calculate growth parameters. At the end of the trial, weight gain, specific growth rate (SGR), feed conversion ratio (FCR), and protein efficiency ratio (PER) were calculated following standard protocols.

Table 1. Composition and proximate analysis of experimental diets (g/kg dry matter basis).

Ingredient	C	CC25	CC50	CC75	CC100
Rice polish	45%	45%	45%	45%	45%
Mustard oil cake (MOC)	20%	15%	10%	5%	0%
Fish meal	30%	25%	20%	15%	10%
C. crepidioides meal (24.8% CP)	0%	10%	20%	30%	40%
Wheat flour	4%	4%	4%	4%	4%
Vitamin-mineral mix[1]	1%	1%	1%	1%	1%
Total	100%	100%	100%	100%	100%
Proximate composition (% dry matter)					
Dry matter	92.3 ± 0.3^{a}	92.1 ± 0.4^{a}	92.0 ± 0.3^{a}	91.8 ± 0.4^{a}	91.7 ± 0.3^{a}
Crude protein	30.1 ± 0.5^{a}	30.0 ± 0.4^{a}	30.2 ± 0.5^{a}	30.1 ± 0.6^{a}	29.9 ± 0.5^{a}
Crude lipid	8.6 ± 0.3^{a}	8.5 ± 0.2^{a}	8.4 ± 0.3^{a}	8.3 ± 0.2^{a}	8.2 ± 0.3^{a}
Ash	9.8 ± 0.2^{a}	10.3 ± 0.3^{b}	10.8 ± 0.2^{c}	11.3 ± 0.3^{d}	11.8 ± 0.2^{e}
Crude fiber	5.2 ± 0.2^{a}	6.5 ± 0.3^{b}	7.8 ± 0.2^{c}	9.1 ± 0.3^{d}	10.4 ± 0.2^{e}
NFE[2]	46.3 ± 0.7^{e}	44.7 ± 0.8^{d}	42.8 ± 0.7^{c}	40.9 ± 0.8^{b}	39.0 ± 0.7^{a}
Gross energy (MJ/kg)[3]	18.2 ± 0.2^{a}	18.0 ± 0.3^{a}	17.8 ± 0.2^{a}	17.6 ± 0.3^{a}	17.4 ± 0.2^{a}

Values are mean±SD (n = 3). Different superscripts in the same row indicate significant differences ($p<0.05$). [1], Composition of vitamin-mineral premix (quantity/2.5 kg): Vitamin A, 5500000 IU; Vitamin D_3, 1100000 IU; Vitamin B_2, 2000 mg; Vitamin E, 750 mg; Vitamin K, 1000 mg; Vitamin B_6, 1000 mg; Vitamin B_{12}, 6 mcg; Calcium pantothenate, 2500 mg; Nicotinamide, 10 g; Choline chloride, 150 g; Manganese, 27,000 mg; Iodine, 1000 mg; Iron, 7500 mg; Zinc, 5000 mg; Copper, 2000 mg; Cobalt, 450 mg; L- lysine, 10 g; DL-Methionine, 10 g; Selenium, 50 ppm; Satwari, 2500 mg. [2], Nitrogen-free extract (NFE) = 100 - (crude protein + crude lipid + ash + crude fiber). [3], Gross energy calculated based on 23.6, 39.5, and 17.2 kJ/g for protein, lipid, and carbohydrate, respectively.

Digestive enzyme activities (amylase, lipase, and protease) were measured in the intestine following the methods described by Furne *et al.* (2005).

Blood samples were collected from the caudal vein using heparinized syringes from three fish per tank for analysis of hematocrit, hemoglobin, total protein, albumin, and globulin using standard methods (Blaxhall and Daisley 1973).

Statistical analysis: Data were subjected to one-way ANOVA followed by Tukey's HSD test to determine significant differences among treatments ($p<0.05$). Second-order polynomial regression was performed to determine the optimal replacement level of fish meal and mustard oil cake protein with *C. crepidioides.* All statistical analyses were conducted using SPSS software (version 21.0). A basic economic analysis was conducted at the prevailing market rate to determine the cost of feed per kg of fish weight gain for each treatment.

RESULTS AND DISCUSSION

Growth performance: The growth performance of chocolate mahseer fed diets with varying levels of *C. crepidioides* is presented in Table 2. The slow growth rate observed across all treatments is consistent with the natural growth pattern of chocolate mahseer, as reported by Laskar *et al*. (2009) and Mahapatra and Vinod (2011).

The results indicated that *C. crepidioides* can effectively replace the combined protein from fish meal and mustard oil cake up to 75% without significantly impacting growth performance. Fish fed the CC25 and CC50 diets showed improved weight gain and SGR compared to the control, with CC25 showing statistically significant improvement ($p<0.05$). This enhancement could be attributed to the additional nutrients or bioactive compounds present in *C. crepidioides*, as suggested by Dairo and Adanlawo (2007) in their study on the nutritional properties of this plant.

The improved growth performance with moderate inclusion of *C. crepidioides* may be due to several mechanisms:

Enhanced nutrient profile: C. crepidioides contains a wide array of nutrients, including essential amino acids, vitamins, and minerals (Adjatin *et al.* 2013). This diverse nutrient profile may complement the existing feed ingredients, resulting in a more balanced diet.

Bioactive compounds: C. crepidioides is known to contain various bioactive compounds such as flavonoids and phenolic acids (Silalahi 2022). These compounds may have growth-promoting effects through their antioxidant properties or by modulating gut microbiota.

Improved palatability: The preliminary observation that chocolate mahseer readily consumed *C. crepidioides* suggests that its inclusion may enhance feed palatability, potentially leading to increased feed intake and, consequently, improved growth.

However, complete replacement of fish meal and mustard oil cake protein (CC100) resulted in significantly reduced growth performance ($p<0.05$). This decline could be due to an imbalance in the amino acid profile or the presence of residual antinutritional factors, despite the detoxification process. Similar findings were reported by Nguyen *et al.* (2022) and Gregory *et al.* (2023) when completely replacing conventional protein sources with plant-based alternatives in carp diets.

The second-order polynomial regression analysis of weight gain against *C. crepidioides* inclusion level yielded the following equation:

$$Y = 14.6+0.0532X-0.000434X^2 \ (R^2 = 0.95)$$

where, Y, weight gain and X, inclusion level of *C. crepidioides*.

The optimal replacement level was calculated to be 61.3%, beyond which growth performance began to decline. This finding suggests that while *C. crepidioides* can substantially replace the combined protein from fish meal and mustard oil cake, complete replacement is not advisable for optimal growth.

The FCR values improved with moderate inclusion of *C. crepidioides* (CC25 and CC50), indicating better feed utilization. However, the FCR increased significantly in the CC100 group, suggesting reduced feed efficiency at 100% replacement. The PER followed a similar trend, with the CC25 and CC50 groups showing the highest values, although not statistically different from the control and CC75 groups. The CC100 group had significantly lower PER, indicating less efficient use of dietary protein at full replacement of fish meal and mustard oil cake protein. These results align with findings by Gangmei *et al.* (2018), who reported improved growth performance in *Labeo rohita* fingerlings when *C. crepidioides* leaf meal partially replaced soybean meal, but decreased performance at higher inclusion levels.

Survival rates were high across all treatments, ranging from 94.0% to 97.3%, with no significant differences observed ($p>0.05$) (Fig. 2). This suggests that *C. crepidioides* inclusion did not negatively impact fish

Table 2. Growth performance of chocolate mahseer fed diets with graded levels of *C. crepidioides* for 12 weeks

Parameter	C	CC25	CC50	CC75	CC100
Initial weight (g)	5.2 ± 0.3^{a}	5.2 ± 0.2^{a}	5.3 ± 0.3^{a}	5.2 ± 0.3^{a}	5.2 ± 0.3^{a}
Final weight (g)	19.8 ± 0.9^{b}	20.7 ± 0.8^{c}	20.3 ± 1.0^{bc}	19.5 ± 0.7^{b}	18.1 ± 1.2^{a}
SGR (%/day)	1.42 ± 0.06^{b}	1.48 ± 0.05^{c}	1.44 ± 0.07^{bc}	1.40 ± 0.05^{b}	1.31 ± 0.08^{a}
Biomass yield (kg/m^3)	5.75 ± 0.28^{b}	6.05 ± 0.25^{c}	5.86 ± 0.31^{bc}	5.59 ± 0.23^{b}	5.11 ± 0.36^{a}
FCR	2.05 ± 0.11^{b}	1.96 ± 0.09^{ab}	2.01 ± 0.10^{ab}	2.08 ± 0.08^{b}	2.24 ± 0.14^{c}
PER	1.62 ± 0.08^{b}	1.70 ± 0.07^{b}	1.66 ± 0.09^{b}	1.60 ± 0.06^{b}	1.49 ± 0.10^{a}

Values are mean±SD. Different superscripts in the same row indicate significant differences ($p < 0.05$).

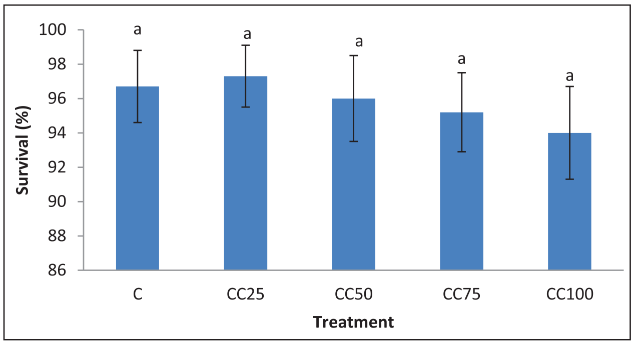

Fig. 2. Survival rate (%) of the experimental fish across different treatments.

survival. Biomass yield, however, showed significant differences among treatments. The CC25 group had the highest yield (6.05±0.25 kg/m³), significantly higher than the control (5.75±0.28 kg/m³) and other treatments ($p<0.05$). The CC100 group had the lowest yield (5.11±0.36 kg/m³), indicating that complete replacement of fish meal and mustard oil cake protein with *C. crepidioides* negatively affected overall production.

Digestive enzyme activity: The digestive enzyme activities showed a similar trend to growth performance (Table 3). Fish fed diets CC25, CC50, and CC75 maintained enzyme activities comparable to the control diet, with CC25 showing slightly elevated levels. This suggests that moderate inclusion of *C. crepidioides* does not negatively impact the digestive capacity of chocolate mahseer and may even slightly enhance it. The increased enzyme activities in CC25 and CC50 groups could be attributed to the presence of certain bioactive compounds in *C. crepidioides* that stimulate digestive enzyme secretion, as observed by Olalekan *et al.* (2013) and Silalahi (2022) in their study on *C. crepidioides*.

The mechanisms behind the maintained or slightly enhanced digestive enzyme activities could include:

Enzyme induction: Certain components in *C. crepidioides* may stimulate the pancreatic secretion of digestive enzymes, leading to increased enzyme activities.

Substrate specificity: The diverse nutrient profile of *C. crepidioides* may provide a wider range of substrates for digestive enzymes, potentially maintaining or enhancing their activities.

Prebiotic effects: Some components of *C. crepidioides* may act as prebiotics, promoting beneficial gut microbiota that contribute to improved digestion and enzyme activities.

However, the CC100 diet resulted in significantly lower enzyme activities ($p<0.05$) compared to other treatments. This reduction could be due to the presence of residual antinutritional factors or an imbalance in dietary components when fish meal and mustard oil cake are completely replaced. Santigosa *et al.* (2008) reported similar findings, where high levels of plant protein sources in fish diets led to reduced digestive enzyme activities.

Blood parameters: Blood parameters are important indicators of fish health and physiological status. Table 4 presents the blood parameters of chocolate mahseer fed the experimental diets. The blood parameters remained within the normal range for mahseer as reported by Nur *et al*. (2023) for all treatments except CC100. Fish fed diets with up to 75% replacement of mustard oil cake with *C. crepidioides* showed no significant differences in blood parameters compared to the control group ($p>0.05$). The slight increase in haemoglobin and total protein in the CC25 group, although not statistically significant, could be attributed to the high protein content and potential hematopoietic factors in *C. crepidioides*, as suggested by Dairo and Adanlawo (2007) in their nutritional analysis of the plant.

However, the CC100 group showed significantly lower values for all blood parameters ($p<0.05$). This reduction could indicate nutritional stress or imbalance when mustard oil cake is completely replaced by *C. crepidioides*. Similar findings were reported by El-Saidy and Saad (2011) when high levels of plant-based proteins were included in fish diets.

Economic analysis: The economic viability of using *C. crepidioides* as a protein source in chocolate mahseer

Table 3. Digestive enzyme activities (U/mg protein) in chocolate mahseer fed diets with graded levels of *C. crepidioides* for 12 weeks

Enzyme	C	CC25	CC50	CC75	CC100
Amylase	$0.85±0.06^{b}$	$0.89±0.05^{b}$	$0.87±0.06^{b}$	$0.86±0.05^{b}$	$0.80±0.07^{a}$
Lipase	$0.42±0.03^{b}$	$0.45±0.02^{b}$	$0.44±0.03^{b}$	$0.43±0.02^{b}$	$0.39±0.04^{a}$
Protease	$1.25±0.08^{b}$	$1.30±0.07^{b}$	$1.28±0.08^{b}$	$1.26±0.07^{b}$	$1.18±0.09^{a}$

Values are mean±SD. Different superscripts in the same row indicate significant differences ($p<0.05$).

Table 4. Blood parameters of chocolate mahseer fed diets with graded levels of *C. crepidioides* for 12 weeks

Parameter	C	CC25	CC50	CC75	CC100
Hematocrit (%)	$32.5±1.8^{b}$	$33.2±1.5^{b}$	$32.8±1.7^{b}$	$32.3±1.6^{b}$	$31.0±2.0^{a}$
Hemoglobin (g/dL)	$7.8±0.4^{b}$	$8.0±0.3^{b}$	$7.9±0.4^{b}$	$7.7±0.3^{b}$	$7.4±0.5^{a}$
Total Protein (g/dL)	$3.5±0.2^{b}$	$3.6±0.2^{b}$	$3.5±0.2^{b}$	$3.4±0.2^{b}$	$3.2±0.3^{a}$
Albumin (g/dL)	$1.8±0.1^{b}$	$1.9±0.1^{b}$	$1.8±0.1^{b}$	$1.8±0.1^{b}$	$1.7±0.1^{a}$
Globulin (g/dL)	$1.7±0.1^{b}$	$1.7±0.1^{b}$	$1.7±0.1^{b}$	$1.6±0.1a^{b}$	$1.5±0.2^{a}$

Values are mean±SD. Different superscripts in the same row indicate significant differences ($p<0.05$).

Table 5. Economic analysis of experimental diets with graded levels of *C. crepidioides*

Parameter	C	CC25	CC50	CC75	CC100
Feed cost (₹/kg)	45.0	42.5	40.0	37.5	35.0
FCR	2.05	1.96	2.01	2.08	2.24
Feed cost per kg weight gain (₹)	92.3	83.3	80.4	78.0	78.4
Cost reduction (%)	-	9.7	12.9	15.5	15.1

feed is crucial for its adoption by resource-poor farmers in Meghalaya. Table 5 presents the economic analysis of the experimental diets.

The economic analysis reveals that incorporating *C. crepidioides* into chocolate mahseer diets can lead to significant cost savings. The feed cost per kg of weight gain decreased as the inclusion level of *C. crepidioides* increased, up to 75% replacement (CC75). The CC75 diet showed the highest cost reduction of 15.5% compared to the control diet. Despite having the lowest feed cost per kg, the CC100 diet did not result in the lowest cost per kg of weight gain due to its poor FCR. This underscores the importance of considering both feed cost and fish performance when evaluating the economic viability of alternative feed ingredients. These findings align with those of Macusi *et al.* (2023), who reported cost savings when partially replacing conventional protein sources with plant-based alternatives in aquaculture.

Justification for high fish meal content in experimental diets: It is important to note that the fish meal content in the experimental diets (10-30%) is higher than typical industrial aquaculture feeds. This decision was based on several factors:

Nutritional requirements of chocolate mahseer: As a carnivorous species, chocolate mahseer has high protein requirements, particularly in the juvenile stage used in this study (Mahapatra and Vinod 2011).

Experimental design: To isolate the effects of *C. crepidioides* replacement, a constant protein level across diets was maintained. The high-quality protein from fish meal helped achieve this while varying the test ingredient.

Study duration: The relatively short duration of the study (12 weeks) required a nutrient-dense diet to observe growth differences.

While it is acknowledged that such high fish meal levels may not be economically or environmentally sustainable for large-scale commercial production, they were necessary for the purpose of this controlled experiment. Future studies should focus on further reducing fish meal content while maintaining optimal growth and health of chocolate mahseer.

This study demonstrates that *Crassocephalum crepidioides* (redflower ragleaf) can serve as a useful ingredient in fish feed for chocolate mahseer, a culturally important fish in North-east India. The research found that replacing up to about 60% of the usual protein sources (fish meal and mustard oil cake) with this weed did not harm the fish's growth or health. Notably, when 25-50% of the usual protein was replaced with the weed, the fish actually grew better than those on the regular diet. The study also revealed that using this plant in fish feed could save farmers up to 15% on feed costs. This finding is particularly significant for small-scale farmers with limited resources. However, completely replacing the usual protein sources with the weed led to poor fish growth and health. This suggests that a balanced approach, using a mix of traditional and new ingredients, is optimal.

These findings could contribute to the development of cheaper, locally-sourced fish feed in Meghalaya and similar regions. By transforming a common weed into valuable fish food, this approach might improve the livelihoods of local fish farmers, while making better use of available resources.

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