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Effect of dietary supplementation of Indian pennywort *Centella asiatica* leaf powder on the growth performance of *Labeo rohita* (Hamilton 1822)

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ABSTRACT

Background & Aim: *Centella asiatica* is considered a nutraceutical and its leaves are rich in carotenoids, vitamins B and C, amino acids, carbohydrates, phenols and terpenoids. The objective of this study was to investigate the effect of dietary supplementation of *C. asiatica* leaf powder (CP) on the growth performance of *Labeo rohita*.

Experimental: *Centella asiatica* leaf powder supplemented feeds in 2 doses (1 g and 5 g) were prepared by mixing the CP in 5 mL vegetable oil, and then admixed with 1 kg each of basal pellet feed and fed to fish for 30 days at 3% body weight twice daily. The parameters like specific growth rate (SGR), feed conversion ratio (FCR) and protein efficiency ratio (PER), serum glucose and creatinine, and the respiratory oxidative burst (ROB) activity were tested.

Results: The results of the growth indices, serum biomarkers and ROB activities indicated that the CP supplementation at 1 g/kg feed was reasonably the best as it gave the higher SGR, PER and ROB activity and lower FCR. The fish fed 5 g CP/kg feed negatively affected the growth in days 30 of feeding.

Recommended applications/industries: Dietary supplementation of CP at 1g/kg feed provided a positive effect on the growth of *L. rohita*. No significant negative effects on the serum biomarkers of stress and kidney function were noted when fed at this level. Further studies are warranted to establish adequate doses of active novel molecules for dietary supplementation rather than using the dried powder.

1. Introduction

Fisheries and aquaculture, the fastest growing food-producing sector, makes substantial contributions to profitable growth, food and nutritional security and the livelihoods of millions of people. Global fish production is estimated to have reached about 179 million tonnes in 2018 of which 82 million tonnes came from aquaculture production. Aquaculture accounted for 46% of the total production and 52% of fish for human consumption (FAO, 2020). India is the second-largest aquaculture nation in the world next to China, contributing 6.3% to the total global fish production (Department of Fisheries, 2020). The total fish production in India was estimated at 14.164 million metric tonnes in 2019-20 (NFDB, 2020). Indian aquaculture has witnessed tremendous growth rates over the last two decades, with freshwater aquaculture contributing over 95% of the total aquaculture production. The freshwater aquaculture comprises the culture of carps, catfish, freshwater prawns, and tilapia. The three Indian major carps (IMCs), namely Catla catla, *Labeo rohita* and *Cirrhinus mrigala* contribute the bulk of production to the extent of 70-75% of the total freshwater fish production (Jayashankar, 2018). Carps form the mainstay of aquaculture practices in the country, supported by a strong traditional knowledge

base and scientific input in various aspects of biology, environment, nutrition, and health management. Several constraints have been put forth in freshwater aquaculture development. Diseases and poor farm management are some of the most noticeable reasons for reduced fish production in Indian aquaculture (Abraham *et al.*, 2020). Modern fish farming with high stocking densities and intensive production units provide ideal conditions for the invasion and persistence of a range of pathogens, which requires appropriate mitigation strategies (Austin and Austin, 2012; Harikrishnan and Balasundaram, 2011; Reverter *et al.*, 2014)

Carps are the major group of freshwater fish that have a global significance as a source of food and as experimental models for research. The beneficial role of plant products particularly the antimicrobial and immunomodulatory properties have been by far the most studied bioactivity with potential application in aquaculture systems. Several plant extracts are reported to stimulate appetite and promote weight gain when they are administered to cultured fish (Citarasu, 2010; Raissy *et al.*, 2022). Indian pennywort *Centella asiatica* (Table 1) is one of the local medicinal plants that has been claimed to have various medicinal effects.

Table 1. Systematic classification of Indian Pennywort *Centella asiatica*

Kingdom	Plantae
Division	Tracheophyta
Subdivision	Spermatophyta
Class	Equisetopsida C. Agardh
Order	Apiales
Family	Apiaceae (Umbelliferea)
Genus	Centella
Species	*Centella asiatica* (L.)

This medicinal plant and its preparations have been in use since ancient times especially in the Ayurvedic medical system of India and in the folk medicine of China and Madagascar (Jaganath and Teik, 2000). *Centella asiatica* is reportedly possessing antibacterial, antifungal, anticancer, wound healing, neuroprotective, immunomodulatory, antiinflammatory, antioxidant, hepatoprotective and insecticidal activities (Inamdar *et al.*, 1996; Punturee *et al.*, 2005; Kalita and Saikia, 2012; Agme-Ghodke *et al.*, 2016), which can be exploited for use in aquaculture as immunomodulators or as an alternative to antibiotics. *Centella asiatica* has been successfully used in controlling columnaris disease caused by Flavobacterium columnare without any inverse impact on fish (Rattanachaikunsopon and Phumkhachorn, 2010) and growth promotion (Sharma *et al.*, 2014; Srichaiyo *et al.*, 2020). This study reports the results of the preliminary investigation on the effect of dietary supplementation of *Centella asiatica* powder on the growth performance of *Labeo rohita*.

2. Materials and Methods

2.1. Experimental fish

The experimental fish *Labeo rohita* (27.25±3.51 g and 13.84±1.18 cm) were procured from a commercial fish farm located in Sonarpur, South 24 Parganas district, West Bengal, India and transported to the laboratory in oxygen-filled bags. On reaching the laboratory, the fish were disinfected by immersion in 5 ppm potassium permanganate solution and acclimated for 15 days in circular fibreglass reinforced plastic (FRP) tanks containing 300-L water at 50 numbers/tank. The fish were fed with commercial pellet feed of 3.0 mm dia (CP Private Limited, India) on demand.

2.2. Preparation of Centella asiatica powder (CP) supplemented diets

Fresh leaves of Indian pennywort *Centela asiatica* (Table 1) were collected from the Faculty of Fishery Sciences, West Bengal University of Animal and Fishery Sciences, Kolkata, (22°47'N, 88°40'E). The leaves were thoroughly washed in running water and air-dried. The leaves were then dried in the hot-air oven at 50°C for 24 h, ground to a fine powder and sieved (pore size: 0.9 mm). Three different types of feeds were prepared with *C. asiatica* for feeding the fish at 3% body weight (BW). *Centella asiatica* leaf powder (CP) in 2 doses, i.e., 1 g and 5 g were added separately in 5 mL vegetable oil, mixed thoroughly and then admixed with 1 kg each of basal pellet feed in airtight plastic containers The control feed without CP was prepared by mixing 5 mL vegetable oil with 1 kg basal pellet feed. After proper mixing, the feeds containing varied concentrations of CP were spread separately, dried under the fan for 24 h and stored in airtight plastic containers at room temperature.

2.3. Experimental design and feeding schedule

Labeo rohita without any gross abnormalities and infections from the acclimatized population were collected and randomly allocated among 9 FRP tanks with 10 fish each. The experimental fish were divided into three groups, i.e., group A, group B and group C, in triplicate. During the 30-day feeding regime, group A was offered the control diet. The treatment groups (groups B (CP1: 1 g CP/kg feed) and C (CP5: 5 g CP/kg feed)) were fed with respective CP diets at 3% BW in two equal portions per day. The wastes and faeces were removed twice weekly followed by an exchange of 25% water. The water quality parameters, viz., water temperature: 25.17-29.17°C; pH: 7.30-7.97; dissolved oxygen: 5.24-6.40 mg/L; nitrite: 0.23-0.66 mg/L and nitrate: 0.25-0.60 mg/L were maintained optimally during the experimental period.

2.4. Assessment of growth indices, serum biomarkers and respiratory oxidative burst activity

Five numbers of fish were carefully caught randomly from each tank during sampling on day 0 and day 30. The total weight, length of sampled fish was recorded on each sampling day to the nearest gram using a digital balance and evaluated the specific growth rate (SGR), feed conversion ratio (FCR) and protein efficiency ratio (PER) (Karnatak *et al.*, 2021). The blood and serum were collected on day 0 and day 30 of feeding. Before the blood collection, two fish from each tank of the respective groups were anaesthetized using clove oil (40 µl/L). The blood was collected by caudal vein puncture (Roberts, 2012) using a 2 mL sterile plastic syringe. An aliquot of blood was heparinized using 2.7% EDTA and processed for measurement within an hour of collection. The blood in the syringe was then allowed to clot by keeping the syringe in a slanting position and then incubated at 4°C overnight. The serum was collected by centrifugation at 600 × g for 15 min and transferred to Eppendorf tubes. The serum samples of two fish from each of the three replicates were pooled separately, labelled and stored at -20 ºC until use. All applicable guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals, Government of India, New Delhi were followed.

Serum glucose, as a stress indicator, was determined by using a glucose test kit (Span Diagnostics Ltd., India) following the GOD-POD method (Trinder, 1996). The serum creatinine level, as a kidney function test, was determined by using a creatinine test kit (DiaSys Diagnostics Systems, GmbH, Germany) following the modified kinetic test without deproteinization according to Jaffe's reaction (Junge *et al.*, 2004) as per the manufacturer's instructions. The non-specific immune parameter was tested by assessing the respiratory oxidative burst (ROB) activity (Sahoo *et al.*, 2011) using the heparinized blood collected on days 0 and 30.

2.5. Statistical analyses

The results were expressed as the mean±standard deviation and analyzed by one way ANOVA (Analysis of variance) followed by Tukey's post-hoc tests for pair-wise comparisons among different treatments using the Statistical Package for Social Sciences (IBM-SPSS) Version: 22.0, considering a probability level of $P<0.05$.

3. Results and discussion

Growth rates in fish may be highly variable and in many cases appear to be limited by food availability (Moyle and Cech, 1996). *Centella asiatica* is considered a nutraceutical due to its biologically active constituents like triterpenes namely asiatic acid and asiaticoside (Inamdar *et al.*, 1996). The leaves are rich in carotenoids, vitamins B and C, amino acids, carbohydrates, phenols, and terpenoids (Idris and Nadzir, 2021; Jantwal *et al.*, 2021). The SGR(W) of *L. rohita* (Figure 1A) of the present study ranged between 0.17±0.02% (CP5 diet) and 0.36±0.01% (CP1 diet), which was comparable to the SGR(W) of pond raised IMCs (0.16-0.41%) (Al Mamunand Mahmud, 2014) and juvenile *L. rohita* (Gandotra *et al.*, 2017). A few studies reported higher SGR(W) in IMCs (Khan *et al.*, 2004; Hosen *et al.*, 2019). The CP1 diet group showed significantly higher SGR(W) compared to the control and CP5 diets (Figure 1A), which indicated a positive effect of CP on the growth of *L. rohita* at the lower dose tried. Possibly, the higher dose of the herbal powder may make the feed unacceptable for fish by changing the texture and taste. Alike, Sharma *et al.* (2014) demonstrated that the administration of *C. asiatica* at 500 mg/kg feed improved the growth and feed utilization of Macrobrachium rosenbergii compared to other experimental diets. In contrast, Srichaiyo *et al.* (2020) used *C. asiatica* powder at 5,

10, and 20 g/kg feed and recorded no significant improvement in the growth rate of Oreochromis niloticus even after 60 days of feeding. The length-wise SGR(L) data, didn't show any significant differences among the experimental groups (Figure 1B).

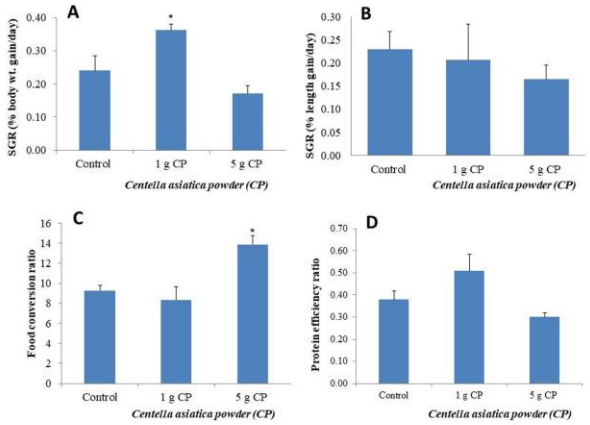

Figure 1. (A) Specific growth rate (SGR) (% body weight gain/day), **(B)** specific growth rate (% length gain/day), **(C)** food conversion ratio and **(D)** protein efficiency ratio of *Labeo rohita* fed with *Centella asiatica* powder (CP) supplemented feeds for 30 consecutive days. Bar with an asterisk (*) differed significantly compared to control (*P<0.05*). Values are expressed as mean ± standard deviation.

The FCR, in its simplest form a comparison of the amount of feed used per unit weight gain of the species being grown, offers a measure of aquaculture production efficiency. Earlier studies reported varied levels of FCR (3.28±0.15-14.02±3.33) in normal diet-fed healthy *L. rohita* depending on the composition of feed and habitat (Gandotra *et al.*, 2017; Khan *et al.*, 2004). In the present study, the FCR of control diet-fed *L. rohita* was 9.27±0.50 after 30 days of feeding (Figure 1C), although high, corroborate the earlier reports (Gandotra *et al.*, 2017; Khan *et al.*, 2004). The FCR values of *L. rohita* fed with CP1 and CP5 diets provided no prudent values. Though the FCR value of CP1 diet-fed fish was a bit lower (8.35±1.31) than the control, the difference was, however, insignificant (Figure 1C). Alike, no significant changes in the FCR were noted in O. niloticus when fed CP at 5, 10, and 20 g/kg feed (Srichaiyo *et al.*, 2020). However, an earlier observation indicated that *C. asiatica* supplemented feed at 500 mg/kg feed offered the best FCR value for M. rosenbergii (Sharma *et al.*, 2014). Varying reports are available on the PER range (0.246±0.002-1.02±0.07) healthy *L. rohita*, as it depends on the food composition and digestibility of food (Gandotra *et al.*, 2017; Khan *et al.*, 2004). In the current study, the PER of control fish was 0.38±0.04, which was within the range of the above studies on *L. rohita* (Figure 1D). The PER value of the CP1 group, though high (0.51±0.07), the difference was insignificant compared to the control (Figure 1D). In contrast, the dietary supplementation of a higher dose (CP5) caused a reduction in PER (0.30±0.02) in *L. rohita*. Similarly, the daily administration of *C. asiatica* at 500 mg/kg as a feed additive offered better growth promotion and feed utilization in *M. rosenbergii* compared to the diets containing 250 and 1000 mg/kg feed (Sharma *et al.*, 2014).

The mean serum glucose levels of control diet-fed *L. rohita* were 61.00±1.00 and 63.50±1.50 mg/dL on day 0 and day 30, respectively (Figure 2A). The results corroborate the findings (60.00±0.05 mg/dL) recorded in healthy *L. rohita* (Kandeepan, 2014). The elevated glucose levels observed on day 30 compared to day 0 could be attributed to the handling stress during capture and blood collection (Figure 2A).

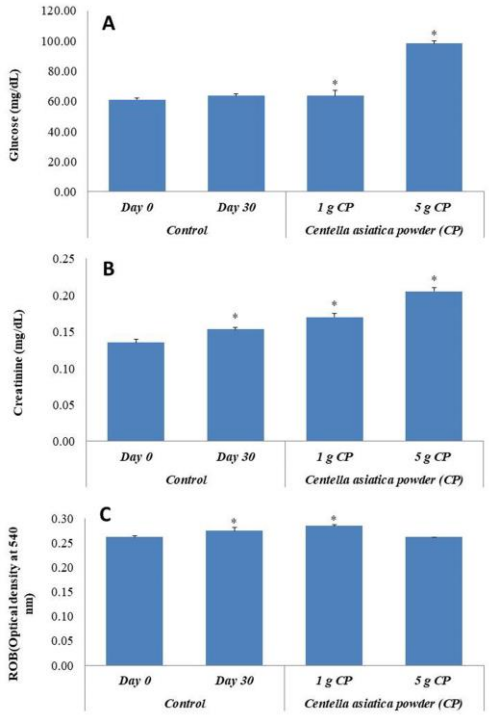

Figure 2. (**A)** Serum glucose, (**B)** serum creatinine levels and (**C)** respiratory oxidative burst (ROB) activity of *Lebeo rohita* fed with *Centella asiatica* powder (CP) supplemented feeds for 30 consecutive days. Bars with an asterisk (*) differed significantly compared to control 0 (*P<0.05*). Values are expressed as mean ± standard deviation.

The CP1 diet-fed groups documented the glucose levels of 63.50±3.5 mg/dL, which indicated that the dietary supplementation of CP at 1g/kg feed did not cause stress to fish. While the fish fed the CP5 diet elevated the glucose levels significantly to 98.33±1.53 mg/dL on day 30 of CP feeding (Figure 2A). It suggested that the CP supplementation above 1g/kg feed may stress the fish. Likewise, higher glucose levels in Cynodon dactylon extract fed Catla catla compared to the control diet-fed fish was reported (Kaleeswaran *et al.*, 2012). The mean serum creatinine levels of control *L. rohita* were in the range of 0.135±0.005-0.140±0.010 mg/dL in 30 days of feeding (Figure 2B), which corroborate the observations of earlier reports, viz., 0.14 mg/dL (Tiwari and Pandey, 2014) and 0.162±0.005 mg/dL (Kulkarni and Pruthviraj, 2016) recorded in healthy *L. rohita*. In the present study, the serum creatinine levels increased significantly to 0.170±0.005 mg/dL in CP1 and 0.205±0.005 mg/dL CP5 group of *L. rohita* (Figure 2B). These results suggested a negative role of CP at the test concentration on the functioning of the kidney .

The ROB activity of fish is related to the secretion of cytokines and inflammatory responses (Reverter *et al.*, 2014). The ROB activities of the control diet-fed *L. rohita* were in the range of 0.261±0.004-0.274±0.007 OD (Figure 2C), which confirms the observations made earlier in *L. rohita* (Sen *et al.*, 2014). The immunostimulating activity of *C. asiatica* on both non-specific cellular immune responses and humoral immune responses were documented earlier (Punturee *et al.*, 2005; Srichaiyo *et al.*, 2020). The ROB activities on 30 days of feeding with CP1 diet (0.284±0.003 OD) and CP5 diet (0.261±0.0005 OD) indicated that only the former showed a significant hike compared to the control on day 0 (Figure 2C). A significant increase in ROB activities in O. niloticus fed with higher doses of *C. asiatica* powder (5 and 10 g/kg) compared to the control was also reported (Srichaiyo *et al.*, 2020). Likewise, Withania somnifera (Ashwagandha) root powder enhanced the ROB activities in *L. rohita* when supplemented through feed at different concentrations viz., 1, 2 and 3 g/kg (Sharma *et al.*, 2010). According to them, the lowest concentration showed the best result. An ample number of researches are available regarding enhanced ROB activity and other immune parameters in different fish species when fed on herbal extract supplemented diets (Bilen *et al.*, 2019; Bulfon *et al.*, 2018; Doan *et al.*, 2019; Raissy *et al.*, 2022).

4. Conclusion

The results of the growth indices, serum biomarkers and ROB activity indicated that the feed supplemented with 1 g CP/kg feed was reasonably the best as it gave the higher SGR, PER and ROB activity and lower FCR. Also, this dose provided no significant negative effects on the serum biomarkers of stress and kidney functioning when fed to *L. rohita* under the controlled condition. Nevertheless, further studies are needed to chemically characterize and quantify the active novel molecules and establish adequate doses for dietary supplementation rather than using the dried powder .

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