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Note

Growth performance and digestive enzyme activities of fringe-lipped carp *Labeo fimbriatus* (Bloch, 1795) in periphyton based nursery rearing system

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ABSTRACT

Nursery rearing of *Labeo fimbriatus* was undertaken in outdoor cement tanks with soil base, where periphyton growth was enhanced by providing sugarcane bagasse as substrate. The growth parameters in terms of final weight of fish, weight gain and biomass were the highest under Substrate+Feed treatment. Growth of fish under Substrate only treatment was comparable with that of the Control. No difference was observed with respect to final length and survival of fish between treatments. Activity of digestive enzymes corroborated the growth pattern. Enhancing periphyton growth can be an alternative for artificial feeding in nursery rearing of *L. fimbriatus.*

Keywords: Digestive enzymes, Fringe-lipped carp, Growth, *Labeo fimbriatus*, Nursery rearing, Periphyton

The strategic goals for research and development to enhance fish production from freshwater aquaculture systems include 'system' and 'species' diversification and prioritisation. Periphyton-based aquaculture is a 'diversified system' which is advantageous in terms of production and has also ensured better resource utilisation as well as eco-friendly protein production (Saikia *et al*., 2011). It is a recent concept and eco-friendly approach in pond aquaculture, which exploits the ability of herbivorous fishes to scrape or graze a two-dimensional layer of periphyton than filtering algae from a three dimensional planktonic environment (Azim *et al*., 2005; Abwao *et al*., 2014). This technology through provision of artificial substrates is gaining importance, particularly in developing countries, since it is a promising technology for resource poor farmers and a way of utilising the otherwise unharvestable nutrients. Growth performance of *Oreochromis mossambicus* (Huchette *et al*., 2000; Keshavanath *et al*., 2004), *Catla catla, Labeo rohita, Cirrhinus mrigala* and *Cyprinus carpio* (Ramesh *et al*., 1999; Umesh *et al*., 1999; Azim *et al*., 2005; Asaduzzaman *et al*., 2009; Gangadhar and Keshavanath, 2012; Keshavanath *et al.*, 2012), *Labeo calbasu* (Wahab *et al*., 1999), mahseer (Keshavanath *et al*., 2002) and *Labeo fimbriatus* (Keshavanath *et al*., 2002; Mridula *et al*., 2003) have been evaluated with different substrates. Among these, browsers with fringed lips such as rohu, calbasu, fimbriatus and tilapia grew better than others and the production ranged from 50 to 77% higher in substrate-based culture systems compared with control systems.

Species diversification has been prioritised as an issue to be addressed for aquaculture development (Ayyappan *et al*., 2009). In recent years, freshwater aquaculture sector of many South-east Asian countries has witnessed inclusion of several new species into the culture systems. In India too, attempts have been made for species diversification in the carp polyculture system by inclusion of new candidates that includes the fringe-lipped carp, *L. fimbriatus*. This species is suitable for cultivation in confined waters (Basavaraju *et al*., 1995). According to van Dam *et al*. (2002), detritus and benthos feeders can also thrive on periphyton, besides specialist herbivores. *L. fimbriatus* is reported to graze on diatoms and algae that grow on submerged rocks and twigs (Bhatnagar and Karamachandani, 1970). Keshavanath *et al*. (2002) reported that *L. fimbriatus* grows well in periphyton based systems. Mohanta *et al*. (2008) have demonstrated that it can be cultured with other major carps in ponds. The species has high potential for composite culture along with other carps, and its culture is slowly picking up in India.

Rearing of spawn in nurseries is an important and crucial step in fish culture. Survival of fish is the most important aspect during nursery rearing and in carp fry rearing, survival is generally around 30% only (Basavaraja, 2007). Adverse rearing conditions and improper management may often lead to severe consequences resulting in mortality of fry to the extent of 90-98% (Jena *et al.*, 1998). No published reports are available on the effect of periphyton enhancement on growth and survival of fish during nursery rearing.

Sugarcane bagasse a byproduct generated by sugar juice vendors is the fibrous residue remaining after sugarcane stalks are crushed to extract juice. Bagasse was evaluated to be one of the good periphyton substrates in terms of periphyton ash free dry matter, chlorophyll *a* and nutrient composition (Gangadhar and Keshavanath, 2008; 2012). Since bagasse is locally available and cheap substrate, it was used for growing periphyton in the present study which was conducted with the objective of evaluating the performance of *L. fimbriatus* in nursery rearing under periphyton enhanced conditions.

The experiment of 20 days duration was conducted in 1000 l outdoor circular cement tanks with 5 cm soil base at the Regional Research Centre of Central Institute of Freshwater Aquaculture, Bangalore, India. Sugarcane bagasse was used as the substrate for periphyton growth. Fresh bagasse procured from local sugarcane juice vending shops was soaked in water for 2 days to get rid of the sugar present and was then dried under sun. Water from a nearby bore well was filled in the tanks to maintain a water column of 90 cm; the evaporation loss, which was very meager, was compensated fortnightly. Cattle dung was applied to each tank @ 600 g tank^{-1}, followed by urea and single super phosphate (SSP) at 1 and 1.5 g tank^{-1} respectively (Jena *et al.*, 2005). Triplicate tanks were allotted for Control; Bagasse; Bagasse+Feed; Bagasse bottom and Bagasse bottom + Feed. While sugarcane bagasse was hung vertically at 2 t ha^{-1} (Keshavanath *et al.*, 2001b) in Bagasse and Bagasse + Feed tanks, maintaining uniform distance between bagasse and tank inner surface, it was applied to tank bottom in the latter two treatments at the same dose. After 10 days, *L. fimbriatus* spawn (mean length 0.68 cm and mean weight 0.48 mg) procured directly from hatchery were stocked @ 2000 tank^{-1} (Jena *et al.*, 2011) in all the tanks. Subsequent fertilisation was done after15 days of initial fertilisation with cattle dung @ 50 g tank^{-1} and urea and SSP @ 1 and 1.5 g tank^{-1} respectively. Fish in all the treatments, except Bagasse and Bagasse bottom, were fed powdered mixture of rice bran and groundnut oil cake at 1:1 ratio at 600 g per lakh spawn for the first 5 days, 1200 g lakh spawn^{-1} per day for the next 5 days and 1800 g lakh spawn^{-1} during subsequent days in two equal installments in the morning (09.00 hrs) and evening (15.00 hrs) (Jena *et al.*, 2005).

Proximate composition of feed was determined following standard methods (AOAC, 1995). Water quality parameters such as pH, temperature, dissolved oxygen (DO), total alkalinity, ammonia, nitrate, phosphate and Secchi disc visibility were analysed once in 5 days, starting from the day of stocking, following standard methods (APHA, 1998). Water samples were collected between 09.00 hrs and 10.00 hrs.

On termination of the experiment, fry were harvested by repeated netting followed by draining the tanks. All surviving fish were counted and mean length of fry was recorded by taking the average of random samples of 30 fry from each tank. The group weight of 30 fry from each replicate tank was taken and the mean weight was calculated.

After completion of the experiment, 30 fry from each treatment group were sacrificed for digestive enzyme activity analyses. The fry were homogenised in ice cold condition with distilled water (4 ml g^{-1}) and centrifuged at 16000 rpm for 20 min at 4ºC. The supernatant (crude enzyme extract) was stored at -20ºC in 1.5 ml aliquots until further use. Total soluble protein of the homogenate was measured using Folin-phenol reagent (Lowry *et al.*, 1951).

Amylase activity was measured following 3, 5-dinitro salicylic acid (DNS) method (Rick and Stegbauer, 1974). Total proteolytic activity was determined by the casein digestion method of Kunitz (1947). Trypsin and chymotrypsin activities were assayed according to Erlanger *et al.* (1961). Carboxypeptidase activity was estimated following Appel (1974), lipase activity using p-nitro phenyl acetate (PNPA) as the substrate (Licia *et al.*, 2006) and cellulase activity was determined based on Miller (1959).

Comparison among treatments for various parameters was done by one way analysis of variance (ANOVA), followed by Duncan's multiple range test (Duncan, 1955; Snedecor and Cochran, 1968).

Rice bran and groundnut cake mixture used as feed in the present study had 4.45±0.11% moisture, 24.88±0.32% crude protein, 11.79±0.13% fat, 10.20±0.25% crude fiber, 7.11±0.46% ash and 41.57±0.02% nitrogen-free extract.

Variation in water quality parameters recorded during the study period is presented in Fig. 1. No significant difference was observed between different treatments in terms of water quality parameters tested, except Secchi disc visibility, ammonia and nitrate levels. Ammonia was less ($p<0.05$) in the treatment tanks with substrate compared to Control. Secchi disc visibility was higher ($p<0.05$) in Control, Bagasse

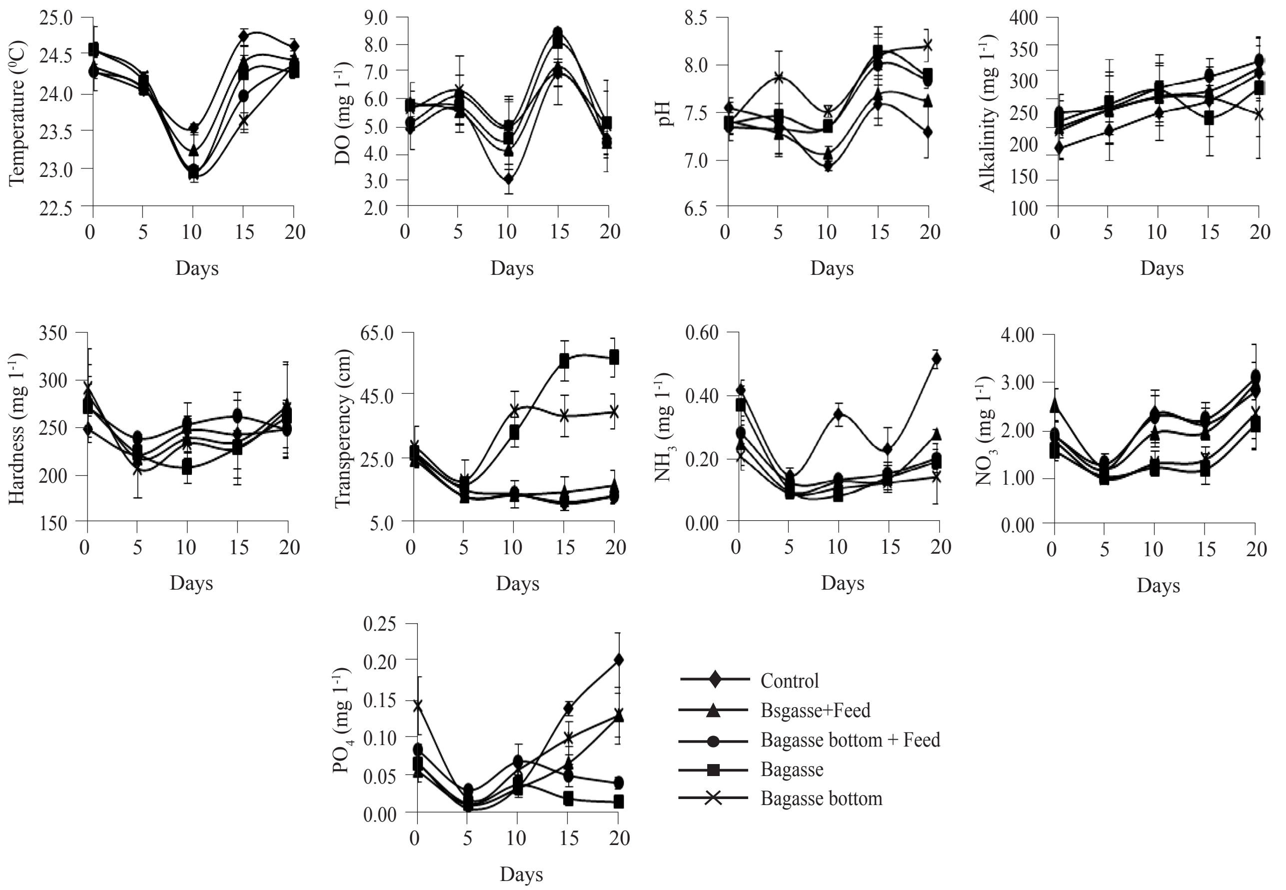

Fig. 1. Water quality parameters (mean ±SD; n=3) recorded in different treatments

and Bagasse bottom tanks. Higher ($p<0.05$) nitrate levels were observed in Bagasse+Feed and Bagasse bottom+Feed tanks. DO and pH showed a direct relationship with temperature. Temperature was lower ($p>0.05$) in Bagasse, Bagasse+Feed, Bagasse bottom and Bagasse bottom+Feed tanks.

Growth parameters in terms of final weight of *L. fimbriatus*, weight gain (%) and biomass were the highest ($p<0.05$) under Bagasse+Feed treatment, followed by Bagasse bottom+Feed (Table 1). Growth of fish under Bagasse and Bagasse column were comparable ($p>0.05$) with that in Control. No difference was observed with respect to final length and survival of fish.

Activity of all the enzymes analysed was highest in fry from Bagasse+Feed treatment (Fig. 2). Fry from Bagasse bottom recorded lower activity of digestive enzymes. Activity of all the enzymes in Control fish was comparable with that in Bagasse treatment. Higher activity of enzymes was recorded when fish was fed in Bagasse bottom treatment. Enzyme activity pattern corroborated the growth pattern. Activity of trypsin in the present study was lower compared to chymotrypsin. Amylase activity was lower than that of protease, however, it was higher than lipase activity.

Water quality parameters were within acceptable limits for the seed rearing of carps (Jena *et al*., 2011).

Table 1. Growth parameters (Mean±SD; n=3) of *L. fimbriatus* fry after nursery rearing

Growth parameters	Control	Bagasse	Bagasse+Feed	Bagasse bottom	Bagasse bottom+Feed
Weight (mg)	81.3^{a}±11.7	96.4^{ab}±15.9	147.2^{c}±28.6	68.6^{a}±12.6	121.2^{bc}±18.1
Length (mm)	19.9^{a}±1.7	22.2^{a}±0.2.7	22.9^{a}±4.1	19.7^{a}±1.9	20.6^{a}±2.6
Survival (%)	49.13^{a}±5.19	56.42^{a}±6.06	52.23^{a}±3.34	50.93^{a}±8.69	48.80^{a}±3.00
Weight gain (mg)	80.8^{a}±11.7	95.9^{ab}±18.6	146.7^{c}±25.9	68.1^{a}±8.8	120.7^{bc}±18.5
Biomass (g tank^{-1})	79.8^{a}± 18.8	100.7^{ab}±18.96	166.04^{c}±20.29	66.96^{a}±14.98	123.41^{b}±18.72

Figures in the same row with different superscripts are significantly different ($p<0.05$)

Initial length and weight of spawn were 0.68±0.08 cm and 0.48±0.03 mg respectively (n=30)

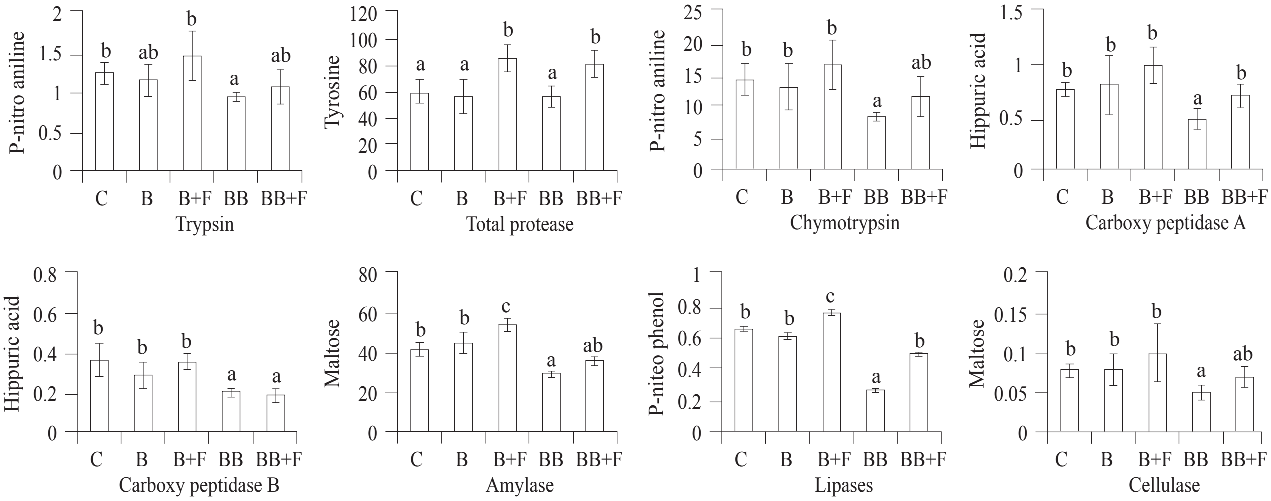

Fig. 2. Digestive enzyme activities (µ moles h^{-1} mg $protein^{-1}$ at $25^{O}C$; mean±SD; n=3) in *L. fimbriatus* fry. Different alphabets on the bar indicate significant difference

pH was in the alkaline range throughout the experimental duration, indicating favourable conditions for biological production. Jhingran (1991) observed that carps thrive well in the temperature range of 18.3 to 37.8°C. Water temperature in the present study ranged from 22.90 to 24.76°C. Temperature was lower ($p>0.05$) in tanks with substrate, attributable to the shading effect of substrates (Keshavanath *et al*., 2002; 2012). Dissolved oxygen content ranged from 3 - 8.75 mg l^{-1}. Generally cyprinids are capable of tolerating low oxygen levels of 3 mg l^{-1} (Huet, 1972). Total alkalinity ranged from 214.66 to 332.38 mg l^{-1}. Periphyton is known to entrap organic detritus, remove nutrients from the water column and help control D.O. concentration and pH of the surrounding water (Azim *et al*., 2002; Bender *et al*., 2004). Waters of higher alkalinity are considered more productive in terms of oxygen production and photosynthesis. Ammonia level was less in tanks with substrate. Enhanced bacterial biofilm developed on bagasse substrate might have brought down the ammonia level by nitrification. Similar observations were recorded earlier in periphyton enhanced culture ponds (Ramesh *et al*., 1999). Comparatively higher nitrate levels were observed in fed tanks which can be attributed to the nitrogen input through feed.

The comparable mean length, weight parameters and survival recorded between Control and substrate treatments indicate that fringe-lipped carp young ones can efficiently utilise periphyton growing on the substrate as a food. It has been documented that nutritional composition of periphyton can be considered as broadly appropriate to fish dietary needs (Azim *et al*., 2002). Holt (1993) suggested that live food facilitates better nutrient absorption as it contains approximately 75% water. Provision of substrate in addition to feed, improved the growth performance ($p<0.05$) of fish. This is attributable to the grazing of periphyton by the fish. Kolkovski *et al*. (1997) noted that live food enhances micro-diet efficiency by promoting assimilation and deposition of dietary nutrients. *L. fimbriatus* is known to feed on diatoms, green algae and blue green algae (Mohanta *et al*., 2008). Species composition of periphyton from bagasse has revealed ample availability of the plankton (Gangadhar and Keshavanath, 2008). Utilisation of periphyton by *L. fimbriatus* has been reported in grow out culture (Dharmaraj *et al*., 2002; Keshavanath *et al*., 2002; Mridula *et al*., 2003). Results of the present study indicate the possibility of exploiting the periphyton technology in carp nursery rearing. Growth of fish under Bagasse and Bagasse column were comparable ($p>0.05$) with that of the control. An earlier study has indicated that sugarcane bagasse can be applied at pond bottom as a substrate for increasing production of rohu (Gangadhar and Keshavanath, 2012), owing to the fact that addition of substrate allows development of autotrophic and heterotrophic populations, besides phytoplankton and bottom microorganisms (Milstein *et al*., 2005). However, in that study, the periphytic biomass and chlorophyll content in periphyton in the substrate applied to the bottom was found to be lower compared to that suspended vertically in the water column. The marginally lower ($p>0.05$) growth of fish in the present study in the Bagasse bottom treatment could be attributed to this fact.

Higher survival of carps was recorded in periphyton-based growth trials compared to systems without substrates (Keshavanath *et al*., 2001a; 2002). Fish larvae fed artificial diets show poor survival due to the under-developed digestive enzyme activity which cannot optimally digest nonliving food (Qin *et al*., 1996). In addition, the ingestion rate of a dry diet in fish larvae is much lower than that of live food (Kolkovski *et al*., 1993). However, in the present study, the survival was not affected (p>0.05) by dietary conditions, because Control fish also received live food through plankton. The survival of *L. fimbriatus* fry ranged from 49-56% when the stocking density was 20 million ha^{-1}. (Jena *et al*. 2011) Jena *et al*. (1998) recorded maximum survival of 61.6, 67.8 and 69.9% in nursery rearing of catla, rohu and mrigal respectively, at a stocking density of 2.5 million ha^{-1}. Rohu spawn reared @ 2.25 to 6.25 million ha^{-1} recorded a survival range of 41-65% when the fry were provided with groundnut oil cake and rice polish (1:1) as supplementary feed (Anon, 1984).

Digestive enzyme activities in fish respond to changes in the quality and quantity of nutrients intake (Coway *et al*., 1981). Activity of all the enzymes, which corroborated the growth pattern, was highest in the fry from Bagasse+Feed treatment. Kamaruddin *et al*. (2011) reported that when larvae of Asian red tail catfish, *Mystus nemurus,* were fed on different diets, the highest enzyme activities were observed among larvae which fed on a combination diet, followed by those fed on live and artificial diets, respectively. This was attributed to the role of exogenous enzymes from live food in larval digestion. Mridula *et al*. (2003, 2005) reported higher digestive enzyme activity in *L. fimbriatus* and *L. rohita* grown in periphyton enhanced culture tanks, compared to those fed with an artificial diet. High digestive enzyme activity coupled with faster growth has been observed in *Etroplus* grown in ponds provided with substrate, followed by those receiving feed and the control (Kumar *et al*., 2009). The insignificant differences in the activity of enzymes between Control and Bagasse treatments observed in the present study could be attributed to the fact that Control tanks in the present study were fertilised, contributing live food. An overall enhancement in protease and amylase activities was observed in the shrimp fed bioflocs (Xu *et al*., 2012). The activity of trypsin in the present study was lower compared to chymotrypsin. Trypsin activity in fish larva is usually lower than chymotrypsin activity (Kumar *et al*., 2009). Herbivorous fish demonstrate greater activity of carbohydrase than protease and a lesser lipase activity compared to carnivorous and omnivorous fish (Opuszynski and Shireman, 1995). But, in the present study, carbohydrase activity was lower than that of protease, however, it was higher than lipase activity.

It is concluded that enhancing periphyton growth through the application of sugarcane bagasse can reduce the cost on artificial feed in nursery rearing of *L. fimbriatus* while provision of substrate in addition to feed can further increase fish weight gain and biomass.

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