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Digestibility and digestive enzyme activity in *Labeo fimbriatus* (Bloch, 1795) fed periphyton grown on sugarcane bagasse

B. GANGADHAR, N. SRIDHAR, H. UMALATHA, H. GANESH, A. R. T. SIMON* AND P. JAYASANKAR

ICAR-Central Institute of Freshwater Aquaculture, Regional Research Centre, Hesaraghatta Lake P.O., Bangalore - 560 089, Karnataka, India

**Federal Institute of Education, Science and Technology, Manaus, Amazonas, Brazil*

e-mail: gbarlaya@yahoo.co.in

ABSTRACT

An experiment of 60 days duration was carried out to compare dry matter and protein digestibility of periphyton grown on sugarcane bagasse bundles and a pelleted feed in *Labeo fimbriatus* (Bloch, 1795). Advanced fingerlings maintained in glass aquaria were allowed to feed on fresh periphyton or pelleted feed (20% crude protein) daily morning, the fecal matter collected following standard procedure and analysed for proximate composition. Acid insoluble ash was used as the reference marker for digestibility estimation. Activity of total protease, trypsin, chymotrypsin, carboxypeptidase - A and B, amylase, lipase and cellulase in the gut of fish was estimated at the end of the feeding trial. Periphyton and pelleted feed showed similar ($p>0.05$) protein digestibility (92.29 and 89.21% respectively), while total dry matter digestibility was higher ($p<0.05$) with peiphyton (85.44 and 75.16% respectively). Among the proteases estimated, activity of total protease and carboxypeptidase B was higher ($p<0.05$) in fish fed periphyton, whereas activity of others showed no difference between those receiving periphyton and pelleted feed ($p>0.05$). Activity of carbohydrases and lipase was higher in fish fed pelleted feed. Results of the study cleary indicated that *L. fimbriatus* can efficiently utilise periphyton.

Keywords: Digestibility, Digestive enzymes, Fringe-lipped carp, *Labeo fimbriatus*, Periphyton, Pelleted feed, Sugarcane bagasse

Introduction

Increase in production of several herbivorous fish species has been reported in periphyton-based aquaculture (Wahab *et al*., 1999; Azim *et al*., 2005; Keshavanath *et al*., 2001b, 2002, 2004; Gangadhar and Keshavanath, 2012). *Labeo fimbriatus* (Bloch, 1795) is considered as a suitable candidate for species diversification in culture. Though slow-growing, this medium sized carp is in good demand due high market value, excellent flavour and meat quality especially in peninsular India (Basavaraju *et al*., 1995). Published work on *L. fimbriatus* is limited (Mridula *et al*., 2003; Saravanan *et al*., 2011; Rao 2011; Swain *et al.,* 2013). *L. fimbriatus* is basically a benthic fish which graze on algae, protozoa, rotifers and diatoms that grow on submerged rocks and twigs (Bhatnagar and Karamachandani, 1970; Talwar and Jhingran, 1991; Das, 2011). In a grow-out study, Jena *et al*. (2011) demonstrated the compatibility of this species in polyculture with major carps. Keshavanath *et al*. (2002) and Mridula *et al*. (2003) have reported *L. fimbriatus* as a good periphyton grazer.

Digestibility is the relative measure of the extent to which ingested food and its nutrient components are digested and absorbed by the animal and is considered as one of the important factors in diet evaluation. The ability of *L. fimbriatus* to digest periphyton and absorb nutrients has not been quantified yet. Hence, the present study was undertaken using sugarcane bagasse, which has been already reported to be a good periphyton substrate (Keshavanath *et al*., 2001a; Mridula *et al*., 2003; Gangadhara and Keshavanath, 2008) that enhances fish growth (Ramesh *et al*., 1999; Keshavanath *et al*., 2001b; Gangadhara and Keshavanath, 2012). Activity of major digestive enzymes *viz*., total protease, trypsin, chymotrypsin, carboxypeptidase A and B, amylase, lipase and cellulase in the gut of fish fed periphyton in comparison with fish fed pelleted feed was estimated to validate the results.

Materials and methods

Growing periphyton

Outdoor cement tanks (6 x 4 x 1.2 m) with 5 cm soil base were used for growing periphyton. Sugarcane bagasse was used as the substrate for growing periphyton. 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. It was made into bundles of 20 (± 2.75) cm perimeter. Water from a nearby bore well was filled in the tanks to maintain a water column of 1 m; the evaporation loss, which was very meager, was compensated fortnightly. Cattle dung was applied to each tank @ 5 t ha^{-1}, followed by urea and single super phosphate (SSP) at 10 and 15 kg ha^{-1}, respectively. Sugarcane bagasse bundles were hung vertically at 2 t ha^{-1} (Keshavanath *et al.,* 2001b), keeping almost uniform distance between them. Subsequent fertilisation was done at fortnightly intervals with cattle dung @ 0.5 t ha^{-1} and urea and SSP @ 10 and 15 kg ha^{-1}, respectively.

Digestibility trial

Dry matter as well as protein digestibility of periphyton by the fish was estimated through a short term trial (Nandeesha *et al*., 1998) conducted in 60 (L) x 37 (W) x 55cm (H) glass aquaria. Ten fingerlings each of *L. fimbriatus* (average weight 5.17±0.19 g) were maintained in six aerated aquaria and acclimatised to laboratory conditions for a period of one week. Every morning at 10 00 hrs, bagasse bundles harbouring periphyton were immersed in three 'test' tanks and allowed for fish to graze on. Fish in three aquaria were fed a pelleted feed having 20% crude protein (Table 1). Fish were allowed to feed for 6 h. The bagasse bundles were removed and the uneaten periphyton/pelleted feed were siphoned out at the end of the feeding period. On the following day, fecal matter was collected from each tank by filtering the water with a fine meshed nylon cloth (15 µm), dried, pooled and stored for proximate analysis. About 50% of water from each aquarium was replaced with freshwater every day after fecal matter collection. This feeding and fecal matter collection trial was conducted for a period of 60 days. Proximate composition of periphyton, pelleted feed and fecal matter was analysed (AOAC, 1995). Dry matter and protein digestibility were determined according to Maynard and Loosli (1972). Acid insoluble ash was used as the reference marker (Goddard and McLean, 2001; Li *et al*., 2008; Bob-Manuel, 2013).

Analyses of digestive enzyme activity

After completion of the experiment, all the fish were weighed and length recorded. Three fish from each tank were sacrificed for digestive enzyme activity analyses. Gut was dissected out and homogenised in ice cooled condition with distilled water (4 ml g^{-1}) and centrifuged at 16,000 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 of Miller (1959). Total proteolytic activity was determined by the casein digestion method of Kunitz (1947). Trypsin and chymotrypsin activities were assayed according to the method of Erlanger *et al.* (1961). Carboxypeptidase activity was estimated following Appel (1974). Lipase activity was determined using p-nitro phenyl acetate (PNPA) as the substrate (Licia *et al.,* 2006). Cellulase activity was determined as per Miller (1959).

Statistical analyses

Data on periphyton digestibility and digestive enzyme activity were compared employing one way ANOVA, followed by t-test.

Results and discussion

The values obtained for proximate parameters of periphyton were: crude protein 19.66%, lipid 1.09%, nitrogen-free extract (NFE) 37.74% and ash 32.63% (Table 1). Periphyton is a complex of sessile biota including algae, invertebrates, detritus and microorganisms. The nutrient quality and availability in periphyton is influenced by several factors like grazing pressure, algal and bacterial species composition, nutrient level of environment, water quality, light intensity and most significantly substrate type (Makarevich *et al*., 1993; Azim *et al*., 2002; Gangadhara and Keshavanath, 2008). Montgomery and Gerking (1980) reported proximate composition of periphyton grown on granite boulders suspended in the Gulf of California. Protein, lipid, carbohydrate and ash contents of these epilithic periphyton were 8-10, 2-5, 52-60 and 25-38% respectively. An average protein content of 15% was estimated in periphyton collected from coral reef (Polunin, 1988). Dempster *et al*. (1995) reported 28-55% protein and 5-18% lipid in some algal species of periphytic nature. Proximate composition of periphyton from different substrates varied from 9 to 32% protein, 2-9% lipid, 25-28% NFE and 16-42% ash (Thompson *et al*., 2002; van Dam *et al*., 2002; Azim *et al*., 2005). Our earlier findings with

Table 1. Proximate composition (% on dry matter basis, mean± SD) of experimental feeds

Sample	Moisture	Crude protein	Fat	Crude fibre	Ash	Nitrogen-free extract (NFE)
Pelleted feed*	5.37±0.37	20.92±0.15	10.44±0.03	16.81±0.11	6.78±0.04	39.68±0.02
Periphyton	2.92±0.26	19.66±0.25	1.09±0.04	5.97±0.40	32.63±0.03	37.73±0.32

Feed comprised groundnut cake, rice bran, finger millet flour and vitamin and mineral mixture at 40, 50, 9 and 1% respectively.

periphyton from sugarcane bagasse revealed the following proximate composition: crude protein 26.06%, lipid 3.08%, NFE 38.02% and ash 17.45% (Gangadhara and Keshavanath, 2008), while Mridula *et al*. (2003) recorded 9.4, 0.33, 38 and 23% values for the respective parameters.

It has been documented that nutritional composition of periphyton can be considered as broadly appropriate to fish dietary needs (Dempster *et al*., 1993; Makarevich *et al*., 1993; Azim *et al*., 2002). Algal proteins in periphyton are also considered to be of good quality (Oser, 1959; Van Der Meeren *et al*., 2007). Apart from being a source of macronutrients, microalgae and heterotrophic bacteria are a rich source of immune enhancers (Supamattaya *et al*., 2005), growth promoters (Kuhn *et al*., 2010), bioactive compounds (Ju *et al*., 2008) and dietary stimulants (Xu *et al*., 2013) which can enhance growth performance of cultured fish. The protein level of 19.66% recorded in periphyton from bagasse (Table 1) compares well with the quality of some of the natural plant feeds that have been used in aquaculture (Yakupitiage, 1993; Dempster *et al.,* 1995). The protein requirement of freshwater fishes especially that of carps, varies between 25-35%, depending upon age and culture conditions (Hossain *et al*., 1997; Keshavanath *et al*., 2002). Studies with rohu have shown that in fertilised ponds, the growth obtained with 20 and 25% protein feeds were similar ($p>0.05$) (Nandeesha *et al*., 1994).

The high ash content of more than 30% in periphyton is attributed to the suspended clay particles (detritus) in the water column that are subsequently trapped on the substrates. Huchette *et al*. (2000) and Yakupitiyage (1993) stated that ash content lesser than 30% is reasonable in fish nutrition. However, ash *per se* is not harmful, but replaces nutritive components in feeds. High ash content of some plant fodders has been found not to impair the growth of tilapia (Yakupitiyage, 1993). Further, Azim *et al*. (2003) and Kaggwa *et al*. (2006) found that the high ash content (55 and 71% of dry matter, respectively) in periphyton had a positive effect on fish yields. Hence, the nutritional quality of periphyton, observed in the present study qualifies it to be considered as a dietary supplement for fish.

The dry matter digestibility values were lesser than the protein digestibility values (Fig. 1). Studies with rohu have also revealed such trends (Salim *et al*., 2004; Gul *et al*., 2007). Dry matter digestibility value recorded with *L. fimbriatus* for periphyton was higher ($p<0.05$) than that of the pelleted feed. Plant proteins are generally less digestible due to the presence of cellulose and other indigestible structural carbohydrates (Boyd and Goodyear, 1971). However, Allan and Rowland (1994) and Singh *et al*. (2003) have reported high apparent

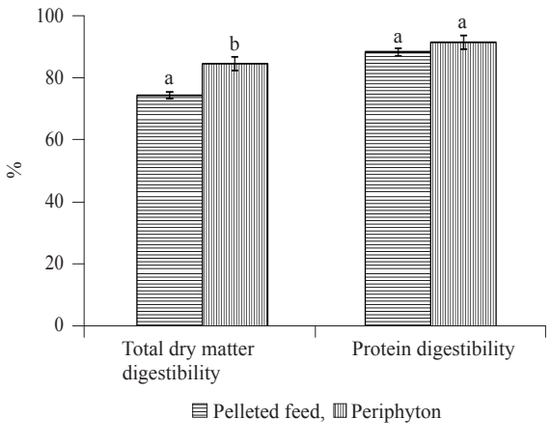

Fig. 1. Digestibility (%, mean± SD) of periphyton in *L. fimbriatus* compared to pelleted feed. Different alphabets on bars for the same parameter indicate significant difference ($p<0.05$)

digestibility coefficients for a number of plant products in the omnivorous carp *Cirrhinus mrigala*. Periphytic assemblage generally consists of chlorophytes, cyanophytes, myxophytes, diatoms, freshwater oligochaetes, protozoa, rotifers, coelenterate hydrozoa, cyanobacteria as well as free bacteria. Hence, periphyton contains substantial percentage of animal components also. Digestibility values recorded in the present study were higher compared to those obtained with other carps (mahseer, grass carp, rohu, mrigal, common carp and catla) and red tilapia, which ranged from 21 to 43% and 49 to 71% respectively, for periphyton grown on bamboo substrate (Gangadhara *et al*., 2004*)*. *L fimbriatus* is a riverine fish that is known to feed on periphyton in its natural habitat. It has fringed-lips for sucking growth on submerged objects. Being an omnivorous fish, it feeds on diatoms, Cyanophyceae, Chlorophyceae, Myxophyceae, Bacillariophyceae, plant tissue, copepods, insects and lower crustacean eggs (David and Rajgopal, 1974). Species composition of periphyton from bagasse has revealed ample availability of varied plankton (Gangadhara and Keshavanath, 2008). Further, live food is known to stimulate digestive enzyme production, contribute enzymes and also facilitate a better nutrient absorption as it contains approximately 75% water (Holt, 1993). The microorganisms present in periphyton represent a complementary food source, providing essential nutrients, particularly amino acids, polyunsaturated fatty acid, sterols, vitamins and pigments (Thompson *et al*., 2002). Composition of periphyton and its impact on enzyme activity would have facilitated higher dry matter digestibility in *L. fimbriatus*.

Protein digestibility values for periphyton and pelleted feed were comparable ($p>0.05$). Generally, the

protein quality of a diet is the leading factor affecting fish performance and protein digestibility is the first measure of its availability to the fish. Though it is reasonable to expect that the quality of protein from periphyton is better than that of the pelleted feed, lower protein content of the two could be the reason for the similar digestibility values recorded.

Ability of any fish to digest a given diet and absorb nutrients depends on the presence and the quality of digestive enzymes. In other words, nutrient digestibility is positively correlated with digestive enzyme activity (Hidalgo *et al*., 1999; De *et al*., 2015). Among proteases estimated in the present study, activity of total protease and carboxypeptidase B were higher ($p<0.05$) in fish fed periphyton, while the activity of others showed no difference between fish fed with periphyton and pelleted feed ($p>0.05$) (Fig. 2). Further, activity of carbohydrases and lipase were higher in fish fed pelleted feed. Digestive enzyme activity is known to be affected by proximate composition of the feed (Hofer, 1979; Fountoulaki *et al*., 2005). It may be noted that the periphyton was fed on wet basis and hence the nutrient content on wet weight basis will be lesser than pelleted feed which was fed dry.

Mridula *et al.* (2003, 2005) reported higher digestive enzyme activity in *L. fimbriatus* and *L. rohita* grown in feed + periphyton treatment, compared to fish fed pelleted feed. High digestive enzyme activity coupled with high growth has been observed in *Etroplus suratensis* grown in ponds provided with substrate, followed by those receiving feed and the control (Kumar *et al*., 2009). An overall enhancement in protease and amylase activities was observed in shrimp fed biofloc (Xu *et al*., 2013). Presence of microalgae and its components/live food even at low concentration in the gut can trigger the production of digestive enzymes in fish (Kamarudin *et al*., 1999; Brito *et al*., 2004). It has been noticed that microalgae enhance trypsin activity (Le Vay *et al*., 1993) as they contain large amounts of free amino acids (Admiral *et al*., 1986). Natural food not only enhances the production of digestive enzymes, but also contributes proteolytic enzymes for the digestion process in fish (Dabrowski and Glogowski, 1977). Munilla-Moran *et al.* (1990) reported that live food contributes significantly (43 to 60% protease, 78 to 88% esterase and 89 to 94% amylase) to the digestive process in *Scopthalmus maximus* larvae. Our studies revealed that periphton contains significant amount of digestive enzymes (Gangadher *et al.,* 2016). Therefore, contribution of digestive enzymes by periphyton to *L. fimbriatus* digestive process cannot be ruled out. Further, small, but measurable quantity of cellulase was recorded in the gut of *L. fimbriatus* (Fig. 2), indicating its endogenous secretion; which is supported by the observations of Jayaram (2013).

Herbivorous fish try to compensate the lower amount of available proteins in their diet by increasing consumption rate and enzyme production (Hofer, 1982). Sabapathy and Teo (1995) also reported similar findings in rabbitfish (*Siganus canaliculatus*). However, this phenomenon could not be ascertained in the present study since feed containing higher protein level was not used.

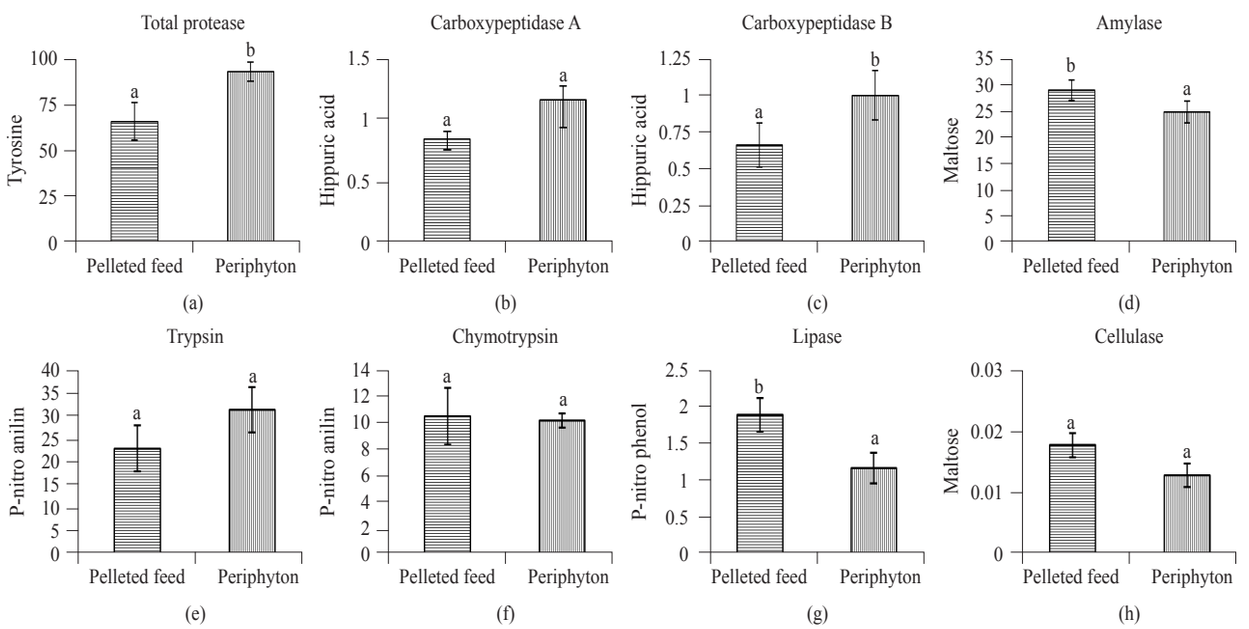

Fig. 2. Activity of digestive enzymes (mean ± SD; µmoles of product liberated h^{-1} mg tissue $protein^{-1}$ at 25°C) in the gut of *L. fimbriatus*. Different alphabets on bars in the same graph indicate significant difference ($p<0.05$)

The results of this study demonstrated that *L. fimbriatus* can utilise periphyton efficiently, which has implications on the economics of its culture.

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