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Digestibility of Dry Matter and Nutrients from *Azolla pinnata* by *Labeo calbasu* (Hamilton, 1822) with a Note on Digestive Enzyme Activity

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Abstract

Digestibility of feed ingredients in the compounded diet is an important factor affecting the growth of fish. A study was conducted in indoor plastic tubs to estimate the *in vivo* digestibility of dry matter and nutrients by a minor carp *Labeo calbasu* for a non-conventional feed ingredient – azolla, incorporated at levels ranging from 0-40% using crude fibre as the marker. The activities of major digestive enzymes were also estimated in the gut of fish fed varied levels of azolla. The digestibility values were the highest ($p<0.05$) at 10% azolla inclusion level. While the total dry matter digestibility and fat digestibility values of control diet were comparable with that of azolla diets up to 30% incorporation, with a decline thereafter; protein and NFE digestibilities were comparable up to 40% incorporation. Activities of majority of digestive enzymes were the highest ($p<0.05$) at 10% azolla incorporation level showing a decreasing trend at still higher levels. The study reveals the usefulness of azolla for inclusion in calbasu diets. Azolla can be used up to 30% without affecting the digestibility.

Keywords: Digestibility, *Labeo calbasu*, azolla, digestive enzyme, non-conventional ingredients

Introduction

Feed constitutes the major expenditure in any fish culture operation (Mukhopadhyay & Jena, 1999) which contributes to cost increase per unit production (New & Csavas, 1993). Farmers and feed manufacturers alike are attempting to reduce feed cost and secure ingredients to produce cost-effective diets. Several studies have been done to make supplementary feeding cost-effective, using less expensive plant protein sources (Jackson et al., 1982; De Silva & Gunasekera, 1989; Shamna et al., 2015; Asadujjaman & Hossain, 2016). Azolla (*Azolla pinnata)*, an aquatic weed, is rich in protein; total protein ranging from 25 to 30%. Other constituents in Azolla are minerals, chlorophyll, carotenoids, amino acids, and vitamins. *A. pinnata* is consumed by some macrophytophagous fish (Cassani, 1981). It is easy to cultivate and has higher productivity and nutritive value. The use of azolla as a fish feed ingredient is well documented (Dutta, 2011, Gangadhar et al., 2014; 2015). Digestibility of individual ingredients in the compounded diet is considered as one of the important factors affecting the growth of fish (De Silva et al., 1990) and hence this study was undertaken to evaluate the digestibility of ingredients before and after its incorporation in diet.

Orange fin labeo, *Labeo calbasu* is an herbivorous fish belonging to *Cyprinidae*, found commonly in rivers and freshwater lakes in and around South Asia and South-East Asia and at several places is referred to as the "Black Rohu". It thrives better in tanks and lakes than in running waters and can also tolerate slightly brackish water. It is a bottom dweller and can tolerate high turbid water during dry season. It is a commercially important species often cultured in South Western China, India, Pakistan, Bangladesh and Thailand. It is a popular food fish next to three Indian major carps *Labeo rohita, Catla catla* and *Cirrhinus mrigala* (Gupta & Banerjee, 2015). The fish is in great demand in the market. Ghosh et al. (1933) stated that its liver oil contains vitamin A. The present study was conducted to estimate the

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digestibility of dry matter and nutrients from the non-conventional feed ingredient azolla, by calbasu. The activity of major digestive enzymes was also estimated in the gut of fish fed varied levels azolla to validate the results.

Materials and Methods

The basal diet consisted of groundnut cake, rice bran, finger millet (binder) and vitamin- mineral mixture. Four experimental diets were formulated for each ingredient replacing groundnut oil cake and rice bran of the basal diet at 10, 20, 30 and 40% levels (Umesh et al., 1994) (Table 1). Whole plants of *A. pinnata* were harvested from fish culture ponds, sun dried for 2-3 days, packed in polythene bags and powdered at requisite quantities before feed preparation. Groundnut oilcake and finger millet were dried and powdered. All the ingredients were sieved through a fine meshed screen (0.5 mm). The required quantity of the ingredients was mixed with hot water to make adough and pressed through a hand pelletiser to get uniform sized pellets (2 mm). The pellets were sundried and packed in polythene bags till further use. Digestibility of the test ingredient was estimated *in vivo* through a short-term trial (Umesh et al., 1994; Nandeesha et al., 1998) conducted in 50 L indoor aerated plastic tubs. Ten fingerlings of calbasu (8.03-10.26 g) were maintained in each aerated tub and acclimated to laboratory conditions for a week. Each diet was fed to fish in triplicate tubs every morning at 10.00 h at 3% body weight. For feeding and faecal matter collection, the method described by De Silva et al. (1990) was adopted. The fish were allowed to feed for 2 h (Xiqin et al., 1994). The pelleted feed was siphoned out at the end of the feeding period. On the following day, fecal matter was collected from each tub 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 tub was replaced with fresh water every day after fecal matter collection. This feeding and fecal matter collection trial was conducted for a period of 45 days. Proximate composition of pelleted feed and fecal matter was analysed (AOAC, 1995). Crude protein, crude fat and crude fibre were estimated using Kel Plus, Soc Plus and Fibra Plus instruments (Pelican, India), respectively. Ash was determined by burning the sample at 550°C in a muffle furnace. Moisture was estimated by drying the sample at 80°C for more than 12 h. NFE was obtained by the 'Difference method' of Hastings (1976). Energy content of the feed ingredients and diets was calculated using values of 22.6 kJ g^{-1} for protein, 38.9 kJ g^{-1} for lipid and 17.2 kJ g^{-1} for carbohydrate

Table 1. Ingredient proportion (%) and proximate composition (%, mean ± S.D.) of experimental feed

Ingredients used	Control	10% Azolla	20% Azolla	30% Azolla	40% Azolla
Groundnut cake	45	40	35	30	25
Rice bran	45	40	35	30	25
Ragi	9	9	9	9	9
Azolla	0	10	20	30	40
Vitamin & mineral mix.	1	1	1	1	1
Proximate composition (%)					
Moisture	5.79±0.08	5.60±0.07	4.87±0.10	4.53±0.06	4.60±0.06
Crude protein	26.23±0.31	25.85±0.43	25.98±0.25	24.96±0.32	24.85±0.68
Fat	7.76±0.43	7.46±0.07	7.12±0.10	6.66±0.06	6.23±0.06
Ash	6.99±0.02	8.51±0.07	10.23±0.02	12.12±0.14	13.55±0.11
Crude fiber	7.81±0.02	7.88±0.30	6.24±1.11	6.41±0.31	6.69±0.85
NFE	45.42±1.51	44.70±0.69	45.56±0.89	45.32±1.83	44.08±2.21
Gross energy (kJg^{-1})	17.07	16.82	16.72	16.53	16.03

NFE – Nitrogen-free extract

Data on digestibility were compared employing one-way analysis of variance by Duncan's multiple range test (p= 0.05) (Duncan, 1955).

as NFE (Mayes, 1990). Crude fibre was used as the reference marker (De Silva et al., 1990; Krontveit et al., 2014). Apparent digestibility values were calculated employing the formula described by Cho et al. (1985). Water samples were collected at fortnightly intervals, before fecal matter collection, between 09.00 and 10.00 h from all the tanks and analysed for pH, temperature, dissolved oxygen and total alkalinity (APHA, 1998). After completion of the experiment, three fish from each tub were sacrificed for digestive enzyme activity analyses. Gut was dissected out and homogenized in ice cooled condition with distilled water (4 m Lg^{-1}) and centrifuged at 16 000 rpm for 20 min at 4°C. The supernatant (crude enzyme extract) was stored at -20°C in 1.5 ml aliquots until further use. Total soluble protein of the homogenate was measured using Folin-phenol reagent (Lowry et al., 1951). 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). Amylase and cellulase were assayed by hydrolyzing starch and carboxy methyl cellulose respectively, followed by estimation of reducing sugars (Nelson, 1944). Lipase activity was determined using p-nitro phenyl acetate (PNPA) as the substrate (Licia et al., 2006). Experimental data was analysed for one-way analysis of varience using Duncan's multiple range test (Duncan, 1955). Significance of differences were established at $p<0.05$.

Results and Discussion

Mean water quality parameters recorded in the tanks during the experimental duration was as follows water temperature 22.64 to 24.52°C, dissolved oxygen 5.18 to 8.84 mg L^{-1}, total alkalinity 91.43 to 121.10 mg L^{-1}, pH 7.84 to 8.39. Crude protein content of azolla-incorporated diets ranged from 24.85-26.23%, while fat and NFE contents varied from 6.23-7.76% and 44.08-45.56% respectively. The total dry matter digestibility (DMD) and major nutrient digestibility values recorded in the trials are given in Table 2. Digestibility values were the highest ($p<0.05$) at 10% azolla inclusion level. While the total DMD and fat digestibility values of control were comparable with that of azolla diets up to 30% incorporation, with a decline there after, protein and NFE digestibilities were comparable up to 40% incorporation.

The activity pattern of digestive enzymes in the gut of calbasu following feeding with azolla diets is given in Fig. 1. Activities of all the enzymes, except cellulase were the highest ($p<0.05$) at 10% azolla in corporation level showing a decreasing trend at still higher levels. In the present study, incorporation of azolla up to 30% did not have any adverse effect on the total DMD and nutrient digestibility in calbasu. *A. pinnata*, has been considered a potential source of nutrients which is directly used as a food component by a number of herbivorous fish (Cassani, 1981; Okeyo, 1989). However, species specific differences in digestibility of nutrients and energy from various feed ingredients have been observed (Refstie et al., 2000). According to Datta (2011), azolla can be incorporated up to 25% in the diet of an Indian major carp, *Labeo rohita*. Azolla inclusion at 40% enhanced growth in *L. rohita* fingerlings (Panigrahi et al., 2014). Asadujjaman & Hosain (2016) reported poor growth of *Catla catla* fed azolla as compared to those fed control diet consisting of rice bran, wheat bran and mustard cake (30:30:40). Gangadhar et al. (2015) indicated the possibility of incorporating azolla in diets of medium carp *L. fimbriatus* up to 40% during fry-to-fingerling rearing. The differences in the

Table 2. Digestibility (%, mean ± S.D.) of dry matter, protein and fat by *L. calbasu* fed experimental feeds

Feeds	Total DMD	Protein Digestibility	Fat digestibility	NFE digestibility
Control	51.73 ± 2.93^{b}	59.57 ± 3.23^{a}	78.05 ± 0.79^{b}	69.34 ± 0.19^{ab}
10% Azolla	62.11 ± 5.42^{c}	74.84 ± 2.02^{b}	90.63 ± 0.83^{c}	81.80 ± 1.38^{c}
20% Azolla	50.29 ± 1.04^{b}	63.11 ± 1.45^{a}	82.18 ± 0.37^{bc}	76.62 ± 0.30^{bc}
30% Azolla	47.20 ± 0.80^{ab}	64.05 ± 0.74^{a}	85.62 ± 2.18^{bc}	76.98 ± 0.03^{bc}
40% Azolla	42.02 ± 2.77^{a}	59.59 ± 2.45^{a}	59.97 ± 8.06^{a}	63.26 ± 0.23^{a}

DMD – Dry matter digestibility;

Different superscripts for values in the same column indicate significant difference ($p<0.05$)

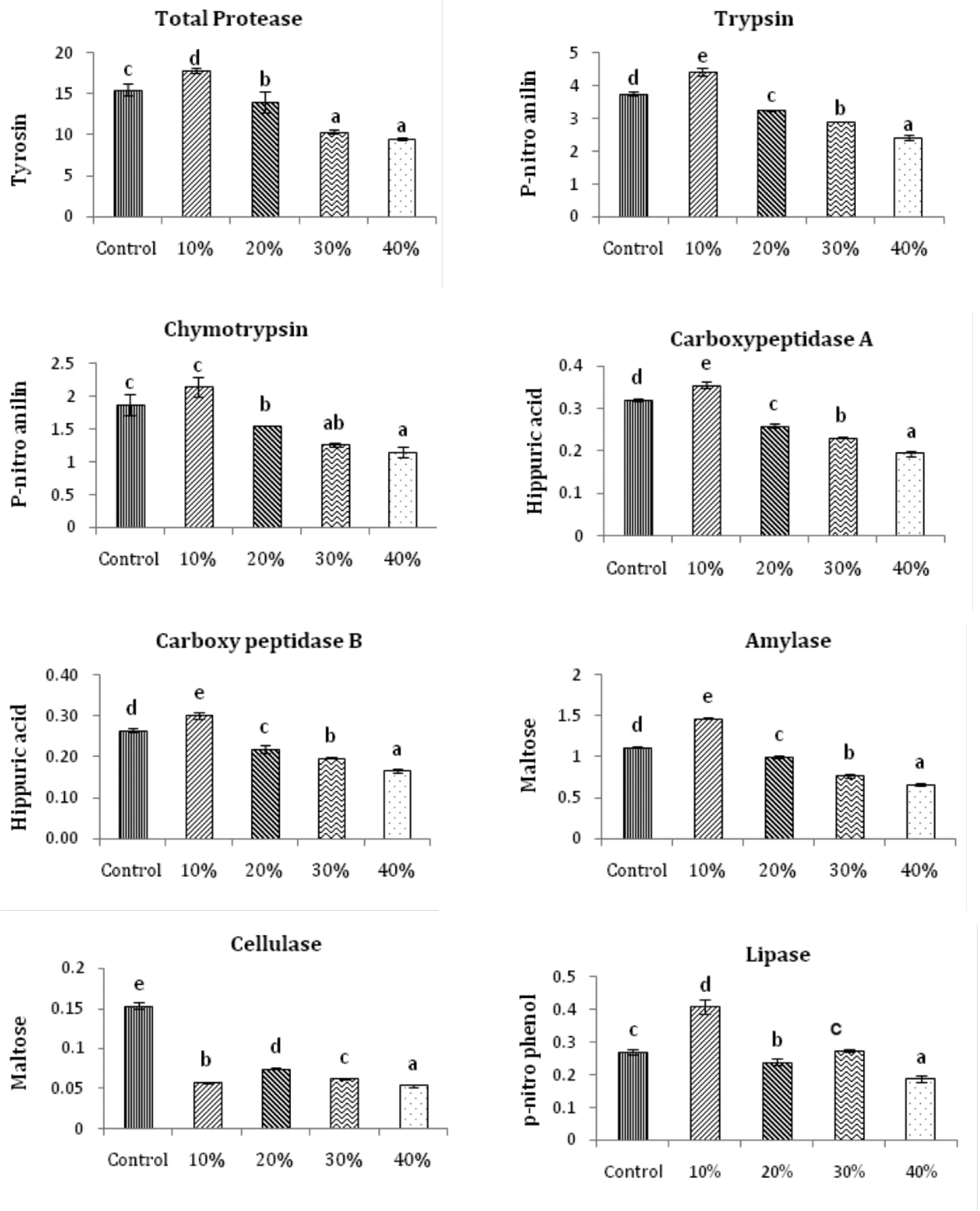

Fig. 1. Activity of digestive enzymes (mean± SD; μmoles of product liberatedhr^{-1}mg tissue $protein^{-1}$ at 25°C) in the gut of *L. calbasu* fed azolla incorporated diets. Different letters on bars in the same graph indicate statistical difference ($p<0.05$).

utilization of azolla by different carps can be attributed to their feeding habits. Generally, calbasu is considered as aherbivorous fish feeding mainly on vegetable matter, followed by crustaceans, insect larvae etc. It feeds on algae (10%), higher plants (48%), protozoa (12%), crustaceans (10%), mollusks (5%), mud and sand (15%) (Bhuiyan, 1964). In the present study, incorporation of azolla above 30% resulted in lower total DMD and fat digestibility of calbasu. Addition of more than 25% azolla in fish diet has led to reduced food utilisation, poor growth performance and altered muscle composition in *Etroplus suratensis* (Joseph et al., 1994). The introduction of plant protein components to carp diets is limited by the content of crude fibre. The quantity of crude fiber in experimental diets tested in the current study did not increase from the level estimated in the control diet. Hence, it is presumed that it did not impact digestibility.

Studies conducted earlier with other plant protein sources such as *Gliricidia maculate*, *Colocasiae sculenta*, *Leucaenal eucocephala* and *Eichornia crassipes* in catlaindicated declining protein and dry matter digestibility when they were incorporated beyond 15% in the reference diet. De Silva et al. (1990) while reviewing the work of Cho et al. (1982), suggested 15-20% inclusion of leaf meal as the most appropriate test level instead of the generally recommended level of 30%. The fact that azolla incorporation up to 30% did not result in any adverse results in calbasu, indicates the superiority of azolla as a feed ingredient over other plant protein sources. Enzymes play an important role in the digestion and utilization of feed (Dabrowski & Glogowski, 1977) and dietary nutrients influence digestive enzyme activity (Gangadhara et al., 1997, Fountoulaki et al., 2005). In general, the activity pattern of the digestive enzymes corroborated the digestibility pattern of the nutrients in the diets in calbasu. The study reveals the usefulness of azolla for inclusion in calbasu diets. Azolla can be used up to 30% without affecting the digestibility.

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