Indian J. Fish., 68(4): 152-156, 2021
DOI: 10.21077/ijf.2021.68.4.112816-18

Note

Apparent digestibility of dried azolla powder incorporated diets in rohu *Labeo rohita* (Hamilton, 1822)

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ABSTRACT

Dry matter and nutrient digestibility of dried azolla powder incorporated in the traditional feed was evaluated in advanced fingerlings of *Labeo rohita* (Hamilton, 1822) reared in aerated indoor plastic tanks. The fish were fed nearly isocaloric diets formulated to contain azolla powder, replacing the main ingredients, groundnut cake and rice bran of the Control diet at 10, 20, 30 and 40% levels. Total dry matter digestibility and major nutrient digestibility were estimated using acid insoluble ash as the marker. Digestibility (%) of dry matter, protein, fat and nitrogen free extract (NFE) showed a declining trend with increased azolla incorporation. Only protein and fat digestibility of 20% azolla diet was comparable with that of the Control, with higher incorporation levels resulting in significant reduction in the digestibility values.

Keywords: Alternate protein source, Feed ingredient, Fish feed, Rohu

The small leafed floating aquatic plants *Azolla* spp. are distributed all over the world in freshwater ecosystems of temperate and tropical regions (Kannaiyan and Kumar, 2006) and can be grown easily with minimal expenditure. These aquatic plants with high biomass and protein levels can be used as feed ingredient owing to higher crude protein (24 to 30% on dry weight basis) and essential amino acid content than the majority of green forage crops and other aquatic macrophytes (Panigrahi *et al*., 2014). Azolla is known to contain other constituents such as minerals, chlorophyll, carotenoids, vitamins (Umalatha *et al*., 2018) and some probiotics and biopolymers (Pillai *et al*., 2002). There are studies on the use of azolla in the culture of catla (Asadujjaman and Hosain, 2016), rohu (Mohanty and Dash, 1995; Maity and Patra, 2008; Datta, 2011; Panigrahi *et al*., 2014; Kumari *et al*., 2017), fimbriatus (Gangadhar *et al*., 2015), silver barb (Das *et al*., 2004), tilapia (Micha *et al*., 1988; Sithara and Kamalaveni, 2008; Youssouf, 2012) and black tiger shrimp (Sudaaryano, 2006). Results of these studies, however, are mostly inconsistent and ambiguous. Dietary azolla supplementation in most cases seems to be beneficial in terms of growth performance of fish and often resulted in reduced feed cost. Considering its ease of cultivation, higher productivity and nutritive value and the increasing interest in ecologically sound integrated farming systems, it is worth exploring the potential of azolla as an ingredient for fish feed.

The feed cost constitutes a major portion of the production cost in aquaculture practices irrespective of whether it is intensive or semi-intensive. A judicious choice of dietary constituents leading to reduced feed cost is the key to profitable fish farming. Several dietary formulations suiting to the feeding habit of herbivorous aquatic species are commercially available. The conventional carp feed is a mixture of rice bran and groundnut cake in equal proportions. The cost of a kilo of groundnut cake varies from ₹30-50/- depending on quality, while that of rice bran ranges from ₹20-25/- per kg. High cost of these feed ingredients leads to increased expenditure on fish production, which in turn reduces the profit margins in aqua-farming. Therefore, the main criterion for commercial fish farming is the availability of appropriate cost effective feed, formulated from locally obtainable ingredients which are able to fulfill the nutritional requirements of the cultured species. The present study was conducted considering this urgent need for alternate, locally available feed ingredients and in view of the limited availability of literature on the use of azolla as a dietary ingredient for the Indian major carps. Digestibility of ingredients being the key to the use of any feed ingredient, this study evaluated the effect of incorporating varied levels of dried azolla (*Azolla microphylla*) powder in the conventional feed of one of the important Indian major carps, rohu *Labeo rohita* (Hamilton, 1822), on the digestibility of dry matter and major nutrients.

The basal diet (Control) was formulated with groundnut oilcake and rice bran (Table 1). Finger millet (ragi) was used at 9% level as the binder. *A. microphylla*

Table 1. Ingredient proportion (%) and proximate composition (%, Mean±SD) of experimental diets

Ingredients	Control	10% Azolla	20% Azolla	30% Azolla	40% Azolla
Groundnut cake	45	40	35	30	25
Rice bran	45	40	35	30	25
Ragi (Finger millet)	9	9	9	9	9
Azolla powder	0	10	20	30	40
Vitamin and mineral mixture*	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	26.02±0.31	25.98±0.43	25.55±0.25	24.96±0.32	24.85±0.68
Fat	8.26±0.43	8.04±0.07	7.86±0.10	7.62±0.06	7.33±0.06
Ash	9.81±0.02	9.88±0.07	8.69±0.02	8.40±0.14	8.24±0.11
Crude fiber	7.81±0.02	7.88±0.30	6.69±1.11	6.41±0.31	6.24±0.85
NFE	42.12±1.51	41.99±0.69	42.80±0.89	42.36±1.83	42.43±2.21
Gross energy (kJ g^{-1})	16.34	16.22	16.19	15.89	15.77

*Each kg has Calcium-25.5%, Phosphorus-12.75%, Mangessium-6000 mg, Suphur-0.72%, Sodium-5.9 mg, Potassium-100 mg, Copper-1200 mg, Cobalt-150 mg, Zinc-9600 mg, Iron-1500 mg, Iodine-325 mg, Selenium-10 mg, Manganese-1500 mg, Vitamin A-700000 IU, Vitamin D3-70000 IU, Vitamin E-250 mg, Nicotinamide-1000 mg, DL-methionine-1929 mg, L-lysine-4400 mg, *Lactobacillus* sp. 1.5×10^{11} CFU, *Saccharomyces cerevisiae* -30000 million CFU.

grown under controlled conditions in circular cement tanks were harvested, sun dried for 3-4 days and powdered at required quantities before feed preparation. Four experimental diets were formulated replacing groundnut oilcake and rice bran in the basal diet with azolla at 10 (10% Azolla), 20 (20% Azolla), 30 (30% Azolla) and 40% (40% Azolla) levels as per Umesh *et al*. (1994). For preparation of feed, groundnut oilcake and finger millet were dried, powdered and all the ingredients were sieved through a 0.5 mm meshed screen. The ingredients at appropriate quantity were mixed with hot water to make a dough, which was then pressed through a hand pelletiser to get uniform sized pellets (2 mm). The pellets were dried under sun and packed in air tight bags till use.

Apparent digestibility of the test diets was evaluated *in vivo* through a feeding experiment carried out in indoor, aerated, plastic tanks of 50 l capacity. Ten advanced rohu fingerlings (8.50-11.00 g) were stocked in 15 aerated tanks and acclimated for ten days with the Control diet. The control and test diets were fed every morning at 10 00 hrs to the fish in the designated triplicate tanks till satiation. Fish were allowed to feed for 6 h and at the end of the feeding period, the unconsumed pellets were siphoned out. On the following day, faecal matter from each experimental tank was collected by filtering through a 15 µm meshed nylon cloth. The faecal matter collected was dried and stored for the analysis of proximate composition. About half of water from each tank was replaced with freshwater daily after faecal matter collection. Feeding and faecal matter collection was undertaken for a period of 45 days.

Feed ingredients, pelleted feed and collected faecal material were subjected to proximate analysis (AOAC, 1995). Gross energy content of the diet was calculated following Mayes (1990). Acid insoluble ash was used as the reference marker (Goddard and McLean, 2001; Li *et al*., 2008; Bob-Manuel, 2013). Dry matter and nutrient digestibility were calculated according to Maynard and Loosli (1972). Water quality parameters *viz,* dissolved oxygen and total alkalinity were analysed following APHA (2005), while pH and temperature were recorded using probes (Orion 2 Star pH meter, Thermo Electron Corporation, USA), at fortnightly intervals.

Data on digestibility were compared employing one-way ANOVA. Further, pair-wise comparison of treatment means was done by Duncan's multiple range test (p=0.05) (Duncan, 1955).

The average temperature of tank water ranged from 24.98 to 25.22°C during the study period. Water showed alkaline conditions with average pH ranging from 7.34 to 8.10 and total alkalinity from 300.16 to 313.01 mg l^{-1}. Dissolved oxygen levels varied between 5.86 and 7.26 mg l^{-1} (Table 2). The major water quality parameters recorded were within the acceptable limit for the culture of carps (Jena *et al.,* 2011) and no significant (p>0.05) difference between the treatments was recorded.

Dutta *et al*. (2011) reported the chemical composition of *A. microphylla* from field condition as 20.2% crude protein, 3.5% crude fat, 15.8% crude fibre and 17.2% ash. Chatterjee *et al.* (2013) reported 24.1% crude protein, 3.3% crude fat, 13.4% crude fibre and 19.5% ash in *A. microphylla* produced in the experimental production unit. The proximate composition values obtained in the present study were close to those reported earlier, being 25.5% crude protein, 7.0% crude fat, 15.8% crude fibre and 20% ash.

Table 2. Water quality parameters (Mean±SD) recorded in the experimental tanks during the study period

Water quality	Control	10% Azolla	20% Azolla	30% Azolla	40% Azolla
Temperature (°C)	25.22±0.53	25.09±0.25	25.06±0.23	24.98±0.21	25.21±0.14
pH	8.10±0.12	7.79±0.31	7.49±0.11	8.00±0.12	7.34±0.03
Dissolved oxygen (mg l^{-1})	7.00±1.32	6.67±1.09	5.86±1.11	7.26±0.48	6.87±1.22
Total alkalinity (mg $CaCO_3$ l^{-1})	308.78±3.81	307.87±7.61	313.01±9.31	311.49±5.18	300.16±4.56

The nutritive value of food depends on the capacity of the animal to digest and absorb the nutrients and the efficiency of digestion of the ingested food largely determines the growth performance of the animal (Deganp and Yehuda, 1999). There was an apparent reducing trend in the digestibility (%) of dry matter, protein, fat and NFE with increased dietary incorporation levels of azolla. Only protein and fat digestibility of 20% Azolla diet was comparable with that of the Control, higher incorporation levels showing significant reduction in the digestibility values (Table 3). Fish belonging to the family Cyprinidae have been experimentally fed diets with different azolla inclusion levels. In earlier studies also, better feed utilisation and increased growth was recorded in rohu at dietary azolla inclusion levels of 10-50% (Datta, 2011; Panigrahi *et al.,* 2014). Kumari *et al.* (2017) recommended 20% azolla supplementation in the diet of *L. rohita,* while according to Datta (2011), it can go up to 25%. Maity and Patra (2008) also recommended 25% level of *Azolla pinnata* for better growth, food conversion and protein efficiency ratio in rohu. Incorporation of *A. pinnata* at 10-25% levels resulted in higher growth of rohu due to the ω-6 fatty acids present in azolla (Mohanty and Dash, 1995).

submerged vegetation (Alam *et al*., 2011), is expected to utilise azolla better compared to other IMCs. The study by Asadujjaman and Hosain (2016) revealed that the growth of catla (*Catla catla*) fed azolla was inferior to those fed control diet consisting of rice bran, wheat bran and mustard cake (30:30:40). Total dry matter digestibility (DMD) and protein digestibility of azolla diets in catla were comparable with the control diet up to 20% incorporation ($p>0.05$), reducing there after ($p<0.05$) (Umalatha *et al*., 2018). In similar studies conducted with *L. fimbriatus* and *Cyprinus carpio*, dry matter and protein digestibility decreased at azolla incorporation levels above 20% (Gangadhar *et al*., 2017a) and that with a medium carp *Labeo calbasu*, total DMD and fat digestibility decreased at levels above 30% (Gangadhar *et al*., 2017b). Silver carp and mrigal (Tuladhar, 2003), grass carp (Majhi *et al*., 2006) and Thai silver barb (Das *et al*., 2018) were also reported to have azolla inclusion levels in the range between 10-25%. Incorporation of more than 25% azolla in the diet led to reduced food utilisation in *Etroplus suratensis* (Joseph *et al*., 1994). Reduction in the digestibility can be attributed to increasing crude fibre content, including lignin level, resulting in swift movement of food through the gut of the

Table 3. Digestibility (%) of dry matter, protein, fat and NFE by rohu in the experimental diets

Diets	Dry matter digestibility	Protein digestibility	Fat digestibility	NFE digestibility
Control	81.70±0.62^{c}	87.97±0.40^{c}	99.39±0.12^{c}	91.33±0.95^{c}
10% Azolla	76.92±1.24^{b}	85.72±0.47^{c}	99.48±0.03^{c}	83.13±1.26^{b}
20% Azolla	76.00±1.66^{b}	85.17±0.74^{c}	96.76±0.79^{c}	84.22±0.94^{b}
30% Azolla	65.42±2.45^{a}	77.59±1.43^{b}	93.60±1.02^{b}	80.46±1.63^{ab}
40% Azolla	64.96±0.66^{a}	73.45±1.68^{a}	93.61±0.65^{a}	77.22±1.30^{a}

Figures in the same column with same superscript do not differ significantly ($p>0.05$).

Sheeno and Sahu (2006) observed that azolla protein concentrate is a good source of protein and can be used to the maximum extent of 16.25% in the diet of *L. rohita*. The same authors also recorded lower protein and carbohydrate digestibility in rohu fed azolla protein concentrate in the diet above 24 and 16% levels respectively.

Species specific differences in nutrient and energy digestibility of various feed ingredients have been observed mainly due to the differences in the feeding habits of fish (Refstie *et al*., 2000). Rohu, being a herbivorous fish consuming mainly phytoplankton and animal, thereby lessening the time available for digestion and absorption of the diet (Ayyappan, 2000).

Based on the results of the present study, it is concluded that dietary incorporation of dried azolla up to 20% level does not affect nutrient digestibility in *L. rohita*. The results of this study add to the knowledge on utilisation of azolla in the diet of *L. rohita.*

Acknowledgements

The authors are grateful to the Director, ICAR-CIFA, Bhubaneswar for providing infrastructure facilities for conducting this experiment.

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Date of Receipt : 19.07.2021
Date of Acceptance : 29.11.2021