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# Effect of azolla-incorporated diets on the growth and survival of *Labeo fimbriatus* during fry-to-fingerling rearing

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**Abstract:** An experiment of 75 days duration was conducted in fertilized outdoor circular cement tanks (1,000 L) with soil base for evaluating the growth and survival of *Labeo fimbriatus* fry fed with pelleted feed containing varied levels of dried azolla (*Azolla pinnata*). The Control feed contained 45% groundnut oilcake plus 45% rice bran and 10% finger millet flour added as binder for pelleting. Dried azolla powder was incorporated into the feed at 10, 20, 30 and 40% levels, replacing the groundnut cake and rice bran proportionately. *L. fimbriatus* (mean length 2.42 cm) fry were stocked in all the tanks at 30 $m^{-3}$. The fish were fed 10% of body weight during the first month, followed by 7% during the second month and 5% during the last 15 days. Incorporation of azolla did not affect ($p > 0.05$) the water quality, growth and survival of fingerlings at harvest. Incorporation of azolla in the diet reduced the cost of feed (Rs. per 100 g biomass; Rs: Indian rupee, INR; 1 INR ≈ 0.015 EUR) from 3.35 to 2.53, with a cost saving of 24.48%. The study indicated the possibility of incorporating azolla in diets of *L. fimbriatus* up to 40% during fry-to-fingerling rearing, resulting in savings on feed cost.

**Subjects: Bioscience; Environment & Agriculture; Food Science & Technology**

**Keywords: azolla; growth; fringe-lipped carp; *Labeo fimbriatus*; seed rearing**

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## ABOUT THE AUTHORS

The research group from Peninsular Aquaculture Division of Central Institute of Freshwater Aquaculture, Bangalore, India, is involved in strategic and applied research for developing sustainable and diversified freshwater aquaculture practices for enhanced productivity, quality, water use efficiency and farm income. The first author G. Barlaya is a Senior Scientist and has been working on various aspects of freshwater aquaculture like breeding of endangered mahseer, evaluation of hormonal and non-hormonal growth promoters and alternate protein sources in fish feed. Currently, he is handling the Department of Biotechnology, New Delhi, India-funded research project on periphyton-based aquaculture of a minor carp, *Labeo fimbriatus*.

## PUBLIC INTEREST STATEMENT

The most commonly used supplementary feed in carp seed rearing is the powdered mixture of rice bran and groundnut oil cake at 1:1 ratio. However, since the cost of these feed ingredients, particularly groundnut oilcake, has increased sharply in recent years in the face of expanded world demand, there is an urgent need to evaluate alternative locally available ingredients. This study aims at evaluating the potential of azolla as an ingredient in the feed of *Labeo fimbriatus* during fry-to-fingerling rearing. The study is the first report on the utilization of azolla in the diet of fish in seed rearing. The results of the present study may benefit the fish farmers, hatchery operators, nutritionists and researchers.

## 1. Introduction

In recent years, utilization of aquatic plants and weeds having high food value as feed ingredients has taken a new dimension in producing the much required animal protein at low cost. Aquatic weeds also have the added advantage of being cultivated in association with farmed fish species using the same water resources and/or farm effluent (Edwards, Hassan, Chao, & Pacharaprakiti, 1992; Gavina, 1994). They constitute dietary items for both herbivorous and omnivorous fish species in semi-intensive and extensive aquaculture production (Leonard, 1995). Azolla, which grows in association with the blue green alga *Anabaena azollae*, is perhaps the most promising in the context of increasing interest in ecologically sound integrated farming systems, ease of cultivation, higher productivity and nutritive value. *Azolla pinnata* is one of aquatic plants found in lakes, paddy fields, freshwater pond areas, rivers and irrigation channels round the year (Lumpkin & Plucknett, 1982). The cost of production of aqua feed can be reduced using this ecofriendly and sustainable resource. The use of azolla as a fish feed ingredient is well documented, but literature on its dietary use in seed rearing is scanty. Santiago, Aldaba, Reyes, and Laron (1988) reported that *Oreochromis niloticus* fry fed rations containing up to 42% *of A. pinnata* outperformed fish fed a fishmeal-based control diet. Growth and feed utilization in *O. niloticus* fry improved with increased dietary inclusion of azolla and the survival was unaffected. Devi and Vishwanath (1993) compared the nutritive value and growth responses of azolla-based diets on advanced fry of the endemic medium carp, *Osteobrama belangeri*.

Earlier studies have reported improved feed utilization in *Tilapia mossambica* (Sithara & Kamalaveni, 2008) and increased growth in rohu (Datta, 2011; Tuladhar, 2003), Nile tilapia (Fiogbe, Micha, & Van Hove, 2004; Ebrahim, Zeinhom, & Abou-Seif, 2007), common carp, silver carp and mrigal (Tuladhar, 2003) have been reported upon inclusion of azolla in feeds. Sivakumar and Solaimalai (2003) have observed the beneficial effects of feeding fresh and dried azolla to *O. niloticus* in integrated rice-fish culture system. According to Majhi, Das, and Mandal (2006), utilization of organic azolla through grass carp is one of the best options for the production of fish biomass from the aquatic habitat.

*Labeo fimbriatus*, a "medium carp", commonly called as "fringe-lipped carp" has been considered as a candidate species for species diversification in carp polyculture system. This species is suitable for cultivation in confined waters (Mohanta, Subramanian, Komarpant, & Saurabh, 2008). Jena et al. (2011) have demonstrated the compatibility of this species with major carps in polyculture. Though slow-growing, this medium-sized carp is in good demand due to its excellent meat quality (Basavaraju, Devaraj, & Ayyar, 1995). The culture of *L. fimbriatus* along with other Indian major carps is now being taken up by farmers. However, large-scale farming of these species would necessitate mass-scale production of their fingerlings.

The most commonly used supplementary feed in carp seed rearing is the powdered mixture of rice bran and groundnut oilcake at 1:1 ratio (Jena, Mukhopadhyay, Sarkar, Aravindakshan, & Muduli, 1996). However, since the cost of these feed ingredients, particularly groundnut oilcake, has increased sharply in recent years in the face of expanded world demand (FAO, 2011), there is an urgent need to evaluate alternative locally available ingredients. This study aims at evaluating the potential of azolla (*A. pinnata*) as an ingredient in the feed of *L. fimbriatus* during fry-to-fingerling rearing.

## 2. Materials and methods

### *2.1. Experimental set-up*

This experiment of 75-day duration was carried out in fifteen 1,000 L outdoor circular cement tanks with 5 cm soil base at the Regional Research Centre of Central Institute of Freshwater Aquaculture, Bangalore, India. Bangalore is positioned at 12.97°N 77.56°E, at an average elevation of 920 m. The temperature in Bangalore ranges from 11°C (52°F) in winter and 36°C (97°F) in summer. The region receives around 12 h daylight. Water from a nearby borewell was filled in the tanks to maintain a water column of 70 cm; the evaporation loss, which was very meagre, was compensated fortnightly. The total volume of water in each tank was 0.886 $m^3$. Cattle dung was applied to each tank at 4 t $ha^{-1}$ (0.35 kg $tank^{-1}$), followed by urea and single super phosphate (SSP) at 10 and 15 kg $ha^{-1,}$ respectively

(0.90 and 1.3 g tank$^{-1}$ respectively) (Jena et al., 2005). Ten days after addition of manures and fertilizers, fry of *L. fimbriatus* (mean length 2.42 ± 0.24 cm (mean ± SE), weight 0.11 ± 0.04 g) was stocked at 300,000 ha$^{-1}$ (27 tank$^{-1}$) (Jena et al., 2005) in all the tanks. Subsequent fertilization was done at fortnightly intervals with cattle dung at 0.5 t ha$^{-1}$ and urea and SSP at 10 and 15 kg ha$^{-1}$, respectively.

### *2.2. Feed preparation and feeding*

The proximate composition of the ingredients used in feed manufacturing is given in Table 1. The ingredient proportion and proximate composition of the experimental feeds are shown in Table 2. The conventional feed used for carp seed rearing is a 50:50 mixture of groundnut oilcake and rice bran (Jena et al., 1996). Finger millet flour at 10% was used as binder to increase the integrity of the feed pellets. Four test diets were prepared by replacing the groundnut oilcake and rice bran from Control feed at 10 (10% A), 20 (20% A), 30 (30% A) and 40% (40% A) with dried and powdered azolla. Whole plants of *A. pinnata* were harvested in sufficient quantities from the fish culture ponds, sun dried for 2–3 days, packed in

**Table 1. Proximate composition (g kg$^{-1}$ as dry matter basis) and cost of ingredients**

| | Groundnut cake | Rice bran | Finger millet | Azolla (*A. pinnata*, dried) |
|---|---|---|---|---|
| Moisture | 88 ± 4.1 | 45 ± 2.3 | 37 ± 2.1 | 41 ± 1.8 |
| Crude protein | 400 ± 2.8 | 208 ± 1.9 | 81 ± 2.2 | 275 ± 2.9 |
| Crude fat | 83 ± 1.9 | 90 ± 1.3 | 15 ± 0.3 | 41 ± 1.1 |
| Ash | 68 ± 4.8 | 117 ± 7.1 | 30 ± 3.3 | 200 ± 4.9 |
| Crude fibre | 129 ± 5.1 | 107 ± 3.1 | 27 ± 1.0 | 116 ± 7.0 |
| NFE (Nitrogen-free extract) | 232 | 433 | 810 | 327 |
| Gross energy (MJ kg$^{-1}$) | 16.26 | 15.65 | 16.35 | 13.43 |
| Cost (Rs. kg$^{-1}$) | 41.22 | 15.00 | 25.00 | 5.00[a] |

Notes: Data expressed as Mean ± SE.

Rs: Indian rupee (INR); 1 INR ≈ 0.015 EUR.

[a]The cost of azolla is calculated for collection from pond, drying and powdering.

**Table 2. Ingredient proportion, proximate composition (g kg$^{-1}$ as dry matter basis) and cost of diets**

| | Diets | | | | |
|---|---|---|---|---|---|
| | Control | 10% A | 20% A | 30% A | 40% A |
| *Ingredients* | | | | | |
| Groundnut oilcake | 450 | 400 | 350 | 300 | 250 |
| Rice bran | 450 | 400 | 350 | 300 | 250 |
| Finger millet | 100 | 100 | 100 | 100 | 100 |
| Azolla (*A. pinnata.*) | 0 | 100 | 200 | 300 | 400 |
| *Proximate composition of diets* | | | | | |
| Moisture | 33.2 ± 1.3 | 29.7 ± 1.2 | 31.9 ± 0.9 | 33.9 ± 1.9 | 37.8 ± 0.8 |
| Crude protein | 280.8 ± 2.8 | 278.3 ± 1.9 | 276.3 ± 3.1 | 274.0 ± 3.7 | 271.8 ± 3.2 |
| Crude fat | 78.3 ± 4.1 | 75.0 ± 5.2 | 71.2 ± 2.5 | 67.2 ± 5.8 | 63.5 ± 3.3 |
| Ash | 95.7 ± 5.1 | 99.8 ± 2.8 | 108.9 ± 3.1 | 117.1 ± 2.1 | 119.8 ± 4.0 |
| Crude fibre | 105.3 ± 2.6 | 109.0 ± 3.8 | 108.9 ± 3.9 | 111.1 ± 4.2 | 114.9 ± 2.4 |
| NFE (Nitrogen-free extract) | 406.7 | 408.2 | 402.8 | 396.7 | 392.2 |
| Gross energy (MJ kg$^{-1}$) | 16.39 | 16.28 | 15.94 | 15.63 | 15.36 |
| Cost (Rs. kg$^{-1}$) | 27.80 | 25.49 | 23.18 | 20.87 | 18.55 |

Notes: Data expressed as Mean ± SE.

Rs: Indian rupee (INR); 1 INR ≈ 0.015 EUR.

polythene bags and powdered at required quantities before feed preparation. Groundnut cake, rice bran and finger millet were procured locally. Groundnut cake 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 a dough and pressed through a hand pelletizer to get uniform-sized pellets (2 mm diameter). The pellets were sun dried and packed in polythene bags till further use. Fish in randomly selected triplicate tanks were fed one of the four experimental diets and the control diet once daily (Biswas, Jena, & Singh, 2006) at 10% of body weight during the first month, followed by 7% during the second month and 5% during the last 15 days (Jena et al., 2005).

### *2.3. Chemical analyses*

Proximate composition of feed ingredients and feed were analysed following AOAC (1995) procedures. Carbohydrate content was calculated as nitrogen-free extract (NFE) by the difference method of Hastings (1976). The energy value of each ingredient as well as feed was obtained by multiplying protein, lipid and carbohydrate contents by factors 22.6, 38.9 and 17.2, respectively, (Mayes, 1990) and expressed in kJ $g^{-1}$. Water quality was monitored by analysing for pH, temperature, dissolved oxygen (DO), total alkalinity and Secchi disc visibility at fortnightly intervals following standard methods (APHA, 1998). Water samples were collected between 09.00 and 10.00 h.

### *2.4. Statistical analyses*

On termination of the experiment, fingerlings were harvested by draining the tanks. All surviving fish were counted and their length and weight recorded. These data were used for calculation of growth parameters like specific growth rate (SGR), percent weight gain, survival and total biomass per tank and the feed efficiency indicator, feed conversion ratio (FCR). Comparison among treatments for fish growth, survival and water-quality parameters was done by one-way analysis of variance using SPSS 16 statistical software.

## 3. Results and discussion

The mean water quality parameters recorded in the different treatment tanks during the two experiments did not vary significantly ($p > 0.05$) with the inclusion of *A. pinnata* in diets at increasing levels (Table 3). pH was in the alkaline range throughout the experimental duration, indicating favorable conditions for biological production. The optimum range of pH value for fish culture is 6.5–9 (Woynarovich, 1975). Jhingran (1991) observed that carps thrive well in the temperature range of 18.3–37.8°C. The mean water temperature in the present study ranged from 25.43 to 25.62°C and DO from 6.24 to 6.98 mg $L^{-1}$. Generally, cyprinids are capable of tolerating low oxygen levels of 3 mg $L^{-1}$ (Huet, 1972). Total alkalinity was in a higher range (291.46–317.83 mg $L^{-1}$). Waters of higher alkalinity are considered more productive in terms of oxygen production and photosynthesis (Elnady, Alkobaby, Salem, Abdel-Salam, & Asran, 2010). Water transparency fluctuated between 60.22 and 63.66 cm.

**Table 3. Water-quality parameters recorded during the experiment with *L. fimbriatus***

| Treatment[a] | pH | Temperature ( C) | Dissolved oxygen (mg $L^{-1}$) | Total alkalinity (mg $L^{-1}$) | Secchi disc visibility (cm) |
|---|---|---|---|---|---|
| Control | 8.25 ± 0.07 | 25.55 ± 0.58 | 6.27 ± 0.44 | 317.83 ± 9.94 | 62.11 ± 2.61 |
| 10% A | 8.36 ± 0.74 | 25.62 ± 0.64 | 6.53 ± 0.53 | 306.28 ± 10.69 | 61.33 ± 2.80 |
| 20% A | 8.40 ± 0.09 | 25.56 ± 0.60 | 6.82 ± 0.47 | 306.72 ± 10.71 | 62.33 ± 2.65 |
| 30% A | 8.41 ± 0.10 | 25.47 ± 0.54 | 6.24 ± 0.55 | 314.55 ± 10.22 | 63.66 ± 2.57 |
| 40% A | 8.35 ± 0.33 | 25.43 ± 0.55 | 6.98 ± 0.51 | 291.46 ± 32.54 | 60.22 ± 2.62 |
| P-value | 0.205 | 0.842 | 0.363 | 0.345 | 0.422 |

Note: Data expressed as Mean ± SE ($n = 3$).

[a]Treatments consisting in diets with increasing levels of substitution of the control diet with *A. pinnata* (A).

Incorporation of azolla in the diet did not affect ($p > 0.05$) the growth, survival and FCR in *L. fimbriatus* (Table 4) in any of the levels incorporated. Incorporation of azolla did not affect the crude protein content of experimental diets significantly (Table 2). The gross energy content of diets ranged from 15.36 (40% A) to 16.40 MJ $kg^{-1}$ (Control). The insignificant differences in the growth parameters of fish receiving azolla-incorporated diets could be attributed to differences in energy content of the experimental diets (Lupatsch, Kissil, Sklan, & Pfeffer, 2001).

In nature, freshwater herbivorous fishes like *L. fimbriatus* feed mainly on unicellular algae, filamentous algae and parts of higher aquatic plants. Though there are no reports on the inclusion of azolla in the diet of *L. fimbriatus* in the wild, the species is expected to utilize azolla in the diet. The nutritive value of azolla for fish was explained by Joseph, Sherief, and James (1994). Utilization of sun-dried azolla by young and adults of other cultivable herbivorous and omnivorous species has been reported earlier (Almazan et al., 1986; Joseph et al., 1994; Santiago et al., 1988). El-Sayed (2008) noted that young Nile tilapia utilized azolla more efficiently than adults. Sheeno and Sahu (2006) reported that azolla protein concentrate is a good source of protein and can be used up to 16.25% by replacing 10% fish meal in the diet of *Labeo rohita* fry. According to Datta (2011), azolla can be incorporated up to 25% in the diet of *L. rohita*. Fiogbe et al. (2004) fed *O. niloticus* with diets containing dry azolla meal at 0, 15, 20, 30, 40 and 45% of diets, with azolla-free diet and diet containing 15% azolla resulting in similar growth performance. They concluded that the least expensive diet containing 45% azolla can be used as a complementary diet for tilapia raised in fertilized ponds. Ebrahim et al. (2007) incorporated sun-dried and ground *Azolla nilotica* at 10.6, 21.2, 31.8 and 42.2% levels in the diet of *O. niloticus*. They observed that growth performance and survival of the treated fish was comparable ($p > 0.05$) with that of the control.

In apparent contrast to the present research, studies of Almazan et al. (1986) (with fingerlings of *O. niloticus*), Antoine, Carraro, Micha, and Van Hove (1986) (with *O. niloticus* and *Cichlasoma melanurum*), Micha, Antoine, Wery, and Van Hove (1988) (with *O. niloticus* and *Tilapia rendalli*) and Joseph et al. (1994) (with *Etroplus suratensis*) revealed lowering of growth performance and food conversion with increasing azolla incorporation in the diet. Similar reduction in growth rate and feed conversion was also recorded in rohu fry fed higher levels of azolla protein concentrate (Sheeno & Sahu, 2006). It was opined that the reduction in growth may be due to the imbalance in amino acid make-up of azolla protein. *A. pinnata* protein is reported to be limiting in tryptophan and slightly deficient in threonine (Almazan et al., 1986), increasing the demand for the deficient amino acids with increased concentration of azolla in the diet. However, no significant ($p > 0.05$) effect of azolla incorporation on growth and FCR of *L. fimbriatus* was observed in the present study.

**Table 4. Effect of azolla (*A. pinnata*)-incorporated diets on the growth and survival of *L. fimbriatus* in fry-to-fingerling rearing**

| Parameter | Diets[a] | | | | | |
|---|---|---|---|---|---|---|
| | Control | 10% A | 20% A | 30% A | 40% A | *p*-Value |
| Length (cm) | 7.93 ± 0.23 | 7.85 ± 0.12 | 7.67 ± 0.11 | 7.29 ± 0.37 | 7.41 ± 0.11 | 0.083 |
| Weight (g) | 5.40 ± 0.35 | 5.36 ± 0.38 | 4.91 ± 0.41 | 4.94 ± 0.50 | 4.56 ± 0.16 | 0.180 |
| Survival (%) | 88.82 ± 2.19 | 92.49 ± 2.06 | 92.49 ± 2.06 | 96.20 ± 2.14 | 93.82 ± 3.27 | 0.073 |
| SGR (Specific growth rate, % $d^{-1}$) | 5.16 ± 0.09 | 5.15 ± 0.09 | 5.03 ± 0.11 | 5.03 ± 0.13 | 4.94 ± 0.05 | 0.181 |
| Total biomass (g $tank^{-1}$) | 130.00 ± 11.49 | 133.81 ± 8.67 | 123.30 ± 13.01 | 128.80 ± 15.69 | 115.33 ± 0.34 | 0.305 |
| Weight gain (%) | 4,721 ± 312 | 4,686 ± 338 | 4,287 ± 365 | 4,308 ± 451 | 3,974 ± 141 | 0.180 |
| FCR (Feed conversion ratio) | 1.22 ± 0.11 | 1.18 ± 0.07 | 1.30 ± 0.14 | 1.25 ± 0.13 | 1.35 ± 0 | 0.299 |
| Cost of feed (Rs. per 100 g fish biomass) | 3.35 | 2.99 | 2.95 | 2.55 | 2.53 | |

Notes: Data expressed as Mean ± SE ($n = 3$).

Rs: Indian rupee (INR); 1 INR ≈ 0.015 EUR.

[a]Treatments consisting in diets with increasing levels of substitution of the control diet with *A. pinnata* (A).

The cost of the control diet worked out to Rs. 27.80 (Rs: Indian rupee, INR; 1 INR ≈ 0.015 EUR). Incorporation of azolla in the diet reduced the cost of feed from 3.35 to 2.53 Rs. per 100 g biomass, with a cost saving of 24.48%.

## 4. Conclusion

The results of this study indicate that incorporation of azolla up to 40% in the diet of *L. fimbriatus* during fry-to-fingerling rearing does not affect fish growth and survival. The incorporation results in saving on feed cost.

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**Competing interests**
The authors declare no competing interests.

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**Cover image**
Source: B. Gangadhar.

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