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Growth Response of *Cirrhinus mrigala* Fry to Azolla (*Azolla pinnata*) - incorporated Diets

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Abstract

This experiment of 75 days duration was conducted in out-door circular cement tanks (1000 l) with added soil bottom. Growth and survival of *Cirrhinus mrigala* fry, fed with pelleted feed containing varied levels of dried azolla was evaluated. Tanks were fertilized initially with cow dung at 4 t ha^{-1} followed by 0.5 t ha^{-1} fortnightly. Additionally, urea and single super phosphate were also applied at 10 and 15 kg ha^{-1} at fortnightly intervals. Control feed contained 45% groundnut oil cake 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 oil cake and rice bran proportionately. *C. mrigala* fry was stocked in all the tanks @ 0.3 milion ha^{-1}, ten days after fertilization. Major water quality parameters were analyzed at fortnightly intervals. Incorporation of azolla did not affect ($p>0.05$) the water quality, growth and survival of fingerlings at harvest. However, 40% azolla incorporated diet showed significant reduction ($p<0.05$) in length of mrigal fingerlings. Incorporation of azolla in the diet reduced the cost of feed (Rs 100 g $biomass^{-1}$) from 3.52 (control feed) to 2.86 (40% azolla feed) with a cost saving of 18.75%. Considering no difference between the control and 40% azolla incorporated diets with regard to fish weight and survival at harvest, incorporation of azolla in diet of *C. mrigala* up to 40% is recommended during fry to fingerling rearing for cost saving.

Keywords: *Cirrhinus mrigala*, mrigal, azolla, fingerling rearing

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Introduction

Supplemental feeding is an important management measure in intensive aquaculture for enhancing fish production. In recent years, utilization of aquatic plants/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 et al., 1992; Gavina, 1994). They constitute dietary items for both herbivorous and omnivorous fish species in semi-intensive and extensive aquaculture production (Leonard, 1995). *Azolla pinnata*, which grows in association with 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. *A. pinnata* is one of the aquatic plants found in lakes, paddy fields, freshwater pond areas, rivers and irrigation channels round the year (Lumpkin & Puckett, 1982). The cost of production of aqua feed can be reduced using this eco-friendly and sustainable resource. Santiago et al. (1988) reported that *Oreochromis niloticus* fry fed rations containing up to 42% *of A. pinnata* out performed fish meal-based control diet. Growth and feed utilization in *O. niloticus* fry improved with increased dietary inclusion of azolla and the survival was unaffected. Devi & Viswanath (1993) compared the nutritive value and growth responses of azolla based diets on advanced fry of endemic medium carp, *Osteobrama belangeri*.

Improved feed utilization in *Tilapia mossambica* (Sithara & Kamalaveni, 2008) and increased growth in rohu (Dutta, 2011), Nile tilapia (Fiogbe et al.,

2004, Ebrahim et al., 2007), common carp, silver carp, rohu & mrigal (Tuladhar, 2003) have been reported upon inclusion of azolla in feeds. Sivakumar & Solaimalai (2003) have observed beneficial effects of feeding fresh and dried azolla to *O. niloticus* in integrated rice-fish culture system. According to Majhi et al. (2006), utilization of organic azolla through grass carp is one of the best options for the production of fish biomass from the aquatic habitat.

Cirrhinus mrigala, commonly called as 'Mrigal' is one of the extensively cultured Indian major carps in India, Bangladesh, Pakistan, Myanmar, the Lao People's Democratic Republic, Thailand and Nepal. The adult fish feeds upon filamentous green algae, diatoms, pieces of higher plants, decayed vegetable, mud and detritus (Pillay & Kutty. 2005).

Powdered mixture of rice bran and groundnut oil cake at 1:1 ratio is the most commonly used supplementary feed in carp seed rearing (Jena et al., 1996). Since the cost of these feed ingredients, particularly groundnut oil cake 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. The main objective of the present study is to evaluate the potential of azolla as an ingredient in the feed of *C. mrigala* during fry rearing.

Materials and Methods

This experiment of 75-day duration was carried out in fifteen out-door circular cement tanks with 1000 l capacity and 5 cm soil base at the Regional Research Centre of Central Institute of Freshwater Aquaculture, Bangalore, India. Water from a nearby bore well was filled in the tanks to maintain a water column of 70 cm; the evaporation loss, which was very meagre, was compensated fortnightly. Cattle dung was applied to each tank @ 4 t ha^{-1}, followed by urea and single super phosphate (SSP) at 10 and 15 kg ha^{-1} respectively as per Jena et al. (2005). Ten days after addition of manures and fertilizers, fry of *C. mrigala* (1.97±0.21 cm and 0.07±0.02 g) was stocked @ 0.3 million ha^{-1} (Jena et al., 2005) in all the tanks. Subsequent fertilization was done at fortnightly intervals with cattle dung @ 0.5 t ha^{-1} and urea and SSP at 10 and 15 kg ha^{-1}, respectively.

Control feed consisted of mixture of groundnut oil cake, rice bran and finger millet flour (45:45:10). Finger millet flour at 10% was used as binder to increase the integrity of feed pellets. Four test diets were prepared by replacing groundnut oil cake and rice bran from control feed (C) at 10 (T1), 20 (T2), 30 (T3) and 40% (T4) with dried and powdered azolla (Table 2). Whole plants of *A. pinnata* were harvested in sufficient quantities from fish culture ponds, sun dried for 2-3 days, packed in polythene bags and powdered at requisite quantities before feed preparation. Groundnut oil cake, rice bran and finger millet were procured locally. Groundnut oil 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 pelletiser to get uniform sized pellets (2 mm). The pellets were sun dried and packed in polythene bags till further use. Fish in randomly selected triplicate tanks were fed with one of the four diets once daily (Biswas et al., 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).

Proximate composition of feed ingredients and feed were analyzed following AOAC (2012) 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 kg^{-1}. Water quality (pH, temperature, dissolved oxygen, total alkalinity and Secchi disc visibility) was monitored fortnightly following standard methods (APHA, 1998). Water samples were collected between 09.00 and 10.00 h.

On termination of the experiment, fingerlings were harvested by draining the tanks. All surviving fish were counted and their length and weight recorded. Comparison among treatments for fish growth, survival and water quality parameters was done by one-way analysis of variance (ANOVA). When a main parameter was significant, pair-wise comparison of treatment means was done by Duncan's multiple range test ($p = 0.05$) (Duncan, 1955).

Results and Discussion

Proximate composition of the ingredients used is given in Table 1. The ingredient proportion and proximate composition of the experimental feeds are shown in Table 2. There was a reducing trend in dietary crude protein, fat, NFE and gross energy levels and a subsequent increase in ash and crude

fibre levels with azolla incorporation. The conventional feed used for carp seed rearing is a 50:50 mixture of groundnut oil cake and rice bran (Jena et al., 1996).

Water quality parameters were within acceptable range for carps (Jhingran, 1991), with no substantial variation between treatments (Table 3). pH was in the alkaline range throughout the experiment indicating favourable conditions for biological production. The optimum range of pH value for fish culture is 6.5 to 9 (Woynarovich, 1975). Carps thrive well in the temperature range of 18.3° to 37.8°C (Jhingran, 1991). The mean water temperature in the present study ranged from 24 to 24.25°C and dissolved oxygen (DO) from 5.65 to 6.05 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 (305.42-317.05 mg L^{-1}). Water of higher alkalinity is considered more productive in terms of oxygen production and photosynthesis (Elnady et al., 2010). Water transparency fluctuated between 58.42 and 63.58 cm.

Table 1. Proximate composition (g kg^{-1}) of ingredients

	Groundnut oil cake	Rice bran	Finger millet	Azolla
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	232	433	810	327
Gross energy (kJ kg^{-1})	16.26	15.65	16.35	13.43
Cost (Rs kg^{-1})*	43.22	15.00	27.00	6.84

* Cost of powdering added to groundnut cake and finger millet; cost of azolla calculated based on actual cost of production including powdering charges.

Table 2. Ingredient proportion and proximate composition (g kg^{-1}) of diets

	Diets				
Ingredients	C	T1	T2	T3	T4
Groundnut oilcake	450	400	350	300	250
Rice bran	450	400	350	300	250
Finger millet	100	100	100	100	100
Azolla	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	406.7	408.2	402.8	396.7	392.2
Gross energy (kJ 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

C-control feed, T1- 10% azolla diet, T2- 20% azolla diet, T3- 30% azolla diet, T4- 40% azolla diet.
Data expressed as mean±SE (n=3).

Incorporation of azolla in the diet did not affect ($p>0.05$) the growth parameters, survival and FCR except a reduction ($p<0.05$) in length of fingerlings at 40% incorporation (Table 4). Significant differences were also observed between T1 and T4 in terms of weight, SGR, total biomass production and percent weight gain (Table 4). FCR was higher ($p<0.05$) in T3 and T4 diets compared to T1 and T2. Incorporation of azolla did not affect the crude protein content of experimental diets significantly. The gross energy content of diets ranged from 15.36 (T4) to 16.39 kJ kg^{-1} (C).

In nature, the food of omnivorous species like *Cirrhina mrigala* consists of a varying percentage of plant and animal material. Hence, the species is expected to utilize azolla in the diet. Nutritive value of azolla for fish was explained by Joseph et al. (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; Santiago et al., 1988; Joseph et al., 1994). El-Sayed (2008) noted that young Nile tilapia utilized azolla more efficiently than adults. Sheeno & 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 Dutta (2001), azolla can be incorporated up to 25% in the diet of *Labeo rohita*. Fiogbe et al. (2004) fed *O. niloticus* with diets containing dry azolla meal at 0, 15, 20, 30, 40 and 45% of diets. 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 *A. nilotica* at 10.6, 21.2, 31.8 and 42.2% levels in the diet of *O. niloticus*. The comparable growth performance and survival of the treated fish

Table 3. Average water quality parameters in the rearing tanks of *Cirrhinus mrigala* for a period of 75 days of culture

Treatment	pH	Temperature (°C)	Dissolved oxygen (mg L^{-1})	Total alkalinity (mg L^{-1})	Secchi disc visibility (cm)
C	8.36±0.17	24.14±0.46	5.92±0.26	305.42±13.16	59.75±3.23
T1	8.56±0.17	24.22±0.44	5.67±0.31	314.53±8.42	61.58±3.27
T2	8.53±0.13	24.25±0.44	5.70±0.31	314.60±7.11	60.25±3.43
T3	8.47±0.14	24.05±0.42	6.05±0.36	317.05±6.86	63.58±2.97
T4	8.51±0.14	24.00±0.44	5.65±0.33	308.14±12.81	58.42±2.18

C-control feed, T1- 10% azolla diet, T2- 20% azolla diet, T3- 30% azolla diet, T4- 40% azolla diet.
Data expressed as mean±SE (n = 3).

Table 4. Effect of azolla incorporated diets on the growth and survival of *C. mrigala* fry

Parameter	Diets C	T1	T2	T3	T4
Weight (g)	$6.52±0.41^{ab}$	$6.80±0.23^{b}$	$6.72±0.11^{ab}$	$5.93±0.46^{ab}$	$5.72±0.07^{a}$
Length (cm)	$9.06±0.21^{b}$	$8.64±0.37^{ab}$	$8.64±0.10^{ab}$	$8.67±0.15^{ab}$	$8.19±0.17^{a}$
Survival (%)	$83.66±1.33^{a}$	$85.33±5.78^{a}$	$85.33±4.33^{a}$	$85.66±2.40^{a}$	$82.66±5.55^{a}$
SGR (% d^{-1})	$6.12±0.08^{ab}$	$6.18±0.04^{b}$	$6.17±0.02^{b}$	$5.99±0.10^{ab}$	$5.95±0.02^{a}$
Total biomass (g $tank^{-1}$)	$147.63±7.37^{ab}$	$157.04±11.71^{b}$	$154.22±5.17^{ab}$	$131.82±7.92^{ab}$	$127.55±6.75^{a}$
Percent wt. gain	$9784±617^{ab}$	$10198±343^{b}$	$10077±171^{ab}$	$8846±725^{ab}$	$8567±105^{a}$
FCR	$1.25±0.08^{ab}$	$1.17±0.04^{a}$	$1.18±0.02^{a}$	$1.40±0.11^{b}$	$1.43±02^{b}$
Cost of feed (Rs 100 g $biomass^{-1}$)	3.52	3.11	2.90	3.08	2.86

C-control feed, T1- 10% azolla diet, T2- 20% azolla diet, T3- 30% azolla diet, T4- 40% azolla diet.
Data expressed as mean±SE (n = 3).
Figure in the same row with same superscript are not significantly different ($p>0.05$).

with that of the control in the present study indicates that mrigal fry also utilses the azolla in the diet at the levels tried.

In apparent contrast, studies of Almazan et al. (1986) (with fingerlings of *O. niloticus*), Antoine et al. (1986) with *O. niloticus* and *Cichlasoma melanurum* and Micha et al. (1988) (with *O. niloticus* and *Tilapia rendalli)* revealed lowering of growth performance and FCR with increasing azolla incorporation in the diet. As seen in the present study, a decreasing trend in the performance of mrigal, in terms of growth parameters and FCR, becoming significant at 40% level of azolla incorporation, is an indication that dietary incorporation of azolla beyond 30% is not desirable in fry to fingerling rearing. Similar reduction in growth rate and food conversion was recorded in rohu fry fed with higher levels of azolla protein concentrate (Sheeno & Sahu, 2006). The reduction in growth may be due to the imbalance in amino acid make-up of azolla protein as tryptophan and threonine are limiting in *A. pinnata* (Almazan et al., 1986). Increased FCR and poor growth due to feeding high levels of azolla has been reported in *Etroplus suratensis* (Joseph et al., 1994), *Clarias gariepinus* (Fasakin & Balogun, 1998), *O. niloticus* (Almazan et al., 1986) and rohu fry (Sheeno & Sahu, 2006).

Cost of the control diet worked out to Rs. 27.80. Incorporation of azolla in the diet reduced the cost of feed (Rs. 100 g biomass^{-1}) from 3.52 (C) to 2.86 (T4) with a cost saving of 18.75%. Since the survival of animals is the most important aspect during nursery rearing (Jena et al., 1998), similar survival recorded in the different treatments indicate the possibility of incorporation of azolla up to 40% in the diet of *C. mrigala* during fry to fingerling rearing.

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