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Ramesh Kumari
Research Scholar, Department of Aquaculture, College of Fisheries, MPUAT, Udaipur, Rajasthan, India

ML Ojha
Assistant Professor, Department of Basic Science, College of Fisheries, MPUAT, Udaipur, Rajasthan, India

VP Saini
Professor, Aquaculture Research and Seed Unit, Directorate of Research, MPUAT, Udaipur, Rajasthan, India

SK Sharma
Professor, Department of Aquatic Environment, College of Fisheries, MPUAT, Udaipur, Rajasthan, India

Correspondence
Ramesh Kumari
Research Scholar, Department of Aquaculture, College of Fisheries, MPUAT, Udaipur, Rajasthan, India

Effect of *Azolla* supplementation on growth of rohu (*Labeo rohita*) fingerlings

Ramesh Kumari, ML Ojha, VP Saini and SK Sharma

Abstract
The present study was conducted to evaluate the *Azolla* supplementation effect of fish *Labeo rohita* fingerlings growth performance. The weight gain, per cent weight gain, specific growth rate (SGR), food conversion ratio (FCR) and gross conversion efficiency (GCE) were significantly improved in treatments as compared to control. Still the highest weight gain (27.450±3.839g), per cent weight gain (45.863±1.677%), SGR (1.024±0.051%) and GCE (0.257±0.010) were recorded in T_2 (200g *Azolla* per kg basal diet). The fishes fed with T_2 have also indicated better food utilization with lesser food conservation ratio (FCR) *i.e.* 3.903±0.163 as compared to other treatments. Therefore, it can be concluded that @200g/kg *Azolla* supplemented diet had a significant role in improving the growth of *Labeo rohita*. Thus the dose of 200 g/kg *Azolla* is recommended for supplementation in the diet of *L. rohita*.

Keywords: Growth, *Azolla*, *Labeo rohita*, Feed.

Introduction
Aquaculture, the fastest growing food producing sector, is perceived as having the greatest potential to meet the growing demand for aquatic food. World aquaculture production is likely to grow continuously, but at slow rate FAO [1]. World production attained another all-time high of 158.08 million tons from which aquaculture contributes about 66.6 million tons. The global trend of aquaculture development gaining importance in total fish supply has remained uninterrupted. Farmed food fishes contributed a record 42.2% of the total 158.08 million tons produced (both capture and culture) which is much higher than 13.4% in 1990 and 25.7% in 2000 [1].

Azolla is becoming an integral part of the aquaculture practices to obtain high production. Indian freshwater aquaculture constitutes the mainly culture of IMC *viz., Catla catla, Labeo rohita* and *Cirrhinus mrigala.* Plant based diet like a mixture of groundnut oil cake and rice bran (1:1) are generally used as supplementary feed for these fishes [2]. Carp culture in India constitutes 87% of total aquaculture production [3]. Rohu *(Labeo rohita)* is the most commercial fish with maximum market demand and acceptability as food by the consumers due to its test and flesh quality. Various kinds of supplementary feeds are being tried to accelerate growth and production of fish per unit area [4].

Azolla is an ideal feed substitute which is an aquatic fern (pteridophyte) floating on the water surface of flooded rice fields, small ponds and canals. *Azolla* has been used as a feed for pig, duck and fish. *Azolla* has high protein content (20-30 per cent on dry weight basis) rich in almost all essential amino acids, vitamin A, vitamin B-complex, beta-carotene and minerals such as calcium, phosphorus, potassium, iron, copper and magnesium [5]. It is also a potential source of nitrogen and feed ingredient for livestock [6, 7]. Considering the nutritional value of *Azolla*, the present study was conducted to evaluate its effect on growth of rohu (*labeo rohita*).

Materials and Methods
Experimental fish: The Indian Major Carp, *Labeo rohita* was selected for the present study. The healthy fingerlings were procured from Aquaculture Research and Seed Unit, DOR, MPUAT, Udaipur.

Experimental diet: A basal diet was prepared using groundnut Oilcake (320 g/kg), rice bran (320 g/kg), Soya-bean meal (300 g/kg), wheat flour (50 g/kg) and mineral mixture (10 g/kg). For the preparation of experimental diet, *Azolla* was mixed in basal diet in different quantities

such as 0.0 in (T_0 control), 100 (T_1), 200 (T_2), 300 (T_3) and 400g/kg (T_4) basal diet (Table 1). The experimental diets were analyzed for the proximate composition viz., moisture, crude protein, fat, carbohydrate and ash contents as per standard methods of AOAC [8].

Table 1: The ingredients used for basal diets (g/kg)

S. No.	Ingredients	T_0 (Control)	T_1	T_2	T_3	T_4
1	Groundnut Oil Cake	320	320	320	320	320
2	Rice Bran	320	320	320	320	320
3	Soyabean Meal	300	300	300	300	300
4	Wheat Flour	50	50	50	50	50
5	Mineral Mixture	10	10	10	10	10
6	*Azolla*	00	100	200	300	400

Table 2: Proximate composition of experimental diet

S. No.	Contents	T_0 (Control)	T_1	T_2	T_3	T_4
1	Moisture (%)	$9.30^{a}\pm0.02$	$8.60^{b}\pm0.02$	$8.34^{b}\pm0.04$	$9.24^{a}\pm0.04$	$9.41^{a}\pm0.01$
2	Crude protein (%)	$29.53^{b}\pm0.04$	$30.52^{a}\pm0.06$	$30.84^{a}\pm0.02$	$29.64^{b}\pm0.02$	$29.05^{c}\pm0.02$
3	Fat (%)	$7.97^{a}\pm0.03$	$7.45^{b}\pm0.04$	$7.82^{b}\pm0.01$	$7.65^{b}\pm0.02$	$7.74^{ac}\pm0.01$
4	Carbohydrate (%)	$41.67^{b}\pm0.02$	$41.66^{b}\pm0.01$	$41.24^{c}\pm0.09$	$40.96^{d}\pm0.11$	$41.94^{a}\pm0.02$
5	Ash (%)	$11.51^{b}\pm0.03$	$11.75^{b}\pm0.01$	$11.74^{b}\pm0.00$	$12.49^{a}\pm0.02$	$11.84^{b}\pm0.01$

Data expressed as Mean ± SE (n=3).
Mean values in the same row sharing different superscripts are significantly different at 5% level of probability *($p<0.05$)*

Experimental design: The experiment was conducted for a period of 60 days at the Aquaculture Research and Seed Unit DoR, MPUAT, Udaipur. Five fingerlings of *Labeo rohita* were randomly distributed in each tank (500 l) with triplicate. The stocked fingerlings were apparently healthy and free from any infection. The fishes were fed @ 4% body weight per day. The growth and growth parameters were monitored @ 15 day intervals. For the assessment of growth parameters following formula were used:

Growth Parameters

The fish growth parameter *viz*. weight gain, per cent weight gain, specific growth rate, food conversion ratio and gross conversion efficiency were analyzed following standard formula as described below:

Weight gain (g): Weight gain was determined by considering the final weight and initial weight of experimental fish.

Weight gain (g) = Final weight (g) - Initial weight (g)

$$\text{Per cent weight gain} = \frac{\text{Final weight(g)} - \text{Initial weight(g)}}{\text{Initial weight(g)}} \times 100$$

Specific growth rate (SGR)

$$\text{SGR (\%)} = \frac{(\ln Wt - \ln Wo)}{D} \times 100$$

Where: W_0 = Initial weight of live fish (g); Wt. = Final weight of live fish (g) and D = Duration of feeding (days)

Feed conversion ratio (FCR)

$$\text{FCR} = \frac{\text{Weight of food given (g)}}{\text{Weight gain of fish (g)}}$$

Gross conversion efficiency (GCE)

$$\text{GCE} = \frac{\text{Weight gained (g)}}{\text{Food given (g)}}$$

Water Quality Analysis: The selected water quality parameters such as water temperature, pH, dissolved oxygen, alkalinity and total hardness were tested by standard methods of APHA [9] on initial day and subsequently on the 15th day of the experimental period.

Statistical Analysis

The statistical analysis of the recorded data was carried out using standard statistical methods to draw meaningful conclusion. The analysis of variance (ANOVA) and standard error were performed using SPSS 16.0 to know the significance of *Azolla* in aquaculture.

Results and Discussion

Fish growth is a complex process governed by many parameters like fish species, nutrient present in the feed, feed additives and rearing environment individually or in combination. In the present study, the weight gain, percentage weight gain, specific growth rate, food conversion ratio and gross conversion efficiency were significantly different ($p<0.05$) in *Azolla* supplemented diet (Table 3). Still, the better growth performance was observed in treatment T_2 (200g/kg feed *Azolla* supplemented diet). In this treatment weight gain was 25.450±3.839g, per cent weight gain (45.863±1.677%) and specific growth rate (SGR) was also highest (1.024±0.051). The lowest FCR (3.903±0.163) was recorded in T_2 as compared to all other treatments and control. The gross conversion efficiency (GCE) in this diet was (0.257±0.010). In control diet the overall growth of the fish was the lowest. The statistical analysis of data has revealed significant variations in the result of weight gain, per cent weight gain, SGR, FCR and GCE.

Table 3: Growth parameters of *L. rohita* fed with *Azolla* supplemented diet

S. No.	Treatments	Parameters				
		Net weight gain (g)	Weight gain (%)	SGR (%)	FCR	GCE
1.	T_0 (Control)	$19.150^{a}\pm0.606$	$39.463^{ab}\pm0.638$	$0.836^{ab}\pm0.176$	$4.631^{ab}\pm0.135$	$0.216^{ab}\pm0.006$
2.	T_1	$19.150^{a}\pm0.202$	$39.706^{a}\pm0.288$	$0.788^{a}\pm0.007$	$4.982^{b}\pm0.049$	$0.200^{a}\pm0.002$
3.	T_2	$27.450^{b}\pm3.839$	$45.863^{b}\pm1.677$	$1.024^{b}\pm0.051$	$3.903^{a}\pm0.163$	$0.257^{b}\pm0.010$
4.	T_3	$24.600^{ab}\pm2.771$	$44.096^{ab}\pm3.624$	$0.976^{ab}\pm0.108$	$4.218^{ab}\pm0.432$	$0.242^{ab}\pm0.024$
5.	T_4	$18.550^{a}\pm1.991$	$40.453^{ab}\pm2.635$	$0.867^{ab}\pm0.073$	$4.664^{ab}\pm0.419$	$0.217^{ab}\pm0.019$

Data expressed as Mean ± SE (n=3).

Mean values in the same column sharing different superscripts are significantly different at 5% level of probability ($p<0.05$)

The results of the present investigation were also found in accordance to several researchers who found better growth performance with aquatic plant supplemented diet. The higher growth and SGR values of *Labeo rohita* fed on Duck weed supplemented were reported [10 11]. The dietary *Azolla* supplementation was found to have a positive effect on growth of fish in the present study.

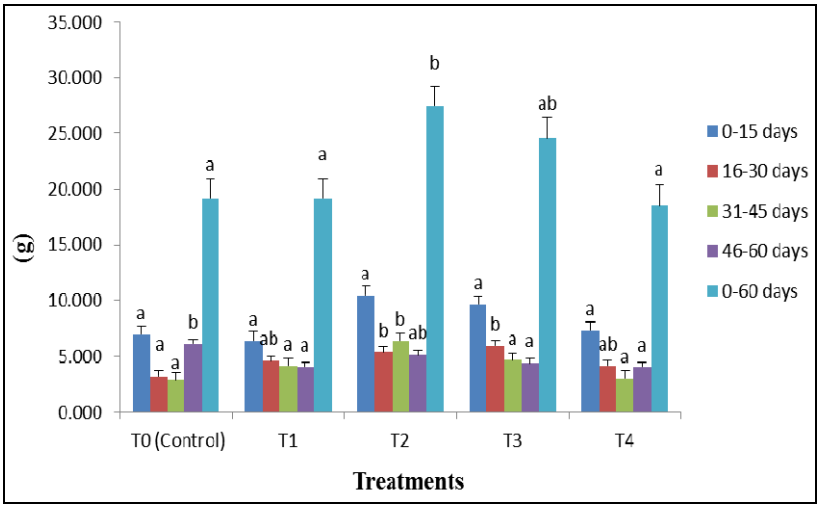

Fig 1: Weight gain of rohu (*L. rohita*) fed with Azolla supplemented diet

Proximate Composition of Fish Carcass

Kalla [12] used sorghum and soybean meals as supplementary feed for *Cirrhinus mrigala* (Ham.) and found significant changes in the proximate composition of fish carcass as compared to control. Nandeesha [13] reported that net protein retention, protein digestibility and carcass protein content in *Cyprinus carpio* (Linn.) and *Cirrhinus mrigala* (Ham.) increased with sodium chloride supplementary feed. In the present study, the proximate composition of fish carcass has been assessed after the completion of the experimental period of 60 days (Fig. 2). The results have shown significant changes in the proximate composition of fingerlings fed with *Azolla* diets. The high protein content was recorded in T_2 (24.846±0.014%) and lowest in initial fish sample (18.870±0.017%). However, the fat content of T_4 was highest (6.723±0.014%) and lowest in initial fish sample (6.260±0.011%). The high moisture content of whole fish was found in T_3 (66.780±0.011%) and lowest in initial fish sample (65.616±0.017%). The carbohydrate content of whole fish was highest in initial fish sample (6.696±0.058%) and lowest in T_3 (0.230±0.055%). The highest ash content was recorded in initial fish sample (3.056±0.012%) and lowest in treatment T_1 (2.480±0.011%).

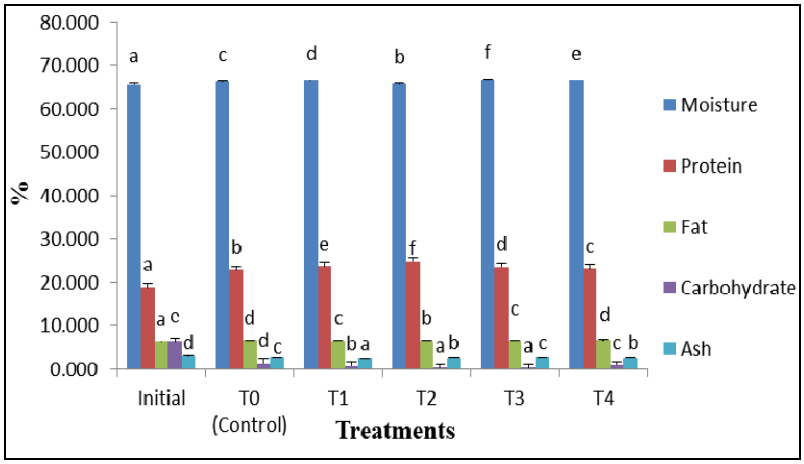

Fig 2: Proximate composition of fish carcass of *L. rohita* fed with *Azolla* supplemented diet

Conclusion

The result of the present study proves significant role of *Azoll*a as an aquatic weed growth promoter when mixed in the basal diet of groundnut oil cake, rice bran, soya bean meal, wheat flour and mineral mixture for fingerlings of *Labeo rohita*. The results of this study indicate a better growth rate, specific growth rate, food conversion efficiency and high gross conversion efficiency of fingerling fed with *Azolla* supplemented diets. In conclusion, the present study revealed that, *Azolla* at a maximum level of 200g/kg (dried pellet form) was suitable as a dietary protein supplement for *Labeo rohita* without any adverse effect on growth performance, survival rate and economical parameters.

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