

Asian Journal of Current Research

Volume 9, Issue 2, Page 173-178, 2024; Article no.AJOCR.12075 ISSN: 2456-804X

# The Potential Effects of Azolla as a Growth Promoter for *Labeo rohita* Fingerlings

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#### Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

#### Article Information

DOI: 10.56557/AJOCR/2024/v9i28678

**Open Peer Review History:** 

This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://prh.ikprress.org/review-history/12075

> Received: 29/02/2024 Accepted: 01/05/2024 Published: 04/05/2024

**Original Research Article** 

# ABSTRACT

Non-conventional aqua feeds are crucial for promoting sustainability, reducing costs, and minimizing environmental impact in aquaculture, fostering industry growth and addressing environmental concerns. The 60 days experiment was conducted for the introduction of Azolla into the diet of *Labeo rohita* fingerlings resulted in significant variations (p<0.05) in several growth parameters, including percentage weight gain, percentage length gain, specific growth rate (SGR),

Asian J. Curr. Res., vol. 9, no. 2, pp. 173-178, 2024

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feed conversion ratio (FCR), and survival rate. While no significant difference in FCR was observed among treatments during the initial 0 to 45-day interval, a notable disparity emerged between 45 to 60 days, with the highest FCR recorded in T3 and the lowest in T3. Similarly, although there were no significant differences in SGR among treatments at various intervals, the highest SGR was observed in T3, while the lowest was in T1. Furthermore, percentages of weight increment were significantly different between the intervals of 15-30 days and 30-45 days, with the highest percentage gain observed in T3 and the lowest in T1. Likewise, percentages of length increment showed significant differences between the intervals of 15-30 days, with T1 exhibiting the highest percentage gain and T1 the lowest. The survival rate % of different treatments at the end of the experiment varied, with T3 showing the highest survival rate and T0 the lowest. Statistical analysis confirmed significant differences in percent length gain, percent weight gain, SGR, and FCR among the treatments. These findings underscore the complex interplay between fish species, feed nutrients, additives, and rearing conditions on the growth process of Labeo rohita fingerlings. The observed variations in growth parameters highlight the potential of Azolla as a growth promoter in aquaculture practices. Further research is warranted to elucidate the underlying mechanisms driving these effects and to optimize Azolla supplementation protocols for enhanced growth performance and sustainability in Labeo rohita aquaculture.

Keywords: Non-conventional aqua feeds; Azolla; fish feed; aquaculture; Labeo rohita.

# 1. INTRODUCTION

Aquaculture is a promising field that can help to meet the food requirements of the growing global population. However, it is crucial to ensure that aquaculture practices are sustainable [1]. In 2020, the production of aquaculture worldwide reached an all-time high of 122.6 million tonnes, with a total value of \$1.5 billion [2, 3]. Aquatic animals made up 87.5 million tonnes of this production, while algae accounted for 35.1 million tonnes [2]. Aquatic plants are essential to the growth and body composition of fish, and they provide vital nutrients for them. In some cases, they can even replace up to 50% of commercial feeds [4,5]. It is recommended to use locally grown aquatic plants because the chemical composition of these plants is significantly impacted by the environment in which they grow [3]. Azolla is an aquatic plant with a high percentage of nutrients, including minerals, chlorophyll, carotenoids, amino acids and vitamins [6]. As a result, it is a useful ingredient for fish ponds and can be used directly or indirectly. Azolla helps macrophytophagous fish gain weight directly and can be fed to fish in Azolla fish pond cultures. Furthermore, Azolla increases the amount of fish feces produced, which is then used as an organic (nitrogenous) fertilizer by bottom dwellers [5]. This leads to an increase in the overall productivity of ponds. Additionally, the rapid decomposition rates of Azolla make it an excellent substrate for microbial processing, such as composting, or for enhancing the detrital food chain before it is applied to ponds [7,8]. Rohu is a preferred Indian

major carp species for cultivation. Various supplementary feeds have been experimented with to increase fish growth and production per unit area [9, 10]. Azolla has been explored as a potential feed supplement, and while it shows promise, it may not be sufficient for high productivity when used alone [6]. Therefore, it can serve as a beneficial supplement to natural feed in low-input aquaculture systems, potentially reducing reliance on fish meal and fish oil sourced from nature [3]. Azolla meal has been found to be significant in the diets of Tilapia species (Oreochromis niloticus, Tilapia mossambica, Tilapia zillii) and members of the Cyprinidae family (Labeo rohita, Catla catla, Labeo calbasu, Labeo fimbriatus. Ctenopharyngodon idella, Barbonymus gonionotus), as observed in the reviewed literature [11-13].

# 2. MATERIALS AND METHODS

# 2.1 Experimental Fish

The present study selected healthy rohu (*Labio rohita*) fingerlings from the fish hatchery of College of Fishery Science, NDVSU, Jabalpur (M.P.).

#### 2.2 Experimental Diets

Experimental diets was formulated comprising mustard oilcake, rice bran, soya-bean meal, wheat flour, carboxymethylcellulose (CMC) and mineral mixture. To create experimental diets, Azolla was replaced with other protein

S. No.	Ingredients	T₀	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>
1.	Azolla	0	200	300	400
2.	Soya-bean meal	350	300	250	200
3.	Mustard oilcake	190	170	190	220
4.	Wheat flour	360	230	170	90
5.	Carboxymethylcellulose	40	40	30	30

Table 1. The ingredients used for the preparation of different experimental diets (g/Kg)

sources into the basal diet at various proportions: 0.0 g/kg (T0 control), 200 g/kg (T1), 300 g/kg (T2), 400 g/kg (T3). Approximately 27% protein-containing diet was prepared for the experiment. The diet preparation is given in Table 1.

#### 2.3 Experimental Design

The experiment lasted for 60 days and was conducted at the Aquaculture Research Laboratory of the College of Fishery Science, NDVSU, Jabalpur. In each tank of 500 liters, five fingerlings of *Labeo rohita* were randomly distributed and triplicated. The stocked fingerlings appeared to be healthy and were fed 5% of their body weight per day. The growth and growth parameters were monitored at 15-day intervals.

#### 2.4 Growth Parameters

Several growth parameters were calculated to analyze the effect of various dietary sources. Initial & final length, initial & final weight, final mean body length, final mean body weight were measured. Specific growth rates (SGR), the percentage increase in length and weight gain, survival rate, feed conversion ratio (FCR) were calculated using following mathematical formulae.

Specific growth rate (SGR) = Log final body weight - log initial body weight  $\times$  100 / Duration of days Percentage increment in length = Mean final length of fish – Mean initial length of fish (cm) $\times$ 10 / Mean initial length of fish

Percentage increment in weight = Mean final weight of fish - Mean initial weight of fish  $(g)\times 100$  / Mean initial weight of fish

Survival rate = Total number of fishes harvested ×100 / Total number of stocked

Feed Conversion Ratio (FCR) = Feed given/ Weight gain

# 3. RESULTS AND DISCUSSION

In the current investigation, the introduction of Azolla in the diet led to significant variations (p<0.05) in parameters such as percentage weight gain, percentage length gain, specific growth rate, feed conversion ratio, and survival rate, highlighting the intricate influence of fish species, feed nutrients, additives, and rearing conditions on the growth process. There is no significant difference in FCR observed in all treatments from 0 to 45 days intervals but between 45-60 days a significant difference was observed between the treatments. The highest value of FCR was found in T<sub>3</sub> (3.26± 0.75) and the lowest was also found in T<sub>3</sub> (1.24±0.08) (Table 2). There is no significant difference was observed in SGR between all treatments at different day intervals. The highest value of SGR was found in  $T_3$  (1.39± 0.37) and the lowest value was found in  $T_1$  (0.57± 0.01) respectively (Table 3).

Table 2. The feed conversion ratio for various treatments was presented at different time
intervals in days

Treatment	0-15 Days	15-30 Days	30-45 Days	45-60 Days
To	2.32± 0.26	2.79± 0.45	2.78± 0.35	1.56 <sup>a</sup> ± 0.19
T <sub>1</sub>	1.88± 0.50	1.90± 0.03	3.23± 0.80	1.51 <sup>ab</sup> ± 0.14
T <sub>2</sub>	1.37±0.10	2.97± 0.81	1.72± 0.31	$1.70^{ab} \pm 0.39$
T <sub>3</sub>	1.24±0.08	1.42±0.12	3.26± 0.75	2.86 <sup>c</sup> ± 0.13

Treatment	0-15 Days	15-30 Days	30-45 Days	45-60 Days
T <sub>0</sub>	0.78± 0.15	0.66± 0.10	0.64± 0.08	0.66± 0.05
T <sub>1</sub>	1.06± 0.31	$0.94 \pm 0.24$	0.57± 0.01	0.57± 0.02
T <sub>2</sub>	1.23± 0.23	$0.74 \pm 0.30$	1.14± 0.43	0.74± 0.13
T <sub>3</sub>	1.39± 0.37	1.19± 0.19	0.67± 0.28	1.07± 0.58

Table 3. Specific growth rate for various treatments was presented at different time intervals indays

Table 4. Percentage increment in weight for various tre	eatments was presented at different time
intervals in day	/S

Treatment	0-15 Days	15-30 Days	30-45 Days	45-60 Days
To	19.99± 2.59	40.71 <sup>a</sup> ± 6.82	64.27 <sup>ª</sup> ± 10.04	77.07±7.51
T <sub>1</sub>	27.59± 7.04	57.77 <sup>ab</sup> ± 8.24	82.16 <sup>ab</sup> ± 10.42	86.37± 8.51
T <sub>2</sub>	33.05± 2.46	55.78 <sup>ab</sup> ± 2.45	99.14 <sup>abc</sup> ± 9.83	92.71±7.65
T <sub>3</sub>	36.49± 2.34	80.34 <sup>c</sup> ± 4.01	107.54 <sup>abc</sup> ± 1.20	76.08± 2.21

 Table 5. Percentage increment in length for various treatments was presented at different time intervals in days

Treatment	0-15 Days	15-30 Days	30-45 Days	45-60 Days
T <sub>0</sub>	6.79±1.58	3.89 <sup>a</sup> ± 1.03	8.83± 0.44	17.54± 1.67
T <sub>1</sub>	9.48± 1.79	$7.62^{ab} \pm 0.46$	7.15± 1.39	16.97± 1.27
T <sub>2</sub>	10.39± 1.43	$9.40^{b} \pm 0.60$	6.01± 0.48	13.77± 0.52
Тз	11.71± 1.10	11.95°± 1.85	7.23± 0.45	15.07± 0.39



Fig. 1. Survival rate (%) of different treatments at the end of the experiment

Percentages of weight increment were found to be significantly different between the intervals of 15-30 days and 30-45 days. Table 4 indicates that the highest percentage gain in weight was observed in T<sub>3</sub> (107.54± 1.20), whereas the lowest value was found in T<sub>1</sub> (19.99± 2.59). Similarly, percentages of length increment were found to be significantly different between the intervals of 15-30 days. Table 5 shows that the highest percentage gain in length was observed in T<sub>1</sub> (17.54± 1.67), whereas the lowest value was also found in T<sub>1</sub> (3.89± 1.03). Fig. 1 illustrates the survival rate % of different treatments at the end of the experiment. It is noteworthy that the highest survival rate was observed in T<sub>3</sub>, whereas the lowest was found in To. The statistical analysis reveals significant differences in percent length gain, percent weight gain, specific growth rate (SGR), and feed conversion ratio (FCR). The results of present investigation were also found in several research. In the study conducted by Kumari et al. (2017). observed that dietarv it was supplementation of Azolla had a beneficial impact on fish growth. Compared to the control the treatments showed significant group, enhancements in weight gain, percent weight gain, specific growth rate (SGR), food conversion ratio (FCR), and gross conversion efficiency (GCE). The researchers also suggested that substituting dietary components with up to 20% Azolla could be a viable feeding strategy for monosex Nile tilapia. This approach has the potential to enhance fish growth, quality, profitability, and sustainability within the tilapia production industry (Refaey *et al.*, 2023).

# 4. CONCLUSION

The findings of the current study indicates the significant role of azolla as a growth promoter for aquatic weed when incorporated into the basal diet consisting of groundnut oil cake, rice bran, sova bean meal, wheat flour, and mineral mixture for Labeo rohita fingerlings. The results indicate improved percentage weight gain, percentage length gain, specific growth rate, feed conversion ratio, and survival rate in fingerlings fed with Azolla-supplemented diets. In summary, the study concludes that Azolla, at a maximum level of 300g/kg and 400g/kg (in dried pellet form), serves as a suitable dietary protein supplement for Labeo rohita, without any detrimental effects on growth performance, survival rate, and economic parameters. By enhancing growth rates, improving feed conversion efficiency, and maintaining economic feasibility, Azolla supplementation offers promising prospects for enhancing the productivity and profitability of Labeo rohita aquaculture. Further research and practical trials are recommended to validate these findings and explore the broader implications of integrating azolla into aquafeed formulations for commercial fish production.

# ACKNOWLEDGEMENTS

On behalf of all authors, we extend our gratitude to the College of Fishery Science, NDVSU, Jabalpur for providing the necessary facilities and support for conducting this research. We would like to express our sincere appreciation to Dr.Vipin Vyas & Dr. S.K. Mahajan,Dr. Sona Dubey, for their invaluable guidance, mentorship, and encouragement throughout the duration of this study. Their expertise and support have been crucial in shaping the direction of our research and contributing to its successful completion.

# **COMPETING INTERESTS**

Authors have declared that no competing interests exist.

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