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RESEARCH ARTICLE

Effect of dietary supplementation of Azolla on growth and survibility of *Labeo rohita* fingerlings

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ABSTRACT..... A feeding trial was conducted to study the effect of Azolla, on growth and survival of *L. rohita* fingerlings. One hundred twenty (4.08 ± 0.02 g) fingerlings were randomly distributed in four treatment groups, each with three replications. Four isonitrogenous (crude protein: 27.75-28.26%) experimental diets were prepared with different percentage of Azolla incorporation except the control group, *viz.*, control (T_0), T_1 (20% Azolla), T_2 (40% Azolla) and T_3 (50% Azolla). The physio-chemical parameters were found in the normal range through out the experimental period. The results revealed that the test animals fed with diet containing 40 per cent Azolla showed better growth (15.45 ± 0.24 g in wt. and 13.73 ± 0.47 cm in length) than the test animals fed with 20 per cent, 50 per cent and control diet. The body weight gain per cent was recorded highest in diet containing 40 per cent Azolla (281.57 ± 7.21) than control (175.62 ± 2.31). The specific growth rate (SGR) was found highest in T_2 group (0.73 ± 0.01) followed by T_3 group (0.67± 0.01) which was ($P < 0.05$) higher than T_1 and control group. The FCR also varied significantly ($P<0.05$) among the treatment group. The better FCR was observed in T_2 group followed by T_3 group. This results suggest that incorporation of Azolla at 40 per cent can enhances growth in *L. rohita* fingerlings and can effectively be used in conventional diet of rohu without any adverse effect on growth and survival.

KEY WORDS..... Azolla sp., Fish, *Labeo rohita*

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INTRODUCTION.....

It is a well- known fact that fish is an important source of protein and also rich in essential amino-acids. It is also a good source of calcium, vitamin A and B_{12} apart from possessing a high content of polyunsaturated fatty acids *i.e.*, omega-3 fatty acids. India blessed with diversified agro-climatic conditions, is endowed with potentially rich and varied aquatic resources. In the Inland sector, there is a wide scope for increasing fish production through aquaculture by utilizing the available resource areas. It has been estimated that at present only 40 per cent of the available cultivable water area has been brought under aquaculture practices. The country also has an important role in global fisheries as the second largest producer of fish in the world and higher enhancement levels as compared to world fish production levels. In India, aquaculture contributed 45 per cent of the country's total fish production of 8.0 million tones. Aquaculture in our country is mainly dominated by Indian major carps accounting around 80 per cent of the total inland production. The increase in cost and demand of protein from conventional resource necessitates fish culturists of the developing countries to incorporate cheap and locally available ingredients in fish feeds. Recently, the utilization of aquatic plants have high food value are used to supplement fish food. A number of formulated diets suiting to the herbivorous feeding habit of aquatic species are commercially available. But, majority of the fish farmers depend on the

conventional feed mixture containing groundnut oil cake and rice bran for feeding fish in culture ponds. Replacement of fish meal by alternative protein sources has long been of interest and will increasingly be important for the development of low-cost highly efficient aqua feeds (Gatlin *et al*., 2007). Fish meal replacement by plant protein has been considered an attractive way to achieve low cost aqua feed formulations (Gatlin *et al*., 2007; Gaylord and Barrows, 2009 ; Lim and Lee, 2009). Azolla sp. is one of the aquatic plants with high biomass and protein production. Inadequate availability of good quality feed is regarded as a major constraint to the prevalent small dominant production system (Anbarasu *et al*., 2004). The conventional feed ingredients are expensive and are not always readily available at affordable prices. Azolla contains 13-30 per cent crude protein, 4.4 per cent - 6.3 per cent crude fat, 5.6 – 15.2 per cent cellulose, 9.8 per cent- 17.9 per cent hemicellulose, 9.3 – 34.8 per cent lignin and 9.7 – 23.8 per cent ash on dry weight basis (Ayyappan, 2000). Azolla sp. has gained its importance in agriculture because it has higher crude protein content ranging from 13 to 30 per cent than most green forage crops and aquatic macrophytes and a rather favourable essential amino acid (EAA) composition for animal nutrition (rich in lysine). It has also attracted the attention of live stock, poultry and fish farmers (Caguan and Pullin,1991). In spite of its attractive nutritional qualities and relative ease of production in ponds and rice fields, reports on the use of Azolla in aquaculture are extremely limited. Therefore, the value of Azolla as a fish feed is still being studied. Some authors like Mandal *et al*. (2010) and Datta (2011) has studied on growth and survivability of tilapia and rohu fingerlings by providing Azolla sp. partially or fully as a component in the fish feed. Central Institute of Freshwater Aquaculture, Kaushalyaganga, Bhubaneswar has also reported (Annual report, 2005-2006) that Azolla has a positive effect on growth of rohu fingerlings. Considering limited information available on the use of Azolla as a feed ingredient for the Indian major carps, the present study was designed to study the effect of dietary supplementation of Azolla on growth and survivility of *L. rohita* fingerlings.

RESEARCH METHODS.....

The present study was undertaken in the laboratory of College of Fisheries, Rangailunda for a period of six months from November 2011 to April 2012. About two hundred fingerlings of *Labeo rohita* of average total length of 7 cm and average body weight of 4 g used for present study, were collected from private fish farm.

Fingerlings were acclimatized in the laboratory condition for two weeks prior to the start of the experiment. Uniform size of *L. rohita* fingerlings were equally distributed in four experimental groups ($T_{0,}$ control ; T_1, 20% Azolla; T_2, 40% Azolla and T_3, 50% Azolla) in triplicate following a Completely Randomized Division (CRD). Each aquarium (24" × 12" × 12") was stocked with 10 fingerlings and about 16 cm water level was maintained. Four different experimental diets were prepared by incorporating different proportions of Azolla in three different concentrations (*i.e.* 20%, 40% and 50%) to the basal diet (GNOC and RB) and a control diet without Azolla. (Table 1). Rice bran and groundnut oilcake were grounded and sieved properly and used in the preparation of diet. Different species of Azolla like *A. pinnata* and *A. caroliniana* were collected from CIFA, Kaushalyaganga for pure culture. The pure strain was inoculated in four equal tubs containing about two litre of water each. Before inoculating Azolla sp., the water was fertilized with single super phosphate @ 2mg/l for the optimum growth of leaves of Azolla sp. and the cultured tubs were put in shady area for rapid multiplication of Azolla sp. The water exchange was done in every week. The cultured Azolla from these tubs were collected, dried, powdered and then used for preparation of experimental diets. The composition of experimental diets is given in Table 1. At 15 days interval, each group of fishes was collectively weighed by using electronic balance having accuracy up to 0.01g to study the following growth parameters :

$$\text{Percentage weight gain} = \frac{\text{Final weight of fish} - \text{Initial weight of fish}}{\text{Initial weight of fish}} \times 100$$

Specific growth rate (%) :

Daily growth rate was calculated by using the following formula :

$$SGR \equiv \frac{\text{Log}_e(BW)_F - \text{log}_e(BW)_I}{T} \times 100$$

where,

BW_I = Initial body weight

BW_F = Final body weight

T= Period of experiment expressed in days.

$$\text{Food conversion ratio} = \frac{\text{Total dry food intake(g)}}{\text{Total live weight gain(g)}}$$

The experimental animals were fed at a rate of 5 per cent of their body weight in each treatment. About 70 per cent of the water was exchanged from the experimental tanks at every two days interval to remove the accumulated faecal matters and left over feed. The various physicochemical parameters such as water depth, temperature, pH, total alkalinity, total hardness, dissolved oxygen, free carbon dioxide were estimated at 15 days interval by following different standard methods (APHA, 1993). The proximate composition of experimental diets like moisture estimation, crude protein content, ash content and crude fat content were done by following standard methods (AOAC, 2006). The data gathered during experimental period were subjected to statistical analysis and analysis of variance.

Table 1 : Percentage composition of different ingredients in experimental diets

Experimental diets	Ingredients		
	Azolla sp. powder	Rice bran	Groundnut oil cake
T_0 feed	Nil	50	50
T_1 feed	20	40	40
T_2 feed	40	30	30
T_3 feed	50	25	25

Table 2 : Proximate composition of different ingredients and different experimental diets on dry weight basis

Proximate composition	Experimental diets				Ingredients		
	T_0 feed	T_1 feed	T_2 feed	T_3 feed	GNOC	Ricebran	*Azolla* powder
Crude protein (%)	28.26	28.06	27.85	27.75	42.63	13.91	27.27
Crude fat (%)	10.18	9.04	8.7	7.34	7.56	12.80	4.50
Moisture (%)	7.75	7.55	7.35	7.26	7.65	7.86	6.79
Ash (%)	10.45	12.03	13.62	14.41	9.40	11.50	18.38

Table 3 : Average body weight (in g) of *Labeo rohita* fingerlings fed with different experimental diets

Days / Treatments	0 day	15 days	30 days	45 days	60 days	75 days	90 days	105 days	120 days	135 days	150 days	165 days	180 days
T_0 feed	4.07 ± 0.02	4.55 ± 0.03	4.7 ± 0.02	5.03 ± 0.02	5.53 ± 0.03	6.32 ± 0.02	7.63 ± 0.02	8.04 ± 0.03	8.59 ± 0.03	9.33 ± 0.15	10.32 ± 0.02	10.92 ± 0.02	11.22 ± 0.22
T_1 feed	4.11 ± 0.02	4.24 ± 0.11	4.67 ± 0.15	4.95 ± 0.03	5.56 ± 0.4	6.26 ± 0.11	6.86 ± 0.11	7.41 ± 0.09	8.31 ± 0.23	9.2 ± 0.15	10.23 ± 0.13	11.1 ± 0.1	12.22 ± 0.22
T_2 feed	4.05 ± 0.02	4.18 ± 0.09	4.47 ± 0.15	5.6 ± 0.09	6.32 ± 0.10	7.17 ± 0.15	7.65 ± 0.11	8.35 ± 0.12	9.81 ± 0.10	10.28 ± 0.22	12.1 ± 0.16	13.96 ± 0.24	15.45 ± 0.24
T_3 feed	4.03 ± 0.03	4.22 ± 0.02	4.61 ± 0.08	5.34 ± 0.72	6.38 ± 0.08	7.14 ± 0.10	7.55 ± 0.03	8.51 ± 0.036	9.31 ± 0.02	10.74 ± 0.04	11.43 ± 0.10	12.78 ± 0.14	13.71 ± 0.08

Table 4 : Average body length (in cm) of *Labeo rohita* fingerlings fed with different experimental diets

Days / Treatments	0 day	15 days	30 days	45 days	60 days	75 days	90 days	105 days	120 days	135 days	150 days	165 days	180 days
T_0 feed	7.03 ± 0.015	7.29 ± 0.03	7.59 ± 0.04	8.18 ± 0.04	8.42 ± 0.13	8.51 ± 0.10	8.87 ± 0.05	9.46 ± 0.66	9.61 ± 0.10	10.14 ± 0.11	10.30 ± 0.08	10.38 ± 0.06	10.65 ± 0.10
T_1 feed	7.04 ± 0.005	7.54 ± 0.10	7.88 ± 0.05	8.27 ± 0.05	8.9 ± 0.08	9.31 ± 0.09	9.45 ± 0.12	10.22 ± 0.09	10.41 ± 0.06	10.88 ± 0.05	11.3 ± 0.07	11.49 ± 0.07	12.14 ± 0.13
T_2 feed	7.03 ± 0.02	7.76 ± 0.12	8.22 ± 0.05	8.7 ± 0.22	9.76 ± 0.38	10.10 ± 0.26	10.52 ± 0.32	11.16 ± 0.10	11.48 ± 0.13	12.02 ± 0.7	12.58 ± 0.43	12.94 ± 0.39	13.73 ± 0.47
T_3 feed	7.06 ± 0.03	7.76 ± 0.12	8.28 ± 0.11	8.43 ± 0.04	8.52 ± 0.03	8.88 ± 0.05	9.42 ± 0.04	10.43 ± 0.02	10.91 ± 0.08	11.6 ± 0.19	11.82 ± 0.18	12.02 ± 0.26	12.37 ± 0.16

Table 5 : The growth parameters and survivility of *L. rohita* fingerlings fed with different experimental diets

Growth parameters	Treatments			
	T_0	T_1	T_2	T_3
Percentage weight gain	175.62 ± 2.31	197.17 ± 6.19	281.57 ± 7.21	239.33 ± 5.24
SGR	0.55 ± 0.01	0.60 ± 0.01	0.73 ± 0.01	0.67 ± 0.01
FCR	4.21 ± 0.09	3.79 ± 0.61	2.93 ± 0.18	3.49 ± 0.11
Survival (%)	76	80	100	100

RESEARCH FINDINGS AND ANALYSIS.....

The water quality parameters like temperature, pH, total alkalinity, total hardness, dissolved oxygen and free carbon dioxide were found in the range of $26^0C – 28.5^0C$, 6.8 – 8.0, 120-157 mg/lt, 125-158 mg/lt , 5.8 – 8.0 mg/lt and negligible – (1.2 mg/lt), respectively. According to Renukaradhya and Verghese (1986), about 30 per cent protein in the diet is enough to meet the dietary protein requirements of both catla and rohu. The formulated feed employed in the present study had 27.75- 28.26 per cent of crude protein, 7.34-10.18 per cent of crude fat, 7.26- 7.75 per cent of moisture and 10.45- 14.41 per cent of ash (Table 2). Chemical analysis of Azolla sp. showed moderate protein content. This level of protein is similar to that reported by (Ayyappan, 2000; Das *et al.*, 2004). Thus, the feed used for rearing the species in the present experiment can be taken as nutritionally balanced.

In the present study the fingerlings fed with dried Azolla powder at 20 per cent, 40 per cent and 50 per cent substitution showed better growth than that of control diet. The highest growth *i.e.* 15.45 ± 0.24 g; 13.73 ± 0.47cm was found in T_2 group fed with 40 per cent Azolla followed by T_3 (13.71 ± 0.08g; 12.37± 0.16 cm) fed with 50 per cent Azolla (Table 3 and 4). The percentage weight gain was found highest (281.57±7.21) in T_2 group followed by T_3 group (239.33±5.24) fed with 40 per cent and 50 per cent Azolla, respectively (Table 5). Mohanty and Dash (1995) reported 168.2 per cent weight gain and good utilization in rohu fry fed with *A. caroliniana* at 60 per cent inclusion level, comparing diets with 30, 40, 50 per cent Azolla incorporation. However, the results of the present study coincide with the findings of Das *et al.* (2004), where they reported significantly increased growth upto 40 per cent level of Azolla inclusion and then significantly decreased growth when the level of Azolla increased to 56.8 and 63.6 per cent in the diets. Inclusion of Azolla above 40 per cent in the present study gave lower growth suggesting that the level of inclusion of Azolla should be carefully determined. A reduction in the fish growth at higher levels of Azolla supplementation could be due to its poor digestibility and higher lignin content (Ayyappan, 2000). A similar trend was also found in SGR values (Table 5). The highest value was recorded in T_2 group (0.73 ± 0.01) followed by T_3 group (0.67 ± 0.01) which was significantly ($P < 0.05$) higher than the control and T_1 group. The feed utilization was found better in T_2 and T_3 groups compared to other treatments. This may be due to the palatable nature of the food with high feeding stimulants in Azolla incorporated diets (Paulraj and Kutty, 1984). Further increase in incorporation of Azolla above 50 per cent may follow the same trend as reported by Ayyappan (2000) and there may be decrease in SGR value. The better FCR value was recorded in T_2 group (2.93 ± 0.18) followed by T_3 group (3.49 ± 0.11) (Table 5). This indicates better utilization of feed by rohu fingerlings up to 40 per cent inclusion of Azolla. Fish fed with 50 per cent Azolla showed poor growth, SGR and FCR compared to group fed with 40 per cent Azolla may be due to the utilization of protein for energy purpose rather than growth as the total lipid content decreased with increase in Azolla level (Table 2). The reduction in lipid content was a result of increase of dry Azolla in the diet. The results are in agreement with Micha *et al*. (1988); El-Sayed (1992) and Ahmad (2003) for different fish species. The FCR (3.49) in T_3 group fed with 50 per cent Azolla suggests a low assimilated value of Azolla above 40 per cent level of incorporation. The survivility of *L. rohita* fingerlings treated with different experimental diets are given in (Table 5). The survival was recorded 100 per cent in T_2 and T_3 groups which may be due to palatability and acceptability of food as compared to T_0 and T_1 groups where the survival percentage was recorded at 76 per cent and 80 per cent, respectively. The low survival percentage might be due to increase in NH_3 concentration as left over feeds deteriorate the water quality. It is thus concluded that the use of Azolla as a feed ingredient may reduce the cost of feed by replacing the protein rich feed ingredients like GNOC and fish meal. It can effectively be used as a substitute to GNOC and rice bran in the conventional diet of rohu of about 40 per cent without any adverse effect on growth and survival.

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