Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2017; 5(5): 715-718

Received: 01-07-2017
Accepted: 02-08-2017

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 proximate composition and digestibility of *Labeo rohita* (Ham.) fingerlings

Ramesh Kumari, ML Ojha, VP Saini and SK Sharma

Abstract
The present study was conducted to evaluate the effect of *Azolla* supplementation on Labeo *rohita* fingerlings proximate composition and digestibility. For this purpose basal diet (T_0, control) was prepared by using Groundnut oil cake (320g/kg), Rice bran (320g/kg), Soya bean meal (300g/kg), Wheat flour (50g/kg) and Mineral mixture (10g/kg). For the preparation of experimental diet, *Azolla* was mixed in basal diet at varying levels, 100g/kg (T_1), 200g/kg (T_2), 300g/kg (T_3) and 400g/kg (T_4). The control and experimental diet was fed to experimental fish @ 4% body weight per day for a period of 60 days. The protein efficiency ratio (PER), apparent net protein utilization (ANPU) and apparent digestibility (AD) were significantly improved in treatments as compared to control. Still the highest protein efficiency ratio (1.998±0.242), apparent net protein utilization (25.975±0.918%) and apparent digestibility (77.990±0.544%) were recorded in T_2 (200g *Azolla* per kg basal diet). Therefore, it can be concluded that @200g/kg *Azolla* supplemented diet had significant role in improving digestibility of *Labeo rohita*. Thus the dose of 200 g/kg *Azolla* is recommended for supplementation in the diet of *L. rohita*.

Keywords: *Azolla*, Digestibility, Feed, *Labeo rohita*, Proximate Composition

Introduction

Aquaculture is developing into a prime industry to top the enormous turnover of bio-energy for the benefit of mankind. This is growing into a giant multi-billion dollar industry in many parts of the world. The global demand for fish is expected to increase to >180 MMT as against the present production of about 160 MMT by the year 2025 AD [1]. The increased fishing pressure has led to over-exploitation and stagnation of fish catch. Therefore, aquaculture is identified as the only viable alternative for any further increase in fish production to meet the ever increasing demand for fish. The demand may be fulfilled by increasing the quality of aquaculture feed. This can be done through improving nutrient contents as well as the digestibility of the low quality feeds by the use of efficient additives. The addition of specific nutrients in feed improves digestion and thereby reduces the feed cost. Indian freshwater aquaculture constitutes mainly culture of IMC *viz.*, *Catla catla*, *Labeo rohita* and *Cirrhinus mrigala.* Plant based diet like 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 kind of supplementary feeds are being tried to accelerate growth and production of fish per unit area. [4] *Azolla* is becoming an integral part of the aquaculture practices to obtain high production.

Aquatic plants are considered to be an important source of nutrients for fish and in many cases could replace up to 50% of commercial feeds without adverse effects on fish growth and body composition. Chemical content of aquatic plants is highly affected by the aquatic environment in which they grow; therefore their use is recommendable in each case on a local level. In India there are only few studies on the evaluation of the nutritional characteristics of locally available aquatic plants. *Azolla* is an ideal feed substitute which is an aquatic fern (pteridophyte) floating on 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 proximate composition and digestibility of rohu (*Labeo rohita*).

Materials and Methods

Experimental fish: For the present study, an Indian Major Carp, *Labeo rohita* was selected as the experimental fish. The healthy fingerlings of this fish were procured from Aquaculture Research and Seed Unit, Directorate of Research, MPUAT, Udaipur.

Experimental diet: A basal diet was prepared using groundnut oil cake (320 g/kg), RB (320 g/kg), soya beam 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 {i.e. 0.0 (T_0 control), 100 (T_1), 200 (T_2), 300 (T_3) and 400(T_4) g/kg basal diet} (Table 1). The experimental diets were analyzed for the proximate composition *viz*., moisture, crude protein, fat and carbohydrate and ash contents as per standard methods of AOAC [8]. As such the results obtained are presented in Table 2.

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 (%)	$9.30^c \pm 0.02$	$8.60^b \pm 0.02$	$8.34^a \pm 0.04$	$9.24^c \pm 0.04$	$9.41^d \pm 0.01$
2	Crude protein (%)	$29.53^b \pm 0.04$	$30.52^c \pm 0.06$	$30.84^d \pm 0.02$	$29.64^b \pm 0.02$	$29.05^a \pm 0.02$
3	Fat (%)	$7.97^d \pm 0.03$	$7.45^a \pm 0.04$	$7.82^c \pm 0.01$	$7.65^b \pm 0.02$	$7.74^c \pm 0.01$
4	Carbohydrate (%)	$41.67^c \pm 0.02$	$41.66^c \pm 0.01$	$41.24^b \pm 0.09$	$40.96^a \pm 0.11$	$41.94^d \pm 0.02$
5	Ash (%)	$11.51^a \pm 0.03$	$11.75^b \pm 0.01$	$11.74^b \pm 0.00$	$12.49^d \pm 0.02$	$11.84^c \pm 0.01$

Data expressed as Mean ± SE (n=3). Mean values in the same column sharing different superscripts are significantly different ($p<0.05$)

Experiment design: This experiment was conducted in 15 FRP tanks of 1000 l. each following complete randomized design (CRD). Total five (5) treatments in triplicate were used. This experiment was conducted for a period of 60 days at Aquaculture Research and Seed Unit DoR, MPUAT, Udaipur during 2017. Ten fingerlings of *L. rohita* were randomly distributed in each tank. The stocked fingerlings were apparently healthy and free from any infection. The fishes were fed @ 4% body weight per day. The digestibility was assessed at 15 days intervals, while the proximate composition was analyzed after termination of experiment.

Digestibility analysis: The fish digestibility parameters *viz*. protein efficiency ratio, apparent net protein utilization and apparent digestibility were analyzed following standard formula as described below:

Protein efficiency ratio (PER)

$$\text{PER} = \frac{\text{Total wet weight gain(g)}}{\text{Crude protein fed (\%)}}$$

Apparent net protein utilization (ANPU)

$$\text{ANUP (\%)} = \frac{100\,(\text{Final tissue protein} - \text{Initial tissue protein})}{\text{Crude Protein fed (\%)}}$$

Digestibility analysis

The unconsumed food and accumulated feacal matter from the bottom of tanks was collected by siphoning and the water level was maintained. 1 per cent Cr_2O_3 was added as an indigestible external marker for digestibility estimations. The samples of feed and feaces were homogenized using a motor and pestle and analyzed following standard methods of AOAC [8]. A 50 mg sample was transferred to a dry 100 ml Kjeldahl flask. 5 ml concentrated nitric acid was added to each. After wait for 5 minutes it was heated with occasional shaking to prevent the particles adhering to the sides until a white precipitate appears (approximately 20 minutes).The flask was removed from the heater and cooled. A 3 ml of perchloric acid was added to each flask and then reheat until the greenish white colour changed to a red orange colour (usually 30-40 minutes). The reaction was completed, the sample was heated for another 10 minutes and cooled and 50 ml of distilled water was added to terminate the reaction. The flask was cooled to room temperature and contents were transferred to a clean 100 ml volumetric flask and the total volume was made 100ml by adding distilled water. After waiting for 10 minutes to allow the formation of precipitates of organic matter present, if any. A suitable aliquot from each flask was taken into a plastic cuvette and optical density was measured at 350 nm on a spectrophotometer.

Apparent digestibility coefficients for crude protein was calculated using following formula:

$$\text{Apparent digestibity (\%)} = 100 - \frac{100\,(\text{\% } Cr_2O_3 \text{ in feed} \times \text{\% nutrient in feaces})}{(\text{\% } Cr_2O_3 \text{ in feaces} \times \text{\% nutrient in feed})}$$

Proximate Composition: The proximate composition of experimental fish was analyzed following standard methods of AOAC [8].

Water Quality Analysis: The selected water quality parameters (i.e. water temperature, pH, dissolved oxygen, alkalinity and total hardness) of experimental water was tested following standard methods of APHA [9].

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.

Results and Discussion

The mean water quality parameters recorded in the different treatment tanks did not vary significantly ($p > 0.05$) with the inclusion of Azolla in diets at increasing levels (Table 3). The 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 [10]. Jhingran [11] 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 25.00 to 25.07 °C and DO from 4.68 to 4.74 mg /l. Generally, cyprinids are capable of tolerating low oxygen levels of 3 mg /l [12]. Total alkalinity was in a higher range (440.01–448.00 mg /l). Waters of higher alkalinity are considered more productive in terms of oxygen production and photosynthesis [13]. Thus, the water quality parameters remained more or less same in different treatments (Table 3). In general all the water quality parameters were congenial for the rearing of experimental fish.

Table 3: Range and average values (in parenthesis) of selected water quality parameters

Parameters	T_0 (Control)	T_1	T_2	T_3	T_4
Temperature (ºC)	22.93-27.20 (25.00)	22.82-27.24 (25.07)	22.61-27.23 (25.04)	22.50-27.28 (25.02)	22.50-27.38 (25.06)
pH	7.80-8.43 (8.17)	7.81-8.45 (8.18)	7.81-8.44 (8.18)	7.79-8.46 (8.17)	7.81-8.48 (8.19)
Dissolved oxygen (mg/l)	4.14-5.46 (4.68)	4.25-5.40 (4.71)	4.27-5.42 (4.74)	4.27-5.38 (4.72)	4.27-5.41 (4.73)
Alkalinity (mg/l)	399.33-490.67 (448.00)	405.90-480.52 (444.80)	403.02-479.47 (442.12)	402.95-476.51 (441.02)	401.85-477.80 (440.01)
Total Hardness (mg/l)	390.00-505.00 (432.57)	394.86-517.43 (441.51)	398.08-525.49 (445.99)	400.33-532.59 (451.54)	404.75-539.03 (456.48)

In the present study, protein efficiency ratio, apparent net protein utilization and apparent digestibility were significantly different ($P<0.05$) in *Azolla* supplemented diets (Table 4). Still, the better digestibility was observed in treatment T_2 (200g/kg feed *Azolla* supplemented diet). The treatment protein efficiency was (1.998±0.242), apparent net protein utilization (25.975±0.918) and apparent digestibility (77.990±0.544). In control diet the overall digestibility of fish was the lowest (Fig.1). The statistical analysis of data has revealed significant variations in the result of PER, ANPU and AD. The presence of the stomach is an important feature from a perspective of increased ability of fish to digest complex proteins and therefore to adapt to variable diets [14], since the gastric glands present in the stomach secret pepsin and hydrochloric acid with the purpose to contribute to the enzymatic degradation of foods in the stomach[15, 16]. Thus, fish species of the genus *Labeo* are desired for using unconventional plant ingredients as nutrient sources.

Fish digestibility 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 [17]. Increased level of starch in the diet decreases its digestibility in carnivorous fish such as turbot [18] and yellowtail [19]. However, Mohapatra [20] reported a significant increase in the carbohydrate digestibility with increasing level of gelatinized carbohydrate (GC) in the diet of *Labeo rohita* fry. Fish fed higher levels (45% and 50%) of GC have higher carbohydrate digestibility (83%), indicating better utilization of carbohydrate by herbivores/omnivores such as carps as compared to control. Similarly, improved digestibility of crude protein and dry matter was also recorded in beluga, *H. huso* by feeding citric acid acidified diet [21]. Improved protein digestibility was also observed in rainbow trout fed formic acid containing diet [22] phytase-supplemented diet to *L. rohita*.

Table 4: Summary data on nutrient retention of *L. rohita* fed with *Azolla* supplemented diet

S. No.	Treatments	Parameters		
		PER	ANPU	AD
1.	T_0 (Control)	1.602^{ab}±0.045	21.096^{a}±0.420	70.284^{a}±0.151
2.	T_1	1.675^{ab}±0.047	23.239^{ab}±0.407	72.852^{b}±0.485
3.	T_2	1.998^{b}±0.242	25.975^{b}±0.918	77.990^{d}±0.544
4.	T_3	1.885^{ab}±0.059	22.679^{a}±1.742	74.668^{c}±0.099
5.	T_4	1.569^{a}±0.067	21.705^{a}±0.576	72.307^{b}±0.122

Data expressed as Mean ± SE (n=3). Mean values in the same column sharing different superscripts are significantly different ($p<0.05$)

Kalla [23] 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 [24] 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* supplemented diets. The highest protein content was recorded in T_2 (24.846±0.014%) and lowest in initial fish sample (18.870±0.017%). However, fat content of T_4 was highest (6.723±0.014%) and lowest in initial fish sample (6.260±0.011%). The highest 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%).

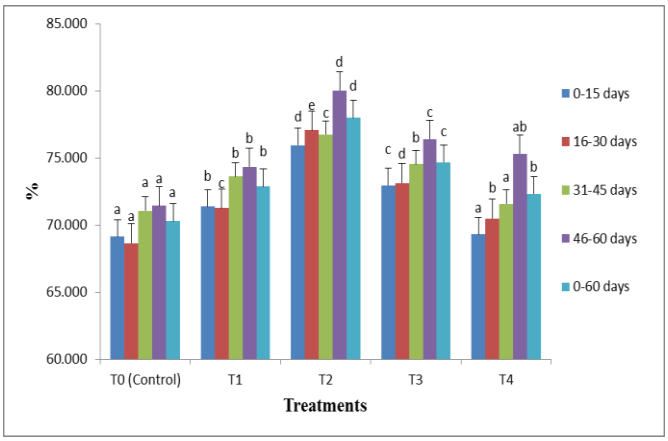

Fig 1: Apparent digestibility (AD) rohu (*l. rohita*) fed with *Azolla* supplemented diet

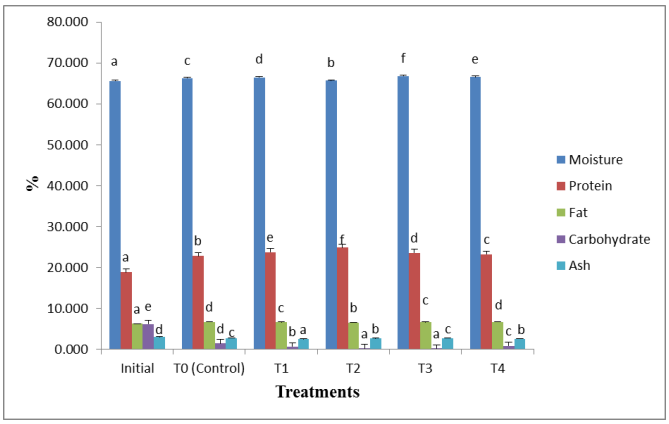

Fig 2: Proximate composition of fish (*L. rohita*) carcass of fed with *Azolla* supplemented diet

Conclusion

The result of the present study prove significant role of *Azolla* as fish diet supplement for better digestibility and proximate compositions. The results of this study indicate a protein efficiency ratio, apparent net protein utilization and apparent digestibility 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) is suitable as a dietary protein supplement for *Labeo rohita* without any adverse effect on digestibility.

References

1. FAO. Food and Agriculture Organization of the United Nation; Eurospan distributor, Rome, Landon. 2014, 39-44.
2. Jhingran VG. Fish and fisheries of India. Hindustan Publication Corporation, India. New Delhi. 1991; 38:129-132.
3. Ayyappan S, Jena JK. Grow-out production of carps in India. Journal of Applied Aquaculture. 2003; 13:251-282.
4. Singh R, Dhawan A. Effect of formulated diets on the growth and ovarian maturation in common carp (*Cyprinus carpio communis* Linn.). Indian Journal of Fisheries. 1996; 43(4):349-353.
5. Biotech A. Animal Nutrition- Products. Cited from Web site. Amit Biotech. 2004, 1-2.
6. Lumpkin TA. Assessing the potential for *Azolla* use in the humid tropics. International Rice Commission News. 1984; 33(6):30-33.
7. Pannaerker S. *Azolla* as a livestock and poultry feed. Livestock Adviser. 1988; 13(6):22-26.
8. AOAC. Official method of analysis. Association of Analytical Chemist Washington D.C., U.S.A, 1980.
9. APHA. (American Public Health Association). Standard methods for examination of water and waste water (12th Ed.). American Water Works Association and Water Pollution Control Federation. Washington, D.C. 1989, 1452.
10. Woynarovich E. Elementary guide to fish culture in Nepal. Rome: Food and Agriculture Organisation of United Nations, 1975.
11. Jhingran VG. Fish and fisheries of India. Delhi: Hindustan Publishing. 1991, 954.
12. Huet M. Textbook of fish culture-Breeding and cultivation of fish. London: Fishing News (Books). 1972, 436.
13. Elnady MA, Alkobaby AI, Salem MA, Abdel-Salam M, Asran BM. Effect of fertilization and low quality feed on water quality dynamics and growth performance of Nile tilapia (*Oreochromis niloticus*). Journal of American Science; 2010; 6:1044-1054.
14. Grabner M, Hofer R. Stomach digestion and its effect upon protein hydrolysis in the intestine of rainbow trout (*Salmogairdneri*).Comparative Biochemistry and Physiology. 1989; 92A:81-83.
15. Kaye G, Romrell L, Ross M. Histology. Text and Atlas.3ed. Williams and Wilkins. Baltimore, 1995, 823.
16. Heath J, Young B. Wheater's functional histology. Text and Atlas.4 ed. Harcourt Publishers. Sydney, Australia, 2000.
17. Kirchgessner M, Kurzinger H, Schwarz FJ. Digestibility of crude nutrients in different feeds and estimation of their energy content for carp *(Cyprinus carpio L.)* Aquaculture. 1986; 58(4):185-194.
18. Inaba D, Ogino C, Talamatsu T, Ueda T, Kurokawa K. Digestibility of dietary components in fishes. II: Digestibility of dietary protein and starch in rainbow trout. Bulletin of the Japanese Society for the Science of Fish. 1963; 29(1):242-244.
19. Shimeno S, Hosokawa H, Takeda M. The importance of carbohydrate in the diet of a carnivorous fish, 1978. FAO/FN: EUFAC/78/SYMP: E/5.
20. Mohapatra M, Sahu NP, Chaudhari A. Utilization of gelatinized carbohydrate in diets in *Labeo rohita* fry. Aquaculture Nutrition. 2002; 8:1-8.
21. Khajepour F, Hosseini SA. Citric acid improve growth performance and phosphorus digestibility in Beluga (*Huso huso*) fed diets where soya bean meal partly replaced fish meal. Animal Feed Science Technology. 2012; 171(1):68-73.
22. Luckstadt C. The use of acidifiers in fish nutrition. CAB Rev: Per Agriculture Veteinary Science Nutrition National Resources. 2008; 3(1):1-8.
23. Kalla A. Growth, survival, digestibility and feed conversion of *Cirrhinus mrigala* fed with sorghum and soya bean meals. M.Sc. Thesis, RAU, Bikaner. 1995, 74.
24. Nandeesha MC, Gangadhar B, Keshavanath P, Varghese TJ. Effect of dietary sodium chloride supplementation on growth, biochemical composition and digestive enzyme activity of young *Cyprinus carpio* (Linn.) and *Cirrhinus mrigala* (Ham.). Journal of Aquaculture Tropical. 2000; 15:135-144.