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The efficacy of dietary supplementation of duck weed (*Lemna minor*) on survival rate and digestive enzyme activity of *Labeo rajasthanicus* (Datta and Majumdar, 1970) fingerlings

MC Meena, BK Sharma, SK Sharma and NK Yadav

Abstract
Duck weed mixed with conventional diet at different levels 10%, 20%, 30%, and 40% and was fed to *Labeo rajasthanicus* fingerlings for 56 days. The experiment was performed with four replications for four test diets and control. The control diet was prepared with rice bran, GNOC, soybean meal, corn flour, wheat flour, and the Mineral mixture. These experimental feeds were given to fishes @ 3-4% of body weight per day. The experimental water has shown average values of different water quality parameters viz. water temperature 25.6-28.0 °C, pH 7.63-8.27, dissolved oxygen 5.10 to 7.40 mg/l, hardness 610 to 638 mg/l, total alkalinity 102.00 to 138 mg/l, and electric conductivity 201 to 246 mS cm-1. The highest protease activity was found in treatment T_2 (0.2044 units/ mg protein/ min) while the lowest was obtained in treatment control (0.1339 units/ mg protein/ min). Highest amylase activity was found in T_2 (0.1337 units/ mg protein/ min.) and lowest in control (0.0936 units/ mg protein/ min.). Highest lipase activity found in T2 (4.09887 units/ mg protein/ min.). The protease and amylase activities of *Labeo rajasthanicus* have shown significant difference ($p<0.05$) among different treatments, while lipase activity did not show any significant difference ($p>0.05$). Survival rate of each fish group at dietary duckweed (*Lemna minor*) levels or among different sizes of *Labeo rajasthanicus* was found unaffected.

Keywords: duck weed, *Labeo rajasthanicus*, lipase, protease, amylase, survival rate

Introduction
Aquaculture is the rearing of aquatic animals and plants in controlled and confined environment for the purpose of food and trade. It is one of the most promising and fast growing component of agriculture sector. The global fish production of 179 million tonnes (contributed by aquaculture and fisheries and aquaculture) in 2018, with aquaculture representing 46% of the total and 53%, of non-food uses (including deficiency to fish meal and fish oil) are renounced. Capture fisheries in the world's inland waters produced 11.6 million tonnes in 2016, with an increase of 2.0 per cent over the previous years (FAO, 2018) [11]. India is the second largest fish producing country in the world after China (FAO, 2018) [11]. The fish production of India in 2018-19 was 13.57 million metric tonnes which get increases by 14.16 MMT in 2019- 20 and the total value of fisheries export was Rs. 46,662.85 crore in 2019-20. In India, the fisheries sector contributes to 1.24 per cent of the GDP and 7.28 per cent of the agriculture GDP (Handbook on fisheries statistics, 2020) [13]. In aquaculture, more than 60% of the input cost of production is contributed by feed alone (Handbook of Fisheries Statistics 2019).

Labeo rajasthanicus, locally known as sarsi, is one of the important minor carp which is native and endemic to South Rajasthan. Among the carps, it is a very important alternate species merits for diversification in freshwater aquaculture in our country as visibly it has good market value in some regions of India. This species also has potential for inclusion in composite culture (Lal *et al*., 2015) [19]. The fish species was reported for the first time in western region of Rajasthan from Jaisamand Lake (Datta and Majumdar, 1970) [9]. The species has been regarded as an important food fish as it has good market value and it has been already raised as brood stock under captivity. Further, the fish seeds produced through induce breeding can be used for aquaculture enhancement (Anon., 2014).

Duckweed Lemna sp. is a group of free floating small aquatic plants belonging to family Lemnaceae (Cheng *et al*., 2002) [4].

Duckweeds are the best known for their excellent productivity and high protein level in temperate climates. They are usually small in size (1-3 mm) and green in colour (Altay *et al.*, 1996). Duckweed stalk easily grows in community and from a thick and constant surface mat (Hasar *et al.*, 2000) [14]. They are used not only for treatment of waste water but also for harvesting nutrients. This is due to their ability of rapid multiplication and high protein biomass (Caicedo *et al*., 2000) [2]. Duckweed wastewater treatment systems were studied in much detail by Korner *et al*., (1998) [17] for a wide range of waste water type. The inclusion of dietary plant sources like papain enzyme suitable for growth enhancement in fishes like rohu (Yadav *et al.*, 2021) [23].

Material and Methods

Experimental Fish: Fingerlings of *Labeo rajasthanicus* (Datta and Majumdar, 1970) [9] were used for the experiment. The *Labeo rajasthanicus* fingerlings of almost similar size were procured from Aquaculture Research and Seed unit of DOR, MPUAT, Udaipur.

Experiment Design: The experiment was conducted for period of 56 days at the Wet Lab, Department of Aquaculture, College of Fisheries, Udaipur. A total 250 healthy and infection free fish seed were stocked in rectangular FRP tanks of 225 liters capacity. After one weeks of acclimatization period, fish with average body weight of 4.11gm (±0.1) were divided into five groups. Before the introduction of fish, every tank was washed and disinfected using $KMnO_4$. In each tank 10 fishes were randomly distributed and tanks were filled with tube well water. The fingerlings were fed @ 3-4% body weight twice a day in morning and evening. Further, the fishes in treatment were fed with diet having graded levels of duckweed *(Lemna minor)* 10, 20, 30 and 40% for experimental period. The survival rate and digestive enzyme activity was analysed end of the experiment. Water quality parameters were analysed at weekly intervals.

Experimental Diet: The fresh *Lemna minor* from a natural water body near Jaisamand was collected for use. The duckweeds so collected were cleaned, air dried and powdered before use. The powdered *Lemna minor* was mixed with basal diet prepared with GNOC, rice bran, soybean meal, wheat flour, corn flour, mineral mixture. The details of different ingredients and the treatment are given in (Table 1). The treatment tank T_0 (control) T_1 (10), T_2 (20), T_3 (30), and T_4 (40) were supplicated with duckweed (*Lemna minor*). The dry ingredients (ground nut cake, rice bran, soybean meal, wheat flour, corn flour, mineral mixture) of the basal diets were thoroughly mixed and formed dough and placed in autoclave at 15 lbs pressure for 30 minutes. To prepare the pellets (spaghetti) hand pelletized were used and feed was air dried and stored in air tight containers for further use.

Table 1: The details of the ingredients used for basal diets (%)

S. No.	Ingredients	Amount (%)
1	Soybean meal	20
2	GnOC	30
3	Rice bran	40
4	Corn flour	5
5	Wheat flour	4
6	Mineral mixture	1
7	Duckweed	0
8	Total	100

Table 2: percentage of different level of *Lemna minor*

Treatment	Basal diet (%)	Ingredients diet (%)
T_1	100	0
T_2	90	10
T_3	80	20
T_4	70	30
T_5	60	40

Water quality analysis: Standard procedures of APHA (2005) [1] were used to determine different water quality parameters viz. water temperature, pH, electrical conductivity, dissolved oxygen, total alkalinity, and total hardness were determined by using

Survival rate: It is the numbers of fish that survive during the experimental period which is expressed as percentage of the stocked fish. It is determined by deducting the dead fish number in the course of culture period from the stocked fish and then it is expressed as percentage (Charo-karisa *et al*., 2006) [3]. Survival rate of experiment fish was predicated according to following formula:

$$\text{Survival rate} = \frac{Nt\ X\ 100}{No}$$

Where, Nt = Final number of fishes
No = Initial number of fishes

Digestive enzyme activity

At the end of experimental period activities viz. Lipase, Protease and Amylase were assessed using standard methods.

Protease: Protease activity was determined by the casein digestion method, (Drapean, 1976) [10]. The enzyme reaction mixture consisted of 1% casein in 0.05 M Tris PO_4 buffer (pH 7.8) and incubation was done for 5 min. at 37 ºC. Further, tissue homogenate was added to that after ten minutes. The reaction was stopped with addition of 10% TCA and the whole content was filtered. The reaction blank was made by adding tissue homogenate just before stopping the reaction of enzyme needed to release acid soluble fragments equivalent to $\Delta 0.001 A_{280}$ per minute at 37 ºC and pH 7.8.

Amylase: Amylase activity was estimated using dinitro-salicylic-acid (DNS) method (Rick and Stegbauer, 1974). The reaction mixture consisted of 1% (w/v) starch solution, phosphate buffer (pH 6.9) and the tissue homogenate. Incubation of reaction mixtures was done at 37ºC for 30 minutes. DNS was added after incubation and kept in boiling water bath for 5 minutes. The dilution of reaction mixture was done with distilled water after cooling and absorbance was measured at 540 nm. Maltose was used as the standard. Amylase activity was expressed as mole of maltose released from starch per minute at 37ºC temperature.

Lipase: The lipase activity (EC 3.1.1.3) was assayed by the method of Cherry and Crandall (1932) [5]. Two test tubes labelled as test (T) and control (C) were taken, and each was filled with 3 ml of distilled water and 1 ml of homogenate were added. The control tube was placed in boiling water for 5 minutes at 100 ºC and cooled. This serves to inactivate the lipase in the control. Than 0.5 ml of phosphate buffer solution (pH 7) and 2 ml of olive oil emulsion were added to both the tubes, shaken well and incubated at room temperature for 24 hours. Then, 3 ml of 95% alcohol and two drops

phenolphthalein indicator solution were mixed. Each of the tube was titrated against 0.05 N NaOH up to the appearance of permanent pink colour. The volume of 0.05 N NaOH required for 100 gm intestinal tissue in the experimental tube minus the volume of 0.05 N NaOH required for the same amount of intestinal tissue in the control tube represents the units of intestinal lipase activity per g tissue.

Statistical Analysis

The mean values of the all examined parameters (such as Growth performance indices, survival, enzyme activity and water quality) and further calculation were done in the MS excel of version 2007 and ANOVA was also performed with the same.

Result and Discussion

The current research work indicated that survival rate during the experiment period of 56 days were unaffected in different dietary levels and 100% survival rate of test fishes was observed owing to good water quality maintained in the experimental tanks. The protease and amylase activities of *Labeo rajasthanicus* have shown significant difference ($p<0.05$) among different treatments, while lipase activity did not show any significant difference ($p>0.05$). The highest protease activity was found in treatment T_2 (0.2044 units/ mg protein/ min) while the lowest was obtained in treatment control (0.1339 units/ mg protein/ min). Highest amylase activity was found in T_2 (0.1337 units/ mg protein/ min.) and lowest in control (0.0936 units/ mg protein/ min). Suzer *et al.*, (2008) in his study investigated the influence of commercial probiotic supplementation on the larval stages of Gilthread sea bream (*Sparus aurata*, L.). Both the digestive enzyme and growth performance activities were found to increase in the treatment as compared to the control. This is similar to the findings of Steffnes (1989) [15]. In the present study, the highest protease and amylase activities were shown by the 20% *Lemna minor* fed fish and lower activity in the control. The results further suggest that the intestinal protease and amylase activities might have reduced by the presence of some ANFs such as polyphenolic compounds (Yan *et al.*, 2011 [24]; Kumar *et al.*, 2014; Chiow *et al.*, 2016 [7]; Garg *et al.*, 2019). Similar results have been observed in *L. rohita* fed with *Mucuna pruriens* leaf extract (Ojha *et al.*, 2014) and *H. cordata* (Garg *et al.*, 2019). Kaleeswaran *et al.* (2011) [16] also reported protease activity to increase in the *Catla catla* fed with *Cynodon dactylon* leaf meal as herbal supplement at 5-50 g/kg in the diet. There was no significant difference observed in the present study in case of lipase activity.

Table 3: Effect of dietary supplementation of duckweed (*Lemna minor*) on Amylase, Protease, and Lipase in the intestine of *Labeo rajasthanicus* fingerling

Treatment	Amylase (units/ mg protein/ min)	Protease (units/ mg protein/ min)	Lipase (unit/mg protein)
Control	0.0936	0.1339	1.18556
T_1	0.1090	0.1792	3.71689
T_2	0.1337	0.2044	4.09887
T_3	0.1258	0.1729	2.60115
T_4	0.0996	0.1636	2.10532
SEm±	0.0047	0.0061	0.1092
CD(P= 0.05)	0.0140	0.0183	0.3291

Data expressed as mean ± SE (n=4)

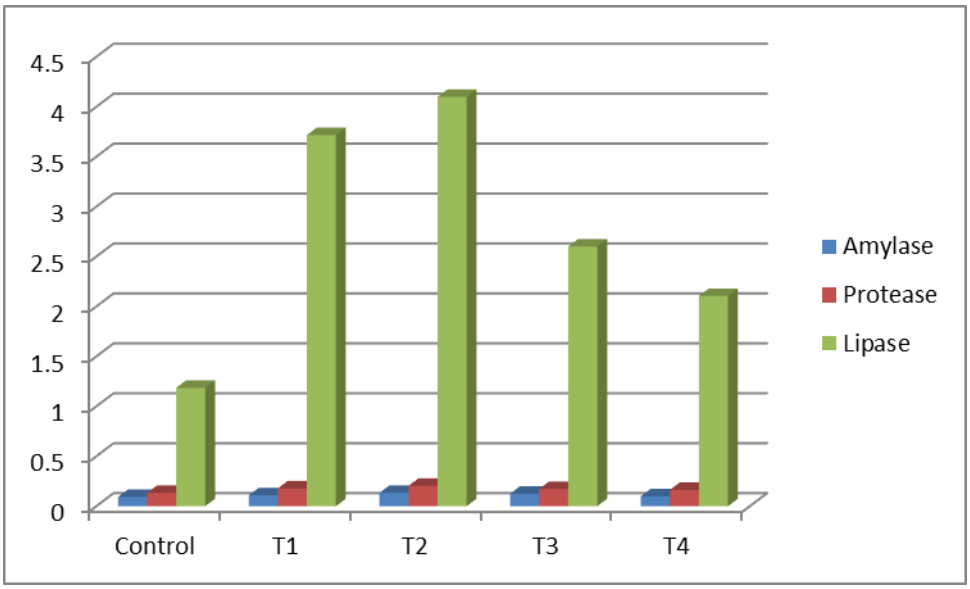

Fig 1: Digestive enzyme activity of Labeo rajasthanicus fingerlings in different treatments

Conclusion

On the basis of the results in the present experiment, it can be concluded that the *Lemna minor* supplementation certainly enhances survival of *Labeo rajasthanicus*. The digestive enzyme activity viz. lipase, protease and amylase also enhanced by inclusion of the duckweed (*Lemna minor*) @ 20% in the diet of *Labeo rajasthanicus*. So duck weed certainly useful to better fish digestive metabolism with aqua friendly rearing environment.

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