E-ISSN: 2347-5129
P-ISSN: 2394-0506
(ICV-Poland) Impact Value: 5.62
(GIF) Impact Factor: 0.549
IJFAS 2019; 7(1): 39-43

www.fisheriesjournal.com
Received: 21-11-2018
Accepted: 25-12-2018

VR Patil
College of Fisheries (Dr. B. S. Konkan Krishi Vidyapeeth), Shirgaon, Ratnagiri, Maharashtra, India

AS Pawase
College of Fisheries (Dr. B. S. Konkan Krishi Vidyapeeth), Shirgaon, Ratnagiri, Maharashtra, India

DI Pathan
College of Fisheries (Dr. B. S. Konkan Krishi Vidyapeeth), Shirgaon, Ratnagiri, Maharashtra, India

RA Pawar
College of Fisheries (Dr. B. S. Konkan Krishi Vidyapeeth), Shirgaon, Ratnagiri, Maharashtra, India

SS Dey
College of Fisheries (Dr. B. S. Konkan Krishi Vidyapeeth), Shirgaon, Ratnagiri, Maharashtra, India

S Swain
College of Fisheries (Dr. B. S. Konkan Krishi Vidyapeeth), Shirgaon, Ratnagiri, Maharashtra, India

Correspondence
AS Pawase
College of Fisheries (Dr. B. S. Konkan Krishi Vidyapeeth), Shirgaon, Ratnagiri, Maharashtra, India

Effect of exogenous carbohydrases on growth and survival of catla fry

VR Patil, AS Pawase, DI Pathan, RA Pawar, SS Dey and S Swain

Abstract
Catla, *Gebelion catla* is one of the most important Indian major carp species that involves supplementary feeding comprising a mixture of rice bran and groundnut oil cake. Since, the plant origin feed sources are not utilized to the fullest extent by the fishes, inclusion of enzymes in fish diets has been proved to be an effective method to improve the feed digestibility. In the present study, some of the exogenous enzymes such as cellulase, hemicellulase and alpha-amylase each at 0.5. 1 and 1.5% level were incorporated in the diets of catla fry. The experiment was carried out in circular plastic containers of 125 L capacity with twelve treatments and two replicates for a period of 90 days. The catla fry were grown to 35.8 – 47.9 mm in length and 5.53 –4.84 g in weight from the initial length range of 17 – 19 mm and weight range of 0.37 – 0.45 g. Results showed the maximum length, weight gain, specific growth rate and survival in the treatment incorporated with mixture of exogenous enzymes each at 0.5 g 100 g^{-1} level in the catla fry diet.

Keywords: catla, exogenous, enzymes, growth

1. Introduction
The inclusion of exogenous enzymes in fish diets can be termed as efficient an alternative technique to improve the utilization of nutrients coming from plant ingredients (Moura *et al*., 2012) [1]. The use of enzymes to pre-treat the ingredients has received much attention in the food industry to obtain hydrolyzed and more readily digestible products. The current trend of increased use of plant proteins in fish diets has generated much interest in the use of enzymatic supplements in aqua feeds. The role of enzymatic action of cellulase, hemicellulase and alpha-amylase is well known in livestock industry. As such, in the present study, the mixture of these exogenous enzymes in different combinations and levels were used to identify their role and effect on growth of catla fry. Exogenous enzymes either supplemented singly or in combination of enzymes were also used by various researchers such as enzymes combination of amylase, cellulase and other enzymes by Ogunkoya *et al*.(2006) [2], Soltan (2009) [3]; cellulase and other enzyme mixture by Ai *et al*. (2007) [4], Ayhan *et al*. (2008) [5], Xavier *et al*. (2012) [6]; alpha-amylase by Kumar *et al*. (2006a and b) [7-8], only cellulase by Erdogan and Olmez (2009) [9]; a mixture of hemicellulase, cellulase and other enzymes by Yildirim and Turan (2010) [10], Kazerani and Shahsavani (2011) [11]; a mixture of cellulase, hemicellulase, alpha-amylase and other exogenous dietary enzymes by Ghomi *et al*. (2012) [12]. However, literature showing the role of exogenous enzymes in IMC culture is sparsely available. The rearing of catla from fry to fingerling stage is considered as one of the important activities in carp rearing. Thus, the present study was attempted to find out efficacy of some commonly used carbohydrases like cellulase, hemicellulase and alpha-amylase in various combinations on digestion of conventional plant-based feed of catla.

2. Materials and Methods
Fry of catla in the range of 17-19 mm length and 0.37-0.45 g weight were procured from the carp seed production unit. Newly arrived fry were acclimatized in fiberglass tank of 500 L capacity for a period of one week. During the acclimation period, fry were fed with flake feed comprising groundnut oil cake and rice bran in 1:1 ratio. The feed was given *ad libitum* three times a day, before the start of actual experiment. Uneaten food and excreta were siphoned out daily in the evening and new water was added to the tank. The actual experiment was conducted in circular plastic tanks of 125 L capacity.

2.1 Feed ingredients

Flake feed as a basal feed was prepared using locally available ingredients such as groundnut oil cake and rice brain in 1:1 ratio (Jena *et al*., 1998) [13]. Proximate composition of ingredients used for basal diet is given in Table 1.

2.2 Enzyme supplementation

The experimental diets were prepared using the different combinations of commercially available exogenous enzymes such as cellulase, hemicellulase and alpha-amylase. Exogenous enzymes were added to the diet in combination of two or three enzymes in order to make the level of 0.5, 1.0, 1.5 g 100 g^{-1} in the diet. Feed ingredients, combination of enzymes in diets and their proximate composition is also given in Table 1.

2.3 Experimental set up

Circular plastic tanks were filled up with approximately 100 L of freshwater. The fry were stocked at a density of 30 fry 100 L^{-1}. In all, 12 diet treatments and one control treatment were used for the experiment using completely randomized design. The experiment was carried out for a period of 90 days with two replicates for each treatment. The flake feed was supplied twice daily in the morning and evening hours. Throughout the experiment, fry were fed at the rate of 10%, 8% and 6% of body weight for first, second and third month, respectively. The faeces of the fry and uneaten feed were siphoning out daily from the tank and about 20 – 30% of the total water volume was exchanged from the experimental tanks. Aeration was provided to each experimental tank.

Table 1: Ingredients and proximate composition of the experimental diets

Ingredients	Proportion of each ingredient in feed used in experiment (g 100 g $^{-1}$)												
Enzymes levels	Control	0.5 g 100 g^{-1}				1.0 g 100 g^{-1}				1.5 g 100 g^{-1}			
	T_0	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9	T_{10}	T_{11}	T_{12}
Groundnut oil cake	50.00	49.75	49.75	49.75	49.75	49.50	49.50	49.50	49.50	49.25	49.25	49.25	49.25
Rice Bran	50.00	49.75	49.75	49.75	49.75	49.50	49.50	49.50	49.50	49.25	49.25	49.25	49.25
Cellulase	0.00	0.167	0.250	0.00	0.250	0.334	0.500	0.00	0.500	0.500	0.750	0.00	0.750
Hemicellulase	0.00	0.167	0.250	0.250	0.00	0.334	0.500	0.500	0.00	0.500	0.750	0.750	0.00
Alpha-amylase	0.00	0.167	0.00	0.250	0.250	0.334	0.00	0.500	0.500	0.500	0.00	0.750	0.750
Proximate composition													
Moisture (%)	9.40	8.70	8.50	8.60	9.20	8.40	8.80	9.50	8.20	8.80	9.40	9.20	8.30
Crude protein (%)	26.25	25.81	25.81	25.38	25.81	25.38	25.81	25.38	24.94	24.94	25.38	25.38	24.94
Crude lipid (%)	8.10	8.30	8.40	8.60	7.90	8.40	8.60	8.30	8.60	8.80	8.20	8.30	8.40
Crude ash (%)	10.80	11.60	11.80	10.20	9.90	10.40	11.50	10.70	11.00	11.20	10.30	10.20	10.90
Crude fiber (%)	12.80	12.50	12.30	12.00	12.40	12.20	11.80	12.60	12.50	12.30	12.30	12.10	11.90
NFE (%)	32.65	33.09	33.19	35.23	34.79	35.23	33.49	33.53	34.76	33.96	34.43	34.83	35.56

a. Rice bran: crude protein – 11.81%, crude lipid – 6.60%, moisture – 9.60%, total ash – 10.80%, fiber – 12.50% and NFE – 48.69%.
b. Ground nut oil cake: crude protein – 39.81%, crude lipid – 8.30%, moisture – 7.20%, total ash – 10.20%, fiber –8.50% and NFE – 25.99%.

2.4 Feed preparation

Flake feed was formulated by using groundnut oil cake and rice bran in 1:1 ratio. The required quantity of finely powdered and sieved ingredients were weighed and mixed thoroughly. Slurry of mixed ingredients was prepared by adding 350 ml of water for 100 g of feed mixture. The slurry was cooked for 15 minutes and cooled at room temperature. After cooling, desired quantity of various combinations of enzymes were added and mixed thoroughly with the slurry. Slurry was uniformly spread on a polythene sheet with the help of soft painting brush in a thin layer and oven-dried at 40˚C for 3 h (Goda *et al*., 2012) [14]. The flakes were packed in airtight plastic pouches and stored in dry place for further use. Conventional carp feed of groundnut oil cake and rice bran mixture without any enzymes was used as control diet.

2.5 Proximate composition

Proximate composition of all diets was carried out according to the methods given by AOAC (2006) [15]. Moisture was calculated by gravimetric analysis following oven drying to achieve constant weight at 105˚C. The nitrogen content was derived by the Kjeldhal method using KEL PLUS-CLASSIC DX apparatus. Crude protein was calculated by multiplying nitrogen content by a constant 6.25. Crude fat content was determined by the Soxhlet method using SOCS PLUS apparatus and petroleum ether. Ash content was determined gravimetrically by burning in muffle furnace at 550˚C for 6 h. Crude fibre content was determined by using FIBRA PLUS with 0.1% sulphuric acid and sodium hydroxide wash. Further ashing of sample was in muffle furnace at 550˚C for 2 h. The nitrogen free extract (NFE) was calculated by subtracting% crude protein,% crude fat,% crude fibre,% crude ash,% moisture contents from a total of 100.

NFE (%) = (100) – [Crude protein (%) + Crude lipid (%) + Crude fibre (%) + Moisture (%) + Ash (%)]

2.6 Water parameters

Analysis of water quality parameters such as dissolved oxygen (DO), total alkalinity, total hardness, free carbon di-oxide were estimated at weekly interval using methods given by Boyd (1981) [16]. Water temperature and pH of water were recorded by using mercury thermometer and universal indicator respectively.

2.7 Growth parameters

Growth parameters such as length gain (%), weight gain (%), specific growth rate (%) and survival (%) were estimated by following standard methods.

2.7.1 Length gain (%)

The length gain was calculated using the formula given below:

$$\text{Length gain (\%)} = \frac{\text{(Final length - Initial length)}}{\text{Initial length}} \times 100$$

2.7.2 Weight gain (%)

The weight gain was calculated using the formula given below:

$$\text{Weight gain (\%)} = \frac{\text{(Final weight - Initial weight)}}{\text{Initial weight}} \times 100$$

2.7.3 Specific growth rate (%)

Specific growth rate was calculated using the following formula given below:

$$\text{Specific Growth Rate (\%)} = \frac{(\ln Wt - \ln W0)}{dt} \times 100$$

Where, Wt = Final weight
W0 = Initial weight
dt = Rearing period in days

2.7.4 Survival (%)

Survival (%) of fish was calculated using the following formula given below:

$$\text{Survival (\%)} = \frac{\text{Final number of fish}}{\text{Initial number of fish}} \times 100$$

2.8 Statistical analysis

Standard error of the average length, weight, specific growth rate, survival, and feed efficiency ratio of the catla fry fed with the diet for each replicate was calculated. Data obtained from the experiments for growth parameters and survival were analysed by one-way ANOVA. Student-Newman-Keuls test was used to determine the significant difference between the treatments.

3. Results

3.1 Length gain (%)

The average length gain (%) of catla fry are given in Table 2. The maximum average length gain of 171.91±1.41% was observed in T_9 comprising each of 0.5 g 100 g^{-1} diet of cellulase, hemicellulase and alpha-amylase; whereas, the control treatment i.e. T_0 diet showed the minimum average length gain of 103.98±0.65%. One way ANOVA showed significant difference ($P < 0.05$) in the average length gain (%) between treatments containing different levels of enzymes**.** Student-Newman-Keuls test (SNK) indicated that average length gain (%) in T_9 diet was significantly higher ($P < 0.05$) than that of other treatments. The growth in T_5 evinced better growth response next to T_9. Overall, the growth response in percentage length gain in each of the treatments showed significantly better response than that of the control treatment. No significant difference was observed in the length gain between the treatments T_1 and T_{12}; T_{10} and T_{11}; T_2 and T_6.

3.2 Weight gain (%)

The average initial weight, final weight and weight gain of catla fry are given in Table 2. The maximum average weight gain of 1088.22±2.46% was observed in T_9 comprising each of 0.5 g 100 g^{-1} diet of cellulase, hemicellulase and alpha-amylase followed by T_5 (0.334 g of each of cellulase, hemicellulase and alpha-amylase) and T_1,T_{12}, T_8, T_4, T_7, T_{10}, T_6, T_7, T_3, T_2.; while, T_0 diet showed the minimum average weight gain of 522.46±0.94%. One way ANOVA showed significant difference ($P < 0.05$) in the average weight gain (%) between the treatments containing different levels of enzymes. Student-Newman-Keuls test (SNK) indicated that average weight gain (%) in T_9 was significantly higher ($P < 0.05$) than other treatments. The response in the average weight gain of the treatment T_5, T_1 and T_{12} was also better next to T_9.

3.3 Specific growth rate (%)

The average specific growth rate of catla fry is given in Table 2. The maximum average specific growth rate of 2.750±0.08% was observed in T_9 followed by treatment T_5, T_1 and T_{12}. The treatment T_0 diet showed the minimum average specific growth rate of 2.032±0.04%. Statistical analysis showed significant difference ($P < 0.05$) in the average specific growth rate (%) between the treatments containing different levels of enzymes. Student-Newman-Keuls test (SNK) showed that average specific growth rate (%) in T_9 was significantly higher ($P < 0.05$) than other treatments.

Table 2: Average length gain (%), weight gain (%), specific growth rate (%) and survival (%) of catla fry fed with different diets

Sr. No.	Treatment	Length gain (%)	Weight gain (%)	Specific growth rate (%)	Survival (%)
1	T_0	103.98±0.65[a]	522.46±0.94[a]	2.032±0.04[a]	81.67±1.09[ae]
2	T_1	154.65±1.14[b]	963.94±2.09[b]	2.627±0.07[b]	76.67±1.54[bd]
3	T_2	112.99±0.59[cl]	557.65±1.10[cm]	2.093±0.05[cm]	61.67±1.09[f]
4	T_3	115.17±0.89[dl]	568.35±2.64[dm]	2.111±0.11[dm]	66.67±1.54[g]
5	T_4	136.8±1.18[e]	851.2±1.89[e]	2.503±0.06[e]	71.67±1.09[d]
6	T_5	160.47±1.13[f]	1012.63±0.9[f]	2.677±0.03[f]	78.33±1.88[cd]
7	T_6	113.45±0.90[gl]	656.14±1.61[gl]	2.248±0.06[gl]	63.33±1.54[e]
8	T_7	116.15±0.75[hl]	655.64±1.41[hl]	2.247±0.05[hl]	68.33±1.09[g]
9	T_8	148.04±0.89[i]	894.56±1.25[i]	2.552±0.04[i]	73.33±1.54[d]
10	T_9	171.91±1.41[j]	1088.22±2.46[j]	2.75±0.08[j]	83.33±1.54[e]
11	T_{10}	121.33±1.26[k]	711.64±1.92[k]	2.327±0.07[k]	65±1.09[ade]
12	T_{11}	122.35±0.30[k]	724.83±2.51[k]	2.344±0.09[k]	70±1.54[d]
13	T_{12}	153.68±0.78[b]	962.47±1.7[b]	2.626±0.06[b]	75±1.09[d]

Values with different superscripts in same column are significantly different ($P < 0.05$)

3.4 Survival (%)

The average survival of catla fry is given in Table 2. The maximum average survival of 83.33±1.54% was observed in T_9 comprising each of 0.5g 100 g^{-1} diet cellulase, hemicellulase and alpha-amylase followed by T_0, T_5, T_1, and T_{12}; whereas, T_2 diet showed the minimum average survival of 61.67±1.09%. Statistical analysis showed the significant difference ($P < 0.05$) in the average survival (%) between the treatments containing different levels of enzymes. Student-Newman-Keuls test (SNK) revealed that average survival (%) in T_9 was significantly higher ($P < 0.05$) than that of other treatments. The treatment T_0 evinced better survival response next to T_9.

3.5 Water quality parameters
During the experimental period, water parameters such as temperature (°C), pH, dissolved oxygen (mg L^{-1}), free carbon dioxide (mg L^{-1}), total alkalinity (mg L^{-1} as $CaCO_3$) and total hardness (mg L^{-1} as $CaCO_3$) were recorded and given in Table 3.

Table 3: Range of water parameters during fry rearing of catla

Sr. No.	Water quality parameters	Ranges
1	Temperature (°C)	24.5 – 27.6
2	pH	6.5 – 7.5
3	Total hardness (mg L^{-1} as $CaCO_3$)	73.84 – 93.88
4	Total alkalinity (mg L^{-1} as $CaCO_3$)	51.30 – 74.26
5	Dissolved oxygen (mg L^{-1})	4.20 – 4.88
6	Free carbon dioxide (mg L^{-1})	1.30 – 4.33

4. Discussion
In the present study, among the tested exogenous enzymes at different combinations and levels, maximum length gain of 171.91±1.41% and the maximum weight gain of 1088.22±2.46% was observed in catla fry fed with diet consisting mixture of cellulase, hemicellulase and alpha-amylase each at 0.5 g 100 g^{-1} diet. Patnaik *et al*. (2005) [17] noticed that catla fry fed with purified diet and reared in plastic tanks of 50 L capacity for a period of 70 days exhibited higher weight gain of 220.43±16.62%. Xavier *et al*. (2012) [6] evaluated that the rohu fingerlings reared in plastic tubs and fed with the diet containing 0.2% cellulase and other enzyme mixture at different levels exhibited higher weight gain of 189.6±8.3% after a period of 60 days. Thus, the enzyme complex is said to be playing significant role in digestion of cellulose matter in fish diets.

In the present study, the maximum specific growth rate of 2.75±0.08% was observed in catla fry fed with diet consisting mixture of cellulase, hemicellulase and alpha-amylase at 1.5 g 100 g^{-1} level. Patnaik *et al*. (2005) [17] observed maximum specific growth rate of 1.67±0.09% in catla fry fed with purified diet and reared in plastic tanks of 50 L capacity for a period of 70 days. Studies suggested that enzymes concentration plays an important role in diets and probably functioning to promote secretion of endogenous enzymes which directly influence on the growth performance.

With proper pond management survival of 60-70% in fry to fingerlings rearing is normally achieved in rearing ponds at the end of 2-3 months. Among the tested exogenous enzymes combinations and levels, maximum survival of 83.33± 1.54% was in observed catla fry fed diet supplemented with mixture of cellulase, hemicellulase and alpha-amylase each at 0.5 g 100 g^{-1} diet. The second best performance was noticed in the diet with a combination of cellulase, hemicellulase and alpha-amylase at 1.0 g 100 g^{-1} of diet. The study opined that combination of three enzymes such at 0.5g 100 g^{-1} diet gave better results than the feed having combination of two enzymes in the diet.

5. Conclusion
It is concluded that conventional feed mixture of rice bran and ground nut oil cake (GNOC) can be supplemented with mixture of exogenous enzymes each at 0.5% level of cellulase, hemicellulase and alpha-amylase for better growth, survival and feed efficiency ratio for rearing of catla fry.

6. References

1. Moura GS, Lanna EAT, Filer K, Falkoski DL, Donzele JL, Oliveira MGA *et al* Effects of enzyme complex SSF (Solid state fermentation) in pellet diets for Nile tilapia. R. Bras. Zootec. 2012; 41(10):2139-2143.
2. Ogunkoya AE, Page GI, Adewolu MA, Bureau DP. Dietary incorporation of soybean meal and exogenous enzyme cocktail can affect physical characteristics of faecal material egested by rainbow trout (*Oncorhynchus mykiss*). Aquaculture. 2006; 254(1-4):466-475.
3. Soltan MA. Effect of dietary fish meal replacement by poultry by-product meal with different grain source and enzyme supplementation on performance, feces recovery, body composition and nutrient balance of Nile tilapia. Pakistan J Nutri. 2009; 8(4):395-407.
4. Ai Q, Mai K, Zhang W, Xu W, Tan B, Zhang C, Li H. Effects of exogenous enzymes (phytase, non-starch polysaccharide enzyme) in diets on growth, feed utilization, nitrogen and phosphorus excretion of Japanese seabass, *Lateolabrax japonicus*. Comp. Biochem. Physiol. A. 2007; 47(2):502-508.
5. Ayhan V, Diler I, Arabaci M, Sevgili H. Enzyme supplementation to soybean based diet in gilthead sea bream (*Sparus aurata*): Effects on growth parameters and nitrogen and phosphorus excretion, Kafkas. Univ. Vet. Fak. Derg. 2008; 14(2):161-168.
6. Xavier B, Sahu NP, Pal AK, Jain KK, Misra S, Dalvi RS, Baruah K. Water soaking and exogenous enzymes treatment of plant-based diet effect on growth performance, whole-body composition, and digestive enzyme activity enzyme activities of rohu, *Labeo rohita* (Hamilton) fingerlings. Fish Physiol. Biochem. 2012; 38(2):341-353.
7. Kumar S, Sahu NP, Pal AK. Non-gelatinized corn supplemented with microbial α-amylase at sub-optimal protein in the diet of *Labeo rohita* (Hamilton) fingerlings increases cell size of muscle. J Fish. Aquat. Sci. 2006a; 1(2):102-111.
8. Kumar S, Sahu NP, Pal AK, Choudhary D, Mukherjee SC. Studies on digestibility and digestive enzyme activities in *Labeo rohita* (Hamilton) juveniles: effect of microbial α-amylase supplementation in non-gelatinized or gelatinized corn-based diet at two protein levels. Fish Physiol. Biochem. 2006b; 32:209-220.
9. Erdogan F, Olmez M. Effects of enzyme supplementation in diets on growth and feed utilization in Angel fish, *Petrophyllum scalare*. J Anim. Vet. Adv. 2009; 8(9):1740-1745.
10. Yildirim YB, Turan F. Effects of exogenous enzyme supplementation in diets on growth and feed utilization in African catfish, *Clarias gariepinus*. J Anim. Vet. Adv. 2010; 9(2):327-331.
11. Kazerani HR, Shahasavani D. The effect of supplementation of feed with exogenous enzymes on the growth of common carp *(Cyprinus carpio)*. Iranian Journal of Veterinary Research. 2011; 12(2):127-132.
12. Ghomi MR, Shahriari R, Langroudi HF, Nikoo M, Elert EV. Effects of exogenous dietary enzyme on growth, body composition, and fatty acid profiles of cultured great sturgeon *Huso huso* fingerlings. Aquacult. Int. 2012; 20:249-254.
13. Jena JK, Aravindakshan PK, Singh WJ. Nursery rearing of Indian major carp fry under different stocking densities. Indian J Fish. 1998; 45(2):163-168.

14. Goda AMA, Mabrouk HAHH, Wafa MAEH. Effect of using baker's yeast and exogenous digestive enzymes as growth promoters on growth, feed utilization and hematological indices of nile tilapia, *Oreochromis niloticus* fingerlings. J Agri. Sci. Tech. B. 2012; (2):15-28.
15. AOAC. Official Methods of Analysis, Edn 18, Association of Official Analytical Chemist, Washington, DC, 2006, 1094.
16. Boyd CE. Water quality in warm water fish ponds. Auburn University, Alabama, USA, 1981, 359.
17. Patnaik D, Sahu NP, Chaudhari A. Effects of feeding raw soybean meal to fry of Indian major carps, *Catla catla* on growth, survival, and protein digestibility. Isr. J Aquac. Bamidgeh. 2005; 57(3):164-174.