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CHANGES OF ENZYMATIC ACTIVITY DURING LARVAL DEVELOPMENT OF CARP, *CATLA CATLA* (HAM.)

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ABSTRACT

Quantitative assays of trypsin, amylase and alkaline phosphatases were made in relation to age and food during the larval development of the Indian major carp *Catla catla*. The responses of all the test enzymes to age and food were identical. No enzymes were detected from the fertilized eggs. Detectable amount of enzymes were first observed in the first day old hatchlings. All the test enzymes in the group fed normal feed tended to rise gradually with advancement of age till day 22 after which an asymptotic level was attained. Absence of food throughout the rearing period caused the enzymatic activity of the larva to remain at the lowest level throughout. When starvation was followed by feeding, enzymatic activity in the former group was consistently higher than that of latter, suggesting that feeding activity was primarily responsible in maintaining the enzymatic activity of carp larva. The enzymatic activity of zooplankton was significantly higher than carp larva till day 6 to 12 after which the latter exceeded the former implying that carp larva during development utilizes the exogenous enzymes of zooplankton.

INTRODUCTION

The changes of enzyme activity of fish are most profound during the early phase of life. Accumulated evidences suggest that absence of stomach or less elaborate and simply organised intestinal tract in the first few weeks after hatching are the main reasons for low production of digestive enzymes in fish larvae (Kawai and Ikada, 1973a; Stroland and Dabowski, 1979; Dabrowski, 1984, Govoni *et al.*, 1986). Digestive enzymes may exist soon after hatching (Alliot, 1981) or it may occur from the day of exogenous feeding (Kawi and Ikede, 1973b; Cousin *et al.*, 1987). In common carp, the digestive system is not sufficiently developed till the tenth day after hatching, and that the enzyme content in the intestinal extract is two to three times less than that of zooplankton (Ostroumova *et al.*, 1980). Among the plankton, the activity was lowest for rotifers and highest for adult copepods (Soda and Cacace, 1989).

Young artificially fed coregonid larvae is reported to starve while their intestines are full (Eckmann and Kausch, 1976). As a result, fish larvae even those of herbivorous species depend primarily upon zooplankton diet because it is demonstrated that exogenous proteolytic enzymes of food organisms play a role in fish digestion, in addition to the activation of fish's own enzymes (Dabrowski and Glogowski, 1977). Lauff and Hofer (1984) showed that exogenous enzymes of natural zooplankton contributed one fourth of the roach's own proteolytic activity. However, addition of proteolytic enzymes to artificial diets resulted in only small positive effects (Ostroumova, 1977; Dabrowski *et al.*, 1978) possibly due to decreased enzyme production (Dabrowski and Glogowski, 1977) and leaching effects (Grabner *et al.*, 1981). Ilkins and Turetskig (1989) demonstrated that exogenous proteolytic enzymes do not play a significant role in the digestion of young common carp. A recent review by McLean and Donaldson (1990) has primarily focused on absorption of bioactive proteins by gastrointestinal tract of fish with reference to transcellular and paracellular processes.

Despite the significance of digestive enzymes in formulating artificial diet of fish larvae, few studies have been made of the digestive enzymes activity of Indian carp larvae. Because of a distinct shift in enzymatic profiles from the start of larval feeding to adult in response to feeding adaptations, and since fish larvae can modulate their enzyme secretions in response to the nature (Hofer and Uddin, 1985) and quantity of food (Cousin *et al.*, 1987; Pedersen *et al.*, 1987), it is of particular interest to examine the digestive enzymes of carp larvae in relation to their natural diet. The present paper attempts to examine the trypsin and amylase contents of *Catla catla*, an Indian major carp at different stages of ontogenic development in relation to natural diet.

MATERIAL AND METHODS

All fish samples subjected to enzymatic assays were procured from a local fish farm. Hormone-induced spawning of *Catla catla* was performed at a water temperature of 25-30°C in May-July, 1992 and 1993. Fertilized eggs, freshly hatched larvae as well as larvae of varying days of post hatching were procured. The entire batch was grouped into four (Table 1) in quadruplicate. Each batch contained at least 1000 carp larvae. The first batch was fed continuously with microzooplankton consisting primarily of *Brachionus* and *Moina micrura* whereas, the second batch was kept starved throughout the period of rearing. The third batch was fed with microzooplankton initially for 4 days followed by starvation. The fourth batch, on the other hand, was starved

Table 1 : *Experimental conditions followed in the study.*

Conditions	Continuous fed	Initially starved	Continuous unfed then fed	Initially fed then starved
Water volume (*l*)	3	3	3	3
No. of hatchlings	1000	1000	1000	1000
Feeding with microzooplankton	All throughout	From day 5 after hatching	Nil	Initial 4 days of hatching
Fasting	Nil	Initial 4 days of hatching	All throughout	From day 5 after hatching.

for initial 4 days followed by feeding with microzooplankton.

All the fish larvae were reared in 31 glass jars containing ground water (temp : 28-30°C, pH : 7.4-7.6, DO : 7.5-8.5 mg/) and had arrangement for aeration and exchange of water. The day of first feeding was determined by daily examination of the gut contents of carp larvae under microscope.

Fertilized eggs and hatched larvae were collected, blotdried and weighed. At least four samples (40 mg) of fish material or live zooplankon were taken from each jar for the preparation of raw enzymatic solution. Then the tissues were homogenised and cryocentrifuged at 10,000 x g and the supernatant was taken for analysis.

Tryptic activity was determined following the method adopted by Dabrowski and Glogowski (1977) using casein as substrate. Mixture of 1 ml supernatant and 1 ml of 1 % casein in the buffer glycine NaOH with pH 9.5 was incubated at 37°C for 2 hours. Then 3 ml of 5% TCA was added and cryocentrifuged for 5 minutes at 4000 x g and the absorbance of the supernatant was measured spectrophotometrically (Shimadzu-UV-180) at 280 nm. The results were expressed as μg of liberated tryosine/mg of wet organisms per hour as total activity.

Amylase activity was estimated following Bernfeld (1955) using starch as substrate. Mixture of 1 ml of supernatant and 1 ml of 1% starch in phosphate buffer with pH 6.9 was incubated at 37°C for 24 hours. Then 2 ml of 3,5-dinitrosalicylic acid was added and was kept in a boiling water bath for 10 minutes and allowed to cool at room temperature and then measured spectrophotometrically at 540 nm. Results were expressed as μg of maltose liberated per mg of tissue per hour at 37°C.

The results were subjected to statistical analysis. Because of availability of data for four treatments employed, one way analysis of variance and Duncan's multiple range (DMR) test (Montgomery, 1984) were performed on day eight after hatching to test the significant differences of treatment means, if any. The level of statistical significance was accepted as being $p < 0.05$.

RESULTS

Tryptic activity : No tryptic activity (TA) was detected from the fertilized eggs. The TA was first detected from one day old hatchlings (0.24 ± 0.007 units/mg tissue). On day eight after hatching, the TA in the normal fed groups was significantly higher ($f3.44 - 11.969$, $P < 0.001$) than that of unfed groups. The rate of change over time in fed and unfed groups was opposite to each other (Table 2), the lowest activity in the former was displayed much later (between eleventh and twelth day) than the latter group (between

Table 2 : *Mean ±SD of trypsin and amylase activities (units mg 1/1) wet tissue during different days of ontogenic development of Catla catla larvae. Means in eighth row with different superscripts are significantly different (DMR test; $p < 0.05$)*

Days	Continuous fed	Initially starved then fed	Continuous unfed	Initially fed then starved
		Trypsin Activity		
1	0.021 ± 0.007	-	0.024 ± 0.007	-
2	0.097 ± 0.018	-	0.050 ± 0.001	-
3	0.174 ± 0.005	-	0.056 ± 0.005	-
4	0.225 ± 0.020	-	0.068 ± 0.005	-
5	0.300 ± 0.002	0.157 ± 0.018	0.086 ± 0.008	0.102 ± 0.004
6	0.387 ± 0.010	0.212 ± 0.010	0.102 ± 0.004	0.107 ± 0.001
7	0.434 ± 0.013	0.275 ± 0.020	0.142 ± 0.006	0.135 ± 0.012
8	0.456 ± 0.005[a]	0.325 ± 0.008[b]	0.174 ± 0.005[c]	0.180 ± 0.002[d]
9	0.512 ± 0.010	0.395 ± 0.004	-	0.190 ± 0.008
10	0.561 ± 0.005	0.408 ± 0.007-	-	-
11	0.607 ± 0.006	0.475 ± 0.020	-	-
12	0.615 ± 0.001			
		Amylase Activity		
1	0.025 ± 0.001	-	0.025 ± 0.001	-
2	0.052 ± 0.005	-	0.030 ± 0.004	-
3	0.090 ± 0.004	-	0.052 ± 0.004	-
4	0.136 ± 0.004	-	0.085 ± 0.001	-
5	0.168 ± 0.005	0.125 ± 0.005	0.101 ± 0.005	0.114 ± 0.005
6	0.202 ± 0.002	0.145 ± 0.002	0.125 ± 0.004	0.125 ± 0.004
7	0.245 ± 0.005	0.186 ± 0.010	0.136 ± 0.005	0.142 ± 0.001
8	0.262 ± 0.002[a]	0.234 ± 0.005[b]	0.167 ± 0.001[c]	0.167 ± 0.001[d]
9	0.292 ± 0.006	0.240 ± 0.001	-	0.168 ± 0.001
10	0.322 ± 0.006	0.256 ± 0.005	-	-
11	0.330 ± 0.001	0.262 ± 0.001	-	-
12	0.358 ± 0.009	-	-	-

second and third days). Fasting for initial four days followed by feeding or withdrawal of food after initial four days of feeding resulted in TA to be 82 to 107% higher and 60 to 72% lower than those of unfed and fed groups, respectively. The activities attributable to the effect of initial fasting but subsequent feeding amounted to 0.071 to o.151 units/mg tissue, whereas, it was 0.005 to 0.016 units /mg due to the effect of withdrawal of food after initial feeding.

The TA of the microzooplankton used as live food was almost consistent throughout the period of investigation, ranging from 0.50 and 0.55 units/mg tissue. The activity of plankton was 1 to 22 times higher than that of carp larvae until day 10 after which a reverse trend was noticed.

Amylase activity : The responses of amylase activity (AA) to feeding remained the same as that of trypsin. Being absent from the fertilized eggs, the amylase activity was first recorded from the one day hatchings (0.025 ± 0.001 units/mg tissue). Normal feeding induced the amylase activity by 56 to 80% ($F_{3,44} = 6.615$, $p < 0.05$) compared with unfed groups. When feeding was terminated after initial four days, there was reduction of AA by 47 to 73% than the normal fed group. Reintroduction of microzooplankton to initial 4 days unfed group resulted in 16 to 40 % increase to AA against continuously unfed group.

The AA from the samples of plankton showed a trend similar to that of trypsin. Initially, the AA of plankton (0.22 to 0.28 units/mg tissue) was 1 to 9 fold higher than carp larvae, but the trend reversed on and after day eight of hatching.

Ontogenic changes : The trend of ontogenic changes of enzymic activity was different between fed and unfed groups. The trends of changes of both TA and AA in fed groups are expressed in a second order equation (Fig 1). The TA in the unfed group as well as in the group initially fed followed by fasting demonstrated an exponential equation. The trends of TA observed in the batch initially starved followed by feeding and AA in the continuously unfed group were linear and expressed in the first order equation (Fig. 1).

Ontogenic patterns of enzymatic activities suggests that enzymic activities of unfed group tended to rise at a reduced rate with increasing age, whereas, in fed group the intensity of rise was very fast during early age, but relatively less at later ages resulting in almost linear rise.

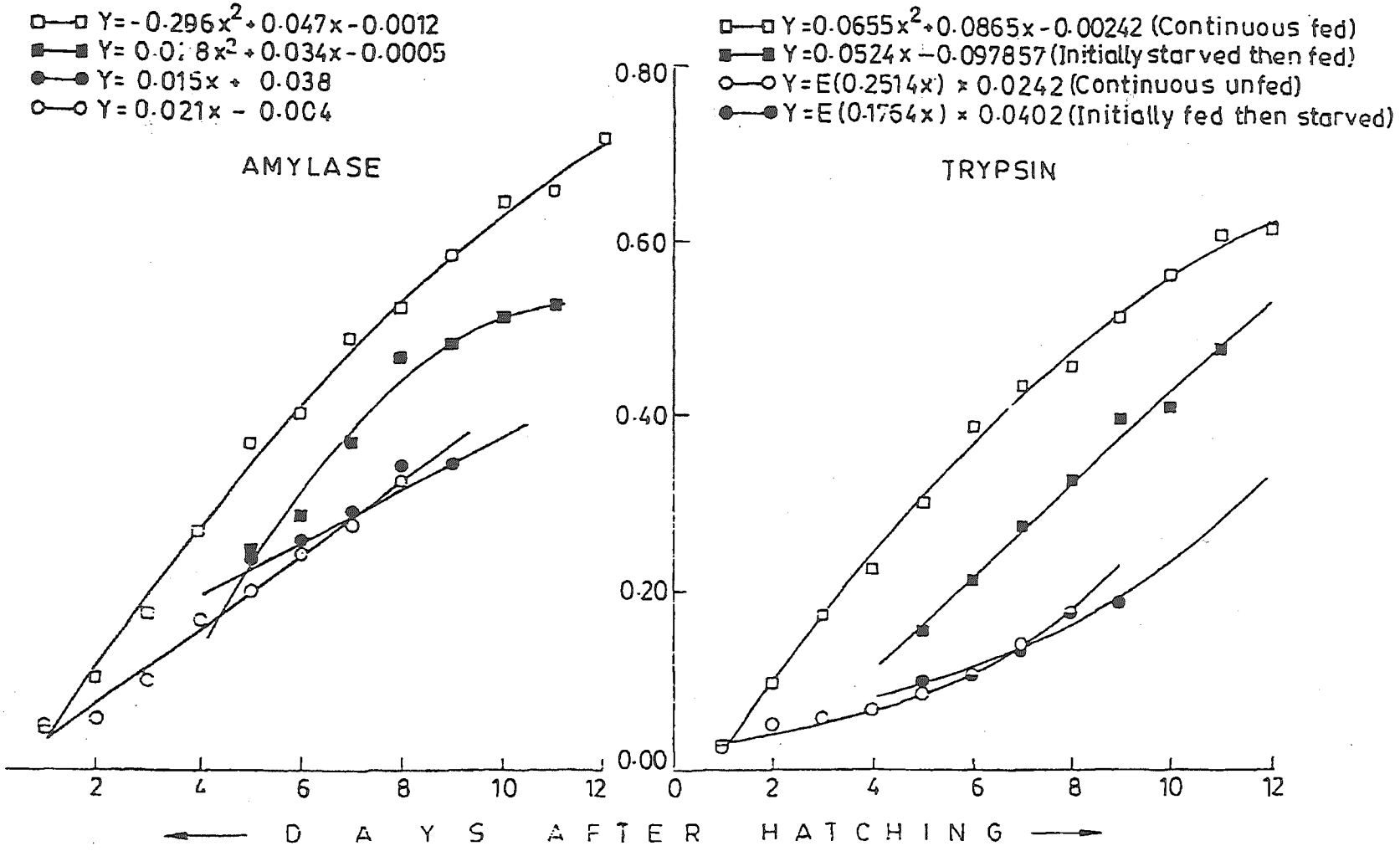

Fig. 1 : *Ontogenic changes of amylase and trypsin of Carp, Catla catla.*

DISCUSSION

Differences in the enzymatic activity over time between fed and unfed groups and consistently high enzyme activity in the former than in the latter, indicate that the digestive systems of test carp larvae were highly regulated by the zooplankton diet, and that the degree of decline in enzymic activities was more profound in absence of food than that of increase in presence of food. It is stated that protease activity in the intestinal contents was maximum 5 hours after feeding in common carp (Onishi *et al.* 1973, 1976), but 20 hours in rainbow trout (Falge and Spannohof, 1976). The specific activity of enzyme patterns differed greatly between rotifer-fed and unfed groups of 6 day larvae and 30 day old turbot juveniles (Munilla-Moran and Stark, 1989).

The shift in enzymic activities of carp larvae in response to zooplankton diet indicates that exogeneous feeding of zooplankton in carp larvae, similar to other species of fish could be an adaptation to compensate deficiency of endogenous enzymes which is either poorly developed or entirely absent prior to natural feeding and this strategy wanes gradually as the fishes grow old with adequate development of digestive system.

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