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Activities of digestive enzymes during ontogenic development of golden mahseer *(Tor putitora)* larvae

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ABSTRACT

Present investigation was undertaken to study the activities of digestive enzymes during ontogenic development of golden mahseer. Different age groups (0 DAH, (0 day after hatching), 3 DAH, 7 DAH, 15 DAH, 21 DAH, 30 DAH and 45 DAH) of mahseer larvae were fed macerated goat liver twice daily. Samples of mahseer larvae of all studied age groups were collected and analyzed for activities of digestive enzymes namely amylase, protease, trypsin, lipase and alkaline phosphatase. Activities of trypsin and lipase showed similar trend expect for the first 7 DAH. Lipase activity showed a linear increase up to 7 DAH, while trypsin activity showed no significant change during this time. Activities of both lipase and trypsin sharply decreased on 15 DAH followed by a significant abrupt increase to a maximum on 21 DAH. Protease activity in mahseer larvae was observed even on 0 DAH and this activity was consistent till 3 DAH and then increased followed by a decrease. Minimum amylase activity was on 0 and 3 DAH and maximum on 7 DAH. Minimum activity of alkaline phosphatase was on 0 DAH and activity remained lower till 7 DAH followed by a sharp increase till 30 DAH and then decreased on 45 DAH. Activities of different digestive enzymes indicated that mahseer larvae are able to digest protein, lipid and carbohydrates at an early stage. In this study, although a sharp increase occurred at the start of exogenous feeding, specific activity of most of digestive enzymes exhibited fluctuations.

Key words: Alkaline phosphatase, Digestive enzymes, Golden mahseer, Ontogeny

Analysis of digestive enzymes activities can be used as indicator of digestive processes and nutritional condition of fish larvae. A better knowledge of the physiology of nutrition during larval development is essential for the understanding of larval nutritional needs (Moyano *et al.* 2005). The ability of larvae to assimilate the required nutrients depends on the composition of the diet and on their capacity to modulate their digestive enzymes and metabolic processes (Zambonino Infante and Cahu 2001). The study of the ontogeny of digestive enzymes may constitute an appropriate parameter to determine the dietary needs of fish larvae.

The natural stocks of Golden mahseer, king of game fish and widely popular throughout the Indian subcontinent, showed drastic declines due to anthropogenic activities and is assessed as endangered in the IUCN Red List of Threatened Species (Basade and Mohan 2012). Therefore, it is need of the hour to conserve this species through artificial breeding, seed rearing and ranching into its natural habitats. But, the major bottleneck in successful seed rearing is unavailability of suitable larval diets. Hence, the present study was carried out to get an insight into the development of the larvae digestive functions to obtain essential data for the formulation of a compound larval diet.

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MATERIALS AND METHODS

Sample collection and tissue homogenate preparation: Samples of mahseer larvae of different age groups (treatments) viz. 0 day after hatching (0 DAH), 3 DAH, 7 DAH, 15 DAH, 21 DAH, 30 DAH and 45 DAH were collected randomly in triplicate from DCFR mahseer hatchery, Bhimtal. There were 15 larvae in each replicate with a total of 45 larvae per treatment/age group. The larvae were fed with macerated goat liver twice a day. Whole larvae (till age 21 DAH) and dissected larvae (30 and 45 DAH) were homogenized with 5% chilled 0.25 M sucrose solution using a mechanical tissue homogenizer. The homogenized samples were centrifuged (6000×g for 10 min, 4°C) and supernatants were collected and stored at –20°C (Prusty *et al.* 2007) for subsequent enzyme assays.

Analytical methods for enzyme assays: Lipase (E.C. 3.1.1.3) activity was determined based on Shihabi and Bishop (1971) and the activity was expressed as micromol

triglyceride bonds hydrolyzed/min/L at 37°C. Protease enzyme activity was determined by casein digestion method of Kunitz (1947) and the activity of protease was expressed as mole of tyrosine released/min/mg protein at 37°C. Amylase (E.C. 3.2.1.1) activity was assayed with 2% (w/v) starch solution as substrate (Rick and Stegbauer 1974) and expressed as mole of maltose released from starch/ min/mg protein at 37°C. The trypsin activity was determined by the method of Holm *et al.* (1988). The activity of trypsin was expressed as U/mg protein at 25°C. Alkaline phosphatase (ALP) (E.C. 3.1.3.1) activity was determined by Garen and Levinthal (1960). ALP activity was expressed as milli moles p-nitrophenol released/ min/mg protein at 37°C.

Statistical analysis: Mean values of all the enzymes were subjected to one-way ANOVA and Duncan's Multiple Range Tests was used to determine the significant differences among the means. Comparisons were made at 5% probability level. All the data were analyzed using statistical package SPSS (Version 19.0).

RESULTS AND DISCUSSION

The activity of lipase (Fig. 1) in the samples of mahseer larvae of different age groups, viz. 0 day after hatching (0 DAH), 3 DAH, 7 DAH, 15 DAH, 21 DAH, 30 DAH and 45 DAH was estimated. Lipase activity showed a rhythmic fluctuation during the course of larval development and the minimum activity was observed on 0 DAH. There was a significant ($P<0.05$) linear increase ($y = 12.15x - 4.95$, $R^2 = 0.98$) in its activity up to 7 DAH. The activity sharply decreased on 15 DAH followed by a significant ($P<0.05$) abrupt increase to a maximum on 21 DAH. Again, lipase activity sharply decreased to a minimum level on 30 DAH. Compared to 30 DAH, there was a slight increase in the activity of this enzyme on 45 DAH. Lipase catalyzes breakdown of triacylglycerol first to diacylglycerol and then to monoacylglycerol. It is produced mainly in pancreas and is thought to play a relatively minor role in lipid digestion in fishes. Results revealed that the lipase activity was present at the 0 DAH even though it was lowest during this time. The observed maximum lipase activity on 21 DAH is in agreement with Martý´nez *et al.* (1999) who observed increased lipase activity in Japanese flounder during 18–27 DAH, which may be due to the metabolism of lipid reserves. Similar rhythmic fluctuations in lipase activities were found in some species such as *Mystus nemurus* (Srichanun *et al.* 2012) larvae.

The protease activity (Fig. 1) in mahseer larvae was observed even on 0 DAH and this activity was consistent till 3 DAH. On 7 DAH, the protease activity increased by 10% and from 7 DAH onward, the activity was sharply decreased (2-fold) to a minimum on 30 DAH. Again, a sharp increase in the protease activity was evidenced on 45 DAH.

Similar pattern of marked reduction in specific activity of proteases was also found in stiped catfish, *Pangasianodon hypopthalamus* (Rangsin *et al.* 2012) and green catfish, *Mystus nemurus* (Srichanun *et al.* 2012).

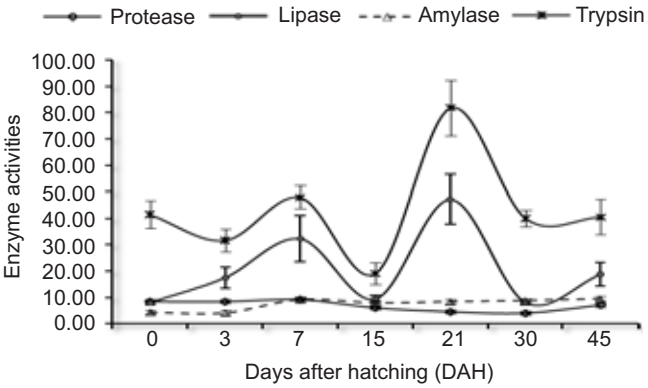

Fig. 1. Activities of digestive enzymes (lipase, protease, amylase and trypsin) during ontogenic development of mahseer larvae (values expressed as mean ± SE, n = 6).

The presence of trypsin activity in mahseer larvae (Fig. 1) was evidenced from 0 DAH and there was no significant difference ($P>0.05$) in trypsin activity till 7 DAH. Activity significantly ($P<0.05$) decreased and become minimum on 15 DAH and then a four-fold increment was observed on 21 DAH. Again there was 2-fold decrease in the trypsin activity on 30 DAH and remained similar till 45 DAH. Trypsin is an endoproteinase which hydrolyses peptide bonds at the carboxyl groups of L-arginine and L-lysine and is directly connected to the protein metabolism and is present in very young fish larvae. It is well known that the secretion of trypsin is known to occur in response to food ingestion and in larval pancreatic tissue most of the trypsin is present as an enzymatically inactive trypsinogen, whereas most of the trypsin in the intestinal tract is enzymatically active (Ueberschär 1995).

In current study, trypsin was firstly detected as early as hatching (0 DAH) and sharply increased from 15 DAH. For instance, this pattern was occurred in some sparids such as *Pagellus auriga* at 3 DAH (Moyano *et al.* 2005) and in green catfish, *Mystus nemurus* (Srichanun *et al.* 2012). In the present study, trypsin specific activity was relatively higher during early stages and then sharply and/or slightly decline was observed about after 20 DAH. Similar pattern about tryptic activity increase in early stages and then relatively reduction is reported in rock bream (He *et al.* 2012).

The minimum amylase activity (Fig. 1) in mahseer larvae was found on 0 DAH and 3 DAH. There was a sharp significant ($P<0.05$) increase in the activity of amylase up to 7 DAH and reached maximum. On 15 DAH, there was a slight decrease in the activity. From 15 DAH till 45 DAH, amylase activity increased linearly ($Y = 0.5053X + 7.475$, $R^2 = 0.9837$). It was documented that amylase is stimulated by glycolytic chains, glycogen, and starch in fish larvae (Pe´res *et al*. 1998). The observed pattern of variation of amylase specific activity in mahseer larvae in the present

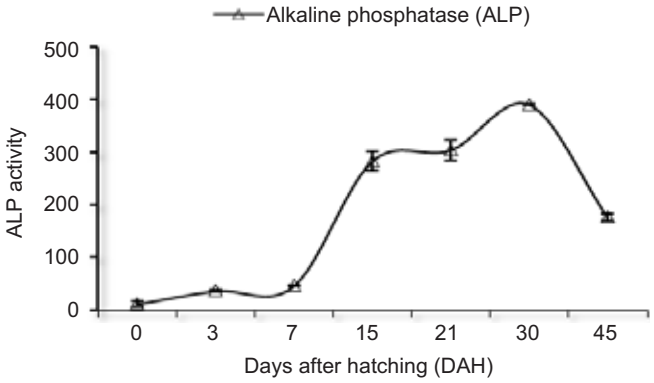

Fig. 2. Activity of alkaline phosphatase during ontogenic development of mahseer larvae. Values expressed as mean ± SE, n = 6.

study was identical to those observed in sturgen (Sanz *et al.* 2011) that is an increase after first feeding, followed by a decrease to a rather constant level. The decrease observed in the specific activity was mainly due to an increase in protein deposition (growth). It was proposed that specific activity of amylase is very high during young larval stages and this activity decreases during the development of larvae (He *et al.* 2012) which suggested that the decrease in amylase specific activity during larvae development may be genetically programmed.

The minimum activity of alkaline phosphatase (ALP) (Fig. 2) in mahseer larvae was found on 0 DAH and activity was remained lower till 7 DAH. A sharp (3-fold) increase in the activity of this enzyme was observed on 15 DAH and further gradually increased to a maximum on 30 DAH. A 2-fold decrease was evidenced on 45 DAH.

Intestinal ALP is produced in the golgi apparatus of the enterocytes (Tengjaroenkul *et al*. 2002), the enzyme then moves to the intestinal brush border which is its active site. Additionally, this enzyme is important in the absorptive process in fish larvae and is considered to be a general marker of nutrient absorption.

Its increased presence in the intestine of larval fishes should indicate more functionally developed enterocytes and maturation of intestinal digestive process. Mai *et al*. (2005) observed an abrupt increase in the activity of ALP in the larvae of yellow croaker, *Pseudosciaena crocea* from day 20 after hatching and in rock bream (He *et al*. 2012). In the present study, we also observed an abrupt increase in ALP activity on 15 DAH.

In summary, the activities of digestive enzymes indicated that mahseer larvae are able to digest protein, lipid and carbohydrate at an early stage. In this study, although a sharp increase occurred at the start of exogenous feeding, the specific activity of most digestive enzymes exhibited fluctuations. This variation in enzyme activities during the early stage may be due to the under developed digestive system. The specific activities gradually increased during the first 1 week of life and reached higher values during this time, and a decline occurred afterwards. This decrease in specific activities of different enzymes like lipase, protease and amylase on 15 DAH might be due to an increase of body protein (as specific activity is the ratio activity per mg protein) and does not reflect a lowering in digestive capacity. Our results revealed that micro-particulate diets could be formulated for rearing of golden mahseer larvae from 7 DAH onwards, more preferably, from 15 DAH taking into account the digestive capacity of larvae.

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