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Digestive Enzyme Complement of *Liza parsia* (Hamilton)

K PALANISAMY*
Central Marine Fisheries Research Institute
P.B. No. 2074, Cochin-682 031

ABSTRACT

An attempt was made to survey various digestive enzymes present in different regions of the degestive tract of the mullet, *Liza parsia*. The crude enzyme extracts from the digestive tract could hydrolyse a wide range of carbohydrate substrates. Amylase and maltase were the major carbohydrases detected throughout the alimentary canal with higher in pyloric caecae and intestine. Cellulase was not detected. Trace of acid protease activity was detected in the cardiac stomach extracts. High alkaline protease activity was observed in the phyloric caecae, followed by anterior intestine. Trypsin and chymotrypsin were the major endoproteases. Carboxypeptidases were detected in trace amounts; leucine aminopeptidase was the important exopeptidase. Considerable quantities of phosphatases and esterases were also detected Full complement of digestive enzymes present in the digestive tract enable mullets to utilize a wide variety of substances.

INTRODUCTION

The importance of a well balanced diet and adequate feeding in producing cultured fish are quite obvious. The production of nutritionally well balanced diets demands intensive research, quality control and biological evaluation. The nutritional value of a particular diet is ultimately determined by the ability of the animal to digest and absorb it. Digestion depends upon the physical state and type of the food and the quality and quantity of enzymes present in the digestive system. As food is not useful until it is absorbed and metabolised, the inclusion of a specific ingredient in a fish diet for purposes other than bulk is partially determined by the ability of the fish to digest it. The efficiency of fish to digest a particular component of diet can be ascertained by investigating the whole complement of digestive enzymes present in the digestive tract. Digestive enzymes of a number of cultured fishes have been investigated (Benitez and Tiro, 1982; Hofer, 1982; Uys and Hecht, 1987). Mullets have good potential for aquaculture (Oren, 1981). Attempts to culture them in cages and pens have been made in India (Prasadam and Kadir, 1988). For the development of an intensive culture technology, knowledge on nutrition, digestion and digestive enzymes are important prerequisites. In the present study, the whole complement of digestive enzymes in different regions of the digestive tract of *L.parsia* were investigated.

MATERIAL AND METHODS

Samples of *L. parsia* in a length range of 160-180 mm TL were collected from Chinese dipnet catches at Vypeen near Cochin and transported in live condition to the laboratory. They were maintained at room temperature ($30^{\circ}\pm2^{\circ}C$) in well aerated filtered sea water. After two days, the fishes were sacrificed, length and weight measured and the digestive tract dissected out carefully ensuring that the contents of different regions are not mixed up. The entire digestive tract was divided into distinct regions. The identical regions dissected out from 10 fishes of uniform size were pooled, homogenised in chilled double distilled water and centrifuged at 25,000 g for 20 min at $0\text{-}5^{\circ}$ C. The clear supernatant was used as crude enzyme extract. All the enzyme activities were assayed following standard methods (Palanisamy, 1989; Palanisamy and Pillai, 1989). The activity of carbohydrases is expressed as µg glucose/mg/hr and in the case of synthetic substrates as µg nitrophenol/mg/hr. The activities of pepsin and total protease are expressed as µ mole tyrosine/mg/hr, while trypsin, chymotrypsin and leucine aminopeptidase as nano moles p-nitroaniline/mg/min. Activities of acid and alkaline phosphatases are expressed as nano moles p-nitrophenol/mg/min. Esterase activity is expressed as µ alpha naphthol/mg/min under the assay conditions. The protein content of the crude enzyme extract was determined by the method of Lowry *et al.* (1951) using bovine serum albumin as the standard.

RESULTS AND DISCUSSION

The hydrolysis of various carbohydrate substrates by the crude enzyme extract from different regions of the digestive tract (Table 1) indicates the presence of enzymes capable of utilising a wide variety of carbohydrates. Majority of this hydrolytic activity centres around pyloric caecae and throughout the length of the intestine. The activity of the extract on substrates with beta-glucosidic linkages was not significant for any region of the digestive tract when compared with that of alpha-glucosidic linkages. The rapid hydrolysis of the substrates with alpha-glucosidic linkages such as maltose, dextrin, sucrose and trehalose suggests that in addition to a alpha-glucosidase, the specific

* Present Address: National Bank for Agriculture and Rural Development T.S.D., P.B.No. 6552, Worli, Bombay-400 018.

Table 1. Hydrolysis of carbohydrate substrates by crude enzyme extracts from different regions of the digestive tract of *L. parsia* a,b

Region / Substrate	Oesophagus	Cardiac stomach	Pyloric stomach	Pyloric caecae	Liver	Gall bladder	Spleen	Anterior intestine	Posterior intestine
Maltose	0	0	0	97 ± 0.5	0	69 ± 18	45 ± 12	154 ± 32	184 ± 34
Dextrin	92 ± 12	248 ± 39	89 ± 11	248 ± 39	0	346 ± 58	0	352 ± 56	368 ± 48
Sucrose	0	0	0	288 ± 54	0	155 ± 14	3 ± 0.31	443 ± 62	521 ± 83
Trehalose	0	31 ± 2	29 ± 3	130 ± 15	0	0	15 ± 1.4	135 ± 27	70 ± 12
PNP α-D glucoside	4.8 ± 0.49	7.08 ± 0.85	2.58 ± 0.23	119 ± 16	6.7 ± 0.61	0	0	30 ± 4	30 ± 4
Raffinose	0	0	0	3.25 ± 0.29	0	0	0	6.2 ± 1.5	9.3 ± 1.02
Cellobiose	36 ± 5	15 ± 2.17	29 ± 4	97 ± 14	0	0	0	129 ± 20	138 ± 19
Salicin	4.68 ± 0.37	15.54 ± 1.39	9.4 ± 0.84	57 ± 6	0	0	7.16 ± 0.85	34 ± 4	27 ± 3
PNP α-D glucoside	3.91±0.46	5.6 ± 0.51	2 ± 0.16	90 ± 13	0	0	0	43 ± 5	12 ± 1.52
PNP β-D galactoside	5.2±0.42	9.3 ± 0.84	2.94 ± 0.32	144 ± 18	12.74	0	4.18 ± 0.33	138 ± 56	64 ± 8
Soluble starch	112 ± 15	124 ± 18	0	228 ± 32	0	36 ± 3	94 ± 11	330 ± 39	437 ± 61
Glycogen	147 ± 20	155 ± 23	74 ± 6	232 ± 30	0	48 ± 4	76 ± 6	337 ± 54	348 ± 62

a Mean ± Standard Deviation

b For units of enzyme activity refer text

enzymes, maltase, dextrinase, sucrase and trehalase are also present in the phyloric caecae and intenstine of *L. parsia*. Since starch is the main storage product of plants, and maltose the breakdown product of starch by hydrolytic action of amylase, the presence of maltase in substantial amounts would ensure complete utilization of starch in *L. parsia*. The maximal sucrase activity recorded in the posterior intestine indicates that this enzyme might be secreted by the intestinal mucosa in mullets. Fange and Grove (1979) also suggested that sucrase is secreted by the intestinal mucosa in fishes. The absence of this enzyme was reported in carnivourous fishes by Kawai and Ikeda (1971). The high amylolytic activity observed in the present study suggests that starch has an important role in the natural diet of the species.

Lactose, melibiose and cellulose were not hydrolysed by any region. Although algae and diatoms form important components in the diet of *L. parsia,* cellulase activity which could hydrolyse the cell walls of algae and diatoms could not be detected in any region of the digestive tract. Most of the earlier studies reported that fishes such as *Tilapia mossambica* (Fish, 1960) and *Chanos chanos* (Chiu and Benitez, 1981) lack indigenous cellulase though algae and diatoms form exclusive or major component of the diet of these fishes. Lobel (1981) in his review of fish herbivory observed that fishes eat plant foods, but are not known to produce cellulase or any other enzyme to digest plant cell wall. The posterior part of the stomach which is transformed into a thick walled gizzard in mullets may act as grinding organ and aid in breaking down the cell wall of ingested plant material (Palanisamy, 1989). The tiny sand grains invariably ingested by mullets (Reddy and Shanbhogue, 1988) may also increase the milling efficiency. Further, the acid secreted by the gastric glands from the cardiac stomach region may also aid in the digestion of algal matter (Palanisamy, 1989). The observations made on the hydroytic activity of enzyme extracts on different carbohydrate substrates suggest that they can hydrolyse a broad range of low molecular weight substrates. Some of the hydrolytic activities recorded for a few substrates appear to have no relevant role in digestion. For example, sucrase activity was observed in most parts of the digestive system. But, it is unlikely that sucrose will ever be present in the natural diet of this species. It is likely that some of the enzyme activities observed are due to general glycosidases with a fairly broad specificity and ability to hydrolyse subsrates which are not usually met within the natural state. This could be of great importance in aquaculture where different ingredients are used in compounding the fish feed.

The present study indicates the presence of endo-proteases and exoproteases normally associated with protein digestion (Table 2). Peptic activity has been demonstrated in the stomach of many teleosts of commercial importance (Fange and Grove, 1979). It is interesting to note that the stomach of *L. parsia* displayed peptic activity. Which suggests that protein digestion is initiated in the stomach itself. Peptic activity has been detected in *Mugil auratus* and *M. capito* also (Albertini-Berhaut and Alliot, 1978). The combined action of acid and pepsin secreted into the stomach and the grinding action of gizzard should render the ingested diet in a commpletely accessible form to the subsequent pancreatic and intestinal enzymes which play a major role in the hydrolysis. Alkaline protease activity was highest in pyloric caecae extracts, followed by intestine. The major

Table 2. Protease, phosphatase and esterase activity of crude enzyme extracts from different regions of the digestive tract of *L. parsia* a,b

Enzyme \ Region	Oesophagus	Cardiac stomach	Pyloric stomach	Pyloric caecae	Liver	Anterior intestine	Posterior intestine
Pepsin	0	2.34 ± 0.28	0.98 ± 0.08	0	0	0	0
Total protease	1.01 ± 0.09	0	0	12.89 ± 1.19	0.42 ± 0.03	9.84 ± 1.08	8.95 ± 0.98
Trypsin	0	0	0	716 ± 85	0	467 ± 65	308 ± 37
Chymotrypsin	0	0	0	138 ± 19	0	98 ± 11	127 ± 19
Lencine amino peptidase	22 ± 2	36 ± 4	12 ± 1.8	212 ± 31	40 ± 5	380 ± 49	4 17 ± 66
Acid phosphatase	3.10 ± 0.38	12.4 ± 1.74	8.74 ± 1.04	10.38 ± 0.73	5.26 ± 0.63	4.49 ± 0.49	2.82 ± 0.25
Alkaline posphatase	67 ± 8	38 ± 5	33 ± 4	134 ± 18	40 ± 6	110 ± 13	128 ± 16
Esterase	3.93 ± 0.35	4.24 ± 0.5	2.48 ± 0.34	15.76 ± 2.36	21 ± 3	12.38 ± 1.98	13.46 ± 2.28

a Mean ± Standard deviation

b For units of enzyme activity refer text

endoproteases detected were trypsin and chymotrypsin which followed the same distribution pattern as that of total protease activity. Although the literature abounds with reports that specific proteolytic activity is proportional to the protein content of the diet, it being higher in carnivorous than in herbivorous species (Hofer, 1982), if the volume of the gut fluid, the number of gut filling per day and the relative length of the gut are also taken into account, besides specific activity, food is exposed to higher proteolytic activity in the digestive tracts of herbivorous fish than carnivorous fish (Hofer, 1982). Thus the extensive lenght of the gut (2.5 times the body length) in *L. parsia* might facilitate the exposure of diet for a longer duration to the digestive enzymes to effect complete hydrolysis. Elastase was not detected in *L. parsia* as in most fish species (Buddington and Doroshov, 1986). The exopeptidases assayed include carboxypeptidase A, B and leucine aminopeptidase. Caroboxypeptidase A and B were detected only in trace amounts. While endoproteases cause the initial hydrolysis of proteins to peptides, sequential cleavage of peptides is effected by exopeptidases. This is an important step as it ensures the availability of free amino acids which can be readily absorbed from the gut lumen. Contribution of acid and alkaline phosphatases to the digestion of food in fishes is not clearly understood. It is generally regarded that these enzymes are involved in nutrient absorptive processes. No lipase activity could be detected in the present study. Similary, lipase activity was not detected in some of the earlier studies also (Moriarty, 1973). However, a strong esterase activity was detected throughout the length of the digestive tract in *L. parsia* (Table 2). Since fatty acids are essential dietary components for fish, some form of lipase or esterase is essential to digest the fat component of the diet. Albertini-Berhuat (1988) detected intra-cellular lipid particles along the intestine in mullets, *Mugil cephalus* and *L. ramada*. This suggests that fat droplets can pass through the mucosa of the intestine and hence lipid digestion could be intracellular not requiring a soluble lipase in *L. parsia*. Thus, the full complement of digestive enzymes present may enable *L. parsia* to utilize efficiently a wide range of substances.

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