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Trypsin activity as a function of variation in shrimp *Penaeus indicus* (Crustacea/Arthropoda)

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The effect of varying ration size on trypsin activity was evaluated in postlarvae, juvenile and adult *P. indicus* maintained on a commercial pelleted feed. Total trypsin activity reported as μm p-nitroanilide produced/minute/g tissue was lower (4.23-6.68 μm) in postlarvae in comparison to juvenile (7.24-8.92 μm) and adult (10.23-12.24 μm) animals. Highest activity was detected at 12%, 8-12% and 4-6% in postlarvae, juveniles and adult *P. indicus* respectively which were the optimum ration sizes, while lowest activity was obtained in the starved animals. Specific activity however exhibited no significant variation with regard to ration size and starvation ($P > 0.05$).

Nutritional studies conducted with shrimp have been confined to emperically designed dietary trials, while investigations of the bioencrgetics and digestive physiology of the organisms have received less emphasis. Numerous investigations[1-3] have examined the affects of diet on the growth rate of shrimp. However, the nutritional suitability of a diet is questionable since many other environmental factors also influence growth[4]. Until recently most investigations concerning the digestive enzymes of shrimp have been qualitative and focussed on the comparative aspects of digestion. Since shrimps are now being evaluated for commercial culture, the changes in enzyme activities during the life cycle and adaptation to new diets are being examined quantitatively. The objective of the present study is to obtain information concerning the changes that occur in the activity of the proteolytic enzyme trypsin (EC 3.4.4.4) in response to stage of animal and amount of food fed (ration size) in *Penaeus indicus* (H. Milne Edwards) maintained on a compounded feed.

The compounded feed used was based on a Japanese shrimp feed formula comprising chiefly of soyabean flour, prawn meal and fish meal and was procured from Higashimaru Feeds (India) Ltd. The same feed base with slight modifications in proximate composition (Table 1) and form (crumbles, pellets 2.2 × 3 mm and pellets 2.2 × 8 mm) and available as starter, grower and finisher feed was used for feeding the postlarvae, juvenile and adult stages.

Four different ration sizes were selected for each stage based on earlier work[5,6]. The chosen ratio sizes (expressed as % body weight) were: 2, 12, 22 and 32% for postlarvae; 2, 8, 12 and 16% for juveniles and 1, 4, 6 and 8% for adults respectively. Feeding experiments were carried out using plastic tubs of 5, 15 and 25 liter capacity respectively for different stages. Individual aeration was provided and each tub was covered with nylon screens to prevent the escape of animals. The post larvae, juveniles and

Table 1—Percentage chemical composition of the three commercial feeds used for feeding postlarvae, juvenile and adult *P. indicus*

Constituent	Types of feeds		
	Starter (crumbles)	Grower (pellets 2.2 × 3 mm)	Finisher (pellets 2.2 × 8 mm)
Moisture	10.83	7.41	8.35
Dry matter (DM)	89.17	92.59	91.65
Crude protein[1]	36.09	34.45	29.53
Ether extract[1]	8.00	7.50	8.50
Crude fibre[1]	2.34	3.20	2.80
Nitrogen free extract[2]	30.12	33.85	39.66
Organic matter (OM)[3]	76.55	79.00	80.49
Ash[1]	12.62	13.59	11.16
Acid insoluble ash	9.88	12.69	10.95
Energy value[4] ($kJ.g^{-1}$)	20.85	20.48	20.47

[1]Calculated on DM basis.

[2]NFE calculated by difference = 100—(moisture) % + crude protein % + crude fat % + crude fibre % + ash %).

[3]OM = dry matter %—ash %.

[4]Energy values calculated as protein 23.4 $kJ.g^{-1}$; fat 39.8 $kJ.g^{-1}$ and carbohydrate 17.2 $kJ.g^{-1}$; fibre was assumed to have zero energetic value[2].

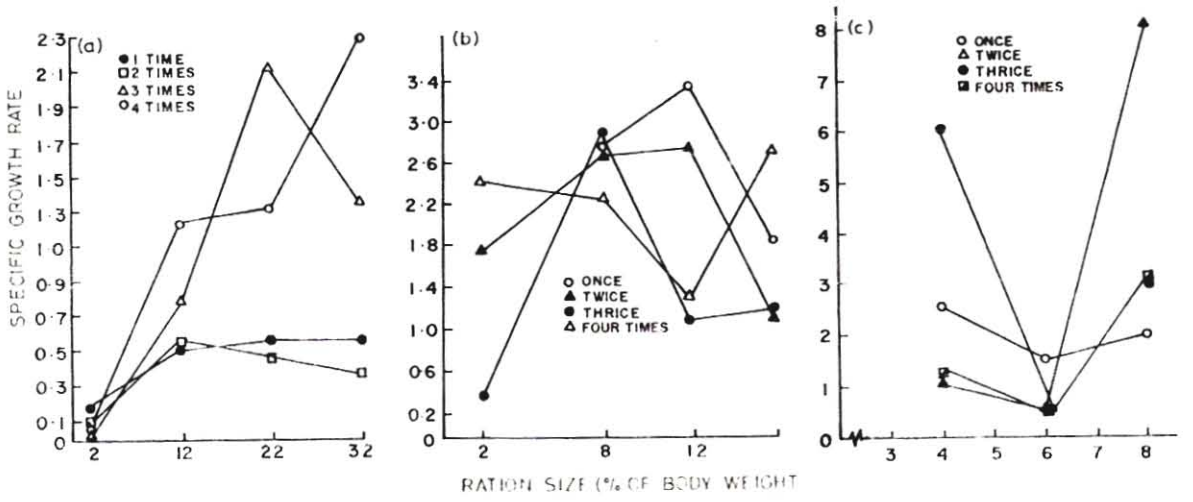

Fig. 1 Specific growth rates for (a) postlarvae, (b) juvenile, and (c) adult *P. indicus* obtained at the different ration sizes after feeding the commercial feed for 30 days

adult animals used for the experiment had an average initial length of 2.98 ± 0.21, 6.353 ± 0.39 and 9.34 ± 1.30 cm respectively and an average initial weight of 0.1313 ± 0.01, 1.49 ± 0.28 and 4.40 ± 1.13 g respectively. After acclimatization to laboratory conditions for a week the animals were segregated at 14 numbers per tub in case of postlarvae and 7 numbers per tub for juveniles and adults with two replicates being maintained for each ration size. Filtered seawater (No. 30 bolting silk) was used and the range of the hydrographic parameters maintained throughout the experimental duration was salinity 30, 20 and 20×10^{-3}; dissolved oxygen 4, 4 and 4 ml/l; *p*H 8.02, 8.05 and 8 and temperature 28.5°, 28° and 28.4°C for postlarvae, juvenile and adult *P. indicus* respectively. The experiment was terminated after 30 days, final lengths and weights recorded and specific growth rates were calculated (Fig. 1).

Upon termination of the growth experiments, digestive tracts of the shrimp from each treatment were assayed for trypsin activity. The digestive gland, stomach and mid gut of each animal was homogenized (n = 6 for postlarvae and juvenile and n = 5 for adults) in appropriate quantity of 0.05 *M* tris buffer (*p*H 7.8) containing 0.02 *M* $CaCl_2$, using a high speed tissue homogenizer. The resulting homogenate was centrifuged (14700 rpm at 0°-5°C) for 60 min and the supernatant was used for assay. Trypsin activity was assayed[7], using benzoyl-DL-arginine p-nitroanilide (BAPNA) as substrate. The total protein content of the extract was determined[8] and protein calculated from a standard curve prepared using Bovine serum albumin. Controls consisted of shrimp from the same initial population which had

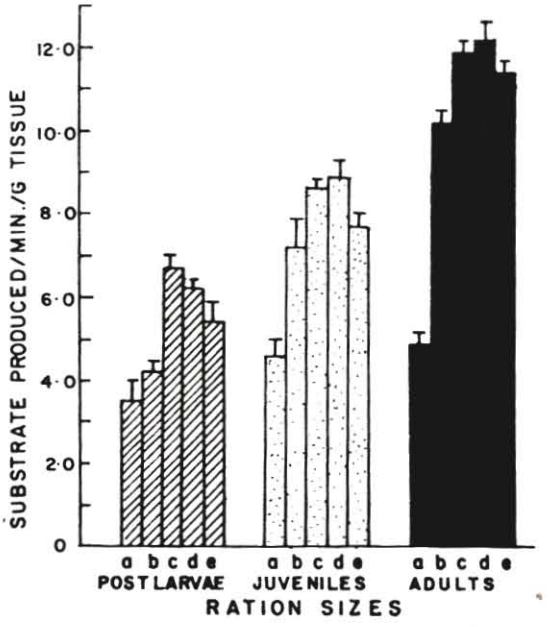

Fig. 2—Total trypsin activity (μm-p-nitroanilide produced/min/g wet tissue) ± SEM in post larvae, juvenile and adult *Penaeus indicus* as a function of ration size. (a = starvation; post larvae, b = 2%, c = 12%, d = 22%, e = 32%, juveniles b = 2%, c = 8%, d = 12%, e = 16%, adults b = 1%, c = 4%, d = 6% and e = 8% ration sizes)

been starved for 2 weeks, a period during which digestive enzyme activity reached a baseline value[9]. Enzyme activity is expressed as total activity (amount of product produced per minute per gram of wet tissue) and specific activity (amount of product produced per minute per mg protein).

Quantitatively the digestive tracts of postlarvae, juvenile and adult *P. indicus* displayed trypsin activity. An increase was observed in total trypsin activity with regard to both ration size and stage of

Table 2—Status of trypsin activity with regard to ration size in postlarvae, juvenile and adult *P. indicus*

Stage of animal	Ration size (% body weight)	Trypsin activity[1] (specific[2])
Postlarvae	Starved	0.068
	2	0.078
	12	0.063
	22	0.072
	32	0.059
Juveniles	Starved	0.110
	2	0.118
	8	0.110
	12	0.112
	16	0.120
Adults	Starved	0.121
	1	0.126
	4	0.109
	6	0.122
	8	0.113

[1]Activities reported as population mean (n = 6 for post larvae and juveniles and n = 5 for adults respectively

[2]Specific activity reported as μm p-nitroanilide produced per minute per mg protein

animal as higher enzyme activities were obtained in juveniles and adults than in postlarvae. Moreover, as the animal grew from postlarvae to adult there was a two fold increase in enzyme activity. However, no statistically significant correlation could be detected with regard to both ration size and stage of animals ($P > 0.05$).

Postlarvae fed at 12 and 22% of their body weight (optimum rations) exhibited higher total trypsin activities (activity expressed as μM p-nitroanilide produced/minute/gram of wet tissue) as also noticed at 8 and 12% for juveniles and 4 and 6% for the adult animals (Fig. 2). These rations were the optimum rations in all the cases. Total trypsin activity in the starved control shrimp was lower in comparison to experimental groups. However, this was not true in case of specific activity (trypsin activity expressed as μM p-nitroanilide produced/minute/mg protein), which was more or less similar in all the 3 stages viz. post larvae, juveniles and adults (Table 2), with regard to ration size and starvation. Trypsin activity of the clam controls could not be measured in all the 3 groups due to excessive mortality on account of cannibalism.

As the same feed was fed to 3 stages of animals no diet induced variation was expected. Little variation observed in the present study in the total trypsin activity amongst the 3 size groups shows that variation may be related to the size of the animal and also the amount of protein in the diet as ration sizes varied. Present observation is in agreement with earlier findings[10], where a differing proteolytic response to protein level and source as a function of size reflecting changes in digestive physiology as the shrimp grew was reported. Moreover, total enzyme activities reflected difference associated with protein levels but not the specific activities as also observed in this study.

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