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Investigations on the Digestive Enzyme Profile of Some Shrimp Feeds

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Abstract

Proteolytic, lipolytic and amylolytic activities were determined in four feeds *viz.*, clam meat (NF1) and shrimp meat (NF2) as natural feeds; *Moina* (LF1) and *Artemia* nauplii (LF2) as live feeds; a laboratory formulated (CF1) and a commercial brand (CF2) and two micro-particulate feeds (MP1 and MP2) as artificial feeds. High protease activity (10.70 units/min) was recorded in the feed CF2 followed by the micro-particulate feeds (9.35 units/min). High amylase activity of 3.72 units/min was recorded in clam meat followed by the agar-gelatin micro-particulate feed in which the activity corresponded to 2.2 units/min. The highest esterase activity of 2.37 units/min was obtained in the commercial (CF2) formulated feed. Activities were recorded in most feeds showing reactivation or incomplete denaturation in the formulated feeds.

Key words: Digestive enzyme, shrimp feeds

Introduction

With the rapid expansion in aquaculture current attempts in nutritional research are focused on the replacement of live feeds with artificial feeds. However, the nutritive value of a formulated feed is dependent not only on the composition but also on the bioavailability of its nutrients which in turn is influenced to a great extent by the digestive enzyme profile of the culture organisms.

The importance of digestive enzymes as an analytical tool in nutrition lies in their specific ability to hydrolyse individual materials in the diet, response to different nutrient sources and levels, bacterial contribution to digestion, cyclic secretion and changes in them with the growth and maturity of the animal. *In vitro* experiments with carp led to assume that exogenous enzymes present in food organisms may support the digestive processes in fish (Jancarik, 1964). In studies with fish, it has been shown that ingestion of exogenous food particles caused elevation of the digestive capacity and enzymes (Uys *et al.*, 1987). In shrimps, artificial diets fed alone generally promoted comparable survival but slower growth in comparison to larvae fed on live diets (Galgani and Aquacop, 1988; Kurmaly *et al.*, 1989; Kamarudhin, 1992). Apart from the few studies on the proteolytic activities of some live feeds and microalgae there is a dearth in literature on the enzyme content of the various feeds used in aquaculture. The present work was therefore designed to measure the enzyme contents of some live and artificial feeds used in shrimp culture in a set of two experiments comprising four feeds in each. The basic information generated would be an indicator for selection of suitable feeds for culture purposes.

Material and Methods

Experiments were conducted under laboratory conditions to assess the profile of a few digestive enzymes in various feeds. The four feeds selected were live feeds, natural feeds, artificial feeds and micro-particulate feeds.

Feed selection, procurement of feeds and ingredients

Eight feeds were selected for the study and four feeds under each of the categories mentioned below analyzed in a set of two separate experiments. These were two live feeds, *viz.*, *Moina* (LF1) and *Artemia* nauplii (LF2); two natural feeds-clam meat (NF1) and shrimp meat (NF2); two artificial feeds-one formulated in the laboratory (CF1) and the other a starter crumbled feed procured from a company of international repute (CF2); and two micro-particulated feeds (MP1 and MP2). The clam meat and shrimp meat used for feeding the post -larvae were purchased from the local market, brought to the laboratory, degutted, cleaned and stored at 4°C. Before feeding, the required quantity was thawed, chopped finely and fed to the post-larvae. The live feed *Moina* was obtained from the live feed culture collection maintained in the Institute. *Artemia* nauplii were cultured in the laboratory. Cysts obtained from the Fisheries harbour laboratory of the Institute were hydrated for 2 hours in sea water and then successsively

treated with NaOCl, sea water, 0.1 N HCl and 4% NaOH. After the treatments, cysts were washed thoroughly in running tap water and transferred to sea water. Vigorous aeration and light were provided. Hatching started within 24 hours with an efficiency of over 80%.

For the micro-bound and compounded feeds the raw materials were purchased from the local market. They were sorted off extraneous material, dried, powdered in a pulverizer and sieved through a sieve of 110 μ mesh size. Wheat flour and soybean flour were in the powdered form. The proximate composition of all the ingredients was determined prior to feed formulation (Table 1). For the

Table 1. Proximate composition of the feed ingredients (% DM basis)

Ingredient	Moisture	Dry matter	Crude protein	Ether extract	NFE*	Crude fiber	Ash
Fish meal	10.00	90.00	62.50	4.00	7.70	1.00	14.80
Shrimp meal	10.00	90.00	40.20	4.00	4.80	14.20	26.80
Clam meal	11.00	89.00	45.80	9.00	28.90	0.10	5.20
Groundnut oil cake	7.00	93.00	47.25	5.80	26.55	6.90	6.60
Soybean meal	10.00	89.20	42.90	5.25	29.15	5.90	6.00
Wheat flour	12.00	88.00	12.70	1.60	69.50	2.50	1.70

*Nitrogen Free Extractives calculated by difference (100 - % Crude protein + % Fat + % Crude fibre + % Ash + % Moisture)

artificial feed, all the feed ingredients and other additives *viz.*, oil, binder, vitamin premixes and cholesterol were weighed according to the formulation given in Table 2. The

Table 2. Percentage composition of the feed ingredients used in the formulation of the artificial feed (CF1)

Ingredients	% Incorporation
Fish meal	15.0
Shrimp meal	15.0
Clam meal	15.0
Groundnut oil cake	15.0
Soybean meal	12.0
Wheat flour	16.5
Gelatin	05.0
Vitamin/Mineral mixes*	02.0
Oil **	04.0
Cholesterol	0.5
Total	100.0

* Mineral mix U.S.P.XIV obtained from S.D. fine chemicals
Composition of Vitamin mix. (Glaxo India Pvt.Ltd)

Vitamin B1	-10 mg	Calcium pantothenate-	10 mg
Vitamin B2	-10 mg	Folic acid	-1500 mg
Vitamin B5	-3 mg	Vitamin B12	-15 mg
Vitamin B6	-100 mg	Vitamin C	-150 mg

**Oil: A combination of 1:1 cod liver and groundnut oil

dry ingredients were weighed and mixed thoroughly to accord uniform mixing. Oil was blended into the feed mix and after the addition of sufficient water the whole mixture was steamed for 10 min in a pressure cooker in order to gelatinize the starch. Vitamin and mineral premix were added to the dough after cooling, followed by pelleting through a meat mincer with a 1.0 mm die. The pellets were dried in an oven at 80±5°C to less than 10% moisture. Feed CF2, a commercial starter shrimp feed wasprocured from a company of international repute. It was in the form of 1.0 to 2.0 mm light brown crumbles and was directly used for analysis.

Formulation of the micro-particulated feeds

Carrageenan micro-bound diet (MP1): 10 g of diet ingredients were weighed and mixed well with 25 ml of distilled water. The mixture was then placed in a water bath at 80°C. Carrageenan (5 g for 100 g diet) was added slowly with constant mixing. Then potassium chloride was added (5 g for 100 g) slowly with constant mixing. The whole diet was cooled in a refrigerator for 30 min. The diet was freeze dried, made into powder and used for analysis.

Agar-gelatin micro-bound diet (MP2): To 10 g of diet ingredients and 7% water mix, 0.3 g agar and 1.2 g gelatin were added. The whole mixture was mixed well and heated over a water bath at 80°C for 5 min, cooled to room temperature and freeze dried. The dried diet was made into powder and sieved to adequate size. The particles were stored at 4°C.

Analytical methods

The proximate composition of the feeds and feed ingredients was determined using standard procedures (AOAC, 1990). Moisture was determined by drying in an oven at 80±2°C. While total nitrogen content was determined by the micro-kjeldahl method. Lipid was determined by Soxhlet extraction method. Crude fibre was determined by acid (H_2SO_4- 1.25 N) and alkali (NaOH- 1.25 N) digestion and washing with acetone. Ash content of samples was estimated by ignition in a muffle furnace at 600±5°C for 6 hours. Carbohydrate content determined as nitrogen free extractives (NFE) was obtained by difference.

Enzyme assays

Crude enzyme extracts were prepared at temperatures ranging between 4 to 8°C. The homogenized samples were centrifuged for 10 min at 12,000 rpm at 4°C and the clear supernatant decanted and stored in vials at 4°C. Assays for proteases, esterases and amylases were carried out within 12 hours and three assays for each sample were carried out and used for calculation of enzyme activity. Total alkaline protease activity was determined by the casein digestion method of Kunitz (1947). The absorbance values were converted into enzyme units by employing a standard curve made with tyrosine and specific activity was expressed as micro-moles of tyrosine liberated/mg protein/h under the stated assay conditions.

Esterase activity was measured by employing alpha-napthyl acetate as substrate according to Nachlas and Seligman (1949) with minor modifications. Enzyme activity was expressed as microgram alpha napthol liberated/mg protein/min under the specified assay conditions. Total amylase activity was measured as the reducing sugar released and measured as glucose employing the Nelson-Somogyi reducing sugar method with minor modifications (Nelson, 1944; Somogyi, 1952). Glucose was used as the standard and specific activity was expressed as the moles of glucose liberated/mg protein/min. Total protein concentration of the crude enzyme extract was determined by the method of Lowry *et al.* (1951).

Results

Experiment I

The four feeds used for this experiment were comprised of clam meat (NF1A&B), *Moina* (LF1A&B), formulated pelleted feed (CF1A&B) and a micro-particulated feed- carrageenan micro-bound (MP1A& B). The enzyme activities of the feeds are presented in Table 3.

protease activity of 3.217 units/min though its specific activity of 0.1072 can be compared well with the specific activity of 0.1063 in formulated feed. *Moina* recorded of protease activity of 1.703 units/min.

Highest amylase activity of 3.72 units/min were obtained in clam meat while other three feeds exhibited comparatively low amylase activities ranging from 0.818 units/min in *Moina* to 0.583 units/min in the case of the formulated feed (Table 3). The micro-particulate feed had 0.750 units/min of amylase activity. Accordingly, clam meat exhibited the highest specific activity of 0.124 followed by *Moina* (0.080) and the micro-particulate feed (0.013). The formulated feed recorded lowest specific activity of 0.006 for amylase.

Among the four feeds used in this experiment, highest esterase activity of 1.737 units/min was recorded in *Moina* which also recorded the highest specific activity of 0.1699. The micro-particulate feed and clam meat showed esterase activities of 0.917 units/min and 0.867 units/min respectively. The formulated feed recorded comparatively

Table 3. Enzyme activities of various live and artificial feeds used in the study (mean±SE)

Feeds	Proteases		Amylases		Esterases	
	Total activity[1]	Specific activity*	Total activity[2]	Specific activity*	Total activity[3]	Specific activity*
Clam meat (NF1 A&B)	3.217±1.224	0107±0.033	3.720±0.979	0.124±0.089	0.867±0.202	0.029±0.007
Shrimp meat (NF2 A&B)	2.772±1.129	0.0498±0.062	0.803±0.218	0.014±0.002	0.066±0.009	0.001
Moina (LF1 A&B)	1.703±0.732	0.1666±0.480	0.818±0.244	0.080±0.022	1.737±0.90	0.1699±0.09
Artemia naupli (LF2 A&B)	2.679±0.954	0.056±0.004	0.278±0.060	0.006±0.001	-	-
Formulated feed (CF1 A&B)	10.70±3.607	0.106±0.097	0.583±0.251	0.006±0.001	0.143±0.070	0.001
Commercial feed (CF2 A&B)	6.530±2.68	0.131±0.0886	1.020±0.917	0.020±0.003	2.37±0.900	0.047±0.012
Micro-particulate feed (MP1 A&B)	9.35±2.46	0.168±0.05	0.750±0.30	0.013±0.04	0.917±0.368	0.017±0.008
Micro-particulate feed (MP2 A&B)	4.285±3.68	0.085±0.033	2.200±1.108	0.086±0.026	1.933±1.004	0.076±0.002

1. activity expressed as mg tyrosine liberated/ml; 2. activity expressed as mg glucose liberated/ml; 3. activity expressed as mg α-naphthol liberated/ml
* Specific activity expressed as enzyme units/mg of protein/min

Highest protease activity of 10.70 units/min was present in the formulated feed, followed by 9.35 units/min in the micro-particulate feed. The micro-particulate feed recorded

low activity of 0.143 units/min with a specific activity of 0.001 (Table 4).

Table 4. Proximate composition of the various feeds (% DM basis) used in the study

Feeds	Designation	Moisture	Crude protein	Ether extract	Ash	Crude fibre	NFE*
Natural feeds	Clam meat (NF1)	11.00	42.00	9.5	5.64	0.10	31.76
	Shrimp meat (NF2)	11.00	40.2	4.00	26.80	14.2	4.8
Live feeds	Moina (LF1)	----	56.69	23.73	6.11	----	13.47
	Artemia nauplii (LF2)	---	55.56	15.2	15.25	0.92	13.03
Artificial feeds	Formulated feeds CF1)	6.27	52.5	9.00	13.3	1.31	17.62
	Commercial feeds (CF2)	4.03	45.5	6.5	12.5	0.58	30.89
Micro-particulate feeds	Micro-particulate feed (MP1)	16.1	45.5	10.5	8.7	0.179	19.19
	Micro-particulate feed (MP2)	9.15	47.00	5.9	13.93	0.152	23.86

* NFE: Nitrogen free extractives calculated by difference (100 - % Crude protein + % Fat + % Crude fibre + % Ash + % Moisture)

highest specific activity of 0.168 followed by 0.166 in the case of *Moina*. Clam meat exhibited comparatively low

The protein content of these four feeds ranged between 42.00% for clam meat to 56.69% in case of *Moina*.

Moina also recorded a high fat content of 23.73%, while the micro-particulate feed, clam meat and the formulated feed exhibited values ranging between 9.0 to 10.5% respectively. Clam meat recorded the highest carbohydrate content (determined as NFE) of 31.76%. *Moina*, formulated feed and micro-particulate feed reported comparatively lower values ranging from 13.47% for the former to 19.19% for the latter (Table 4).

Experiment II

The four feeds used for the second experiment were comprised of shrimp meat (NF2A&B), *Artemia* nauplii (LF2A&B), a commercial pelleted feed (CF2A&B) and a micro-particulated feed - agar gelatin bound (MP2A&B). The enzyme activities recorded in these feeds are presented in Table 3. The highest protease activity of 9.350 units/min was recorded in the micro-particulated feed followed by 6.350 units/min in the commercial feed. The specific activities recorded were 0.085 in case of the micro-particulate feed and 0.1306 in the case of the commercial feed. Similar protease activities of 2.679 and 2.772 units/min were exhibited by both *Artemia* nauplii and shrimp meat, respectively, and correspondingly the specific activities were 0.056 and 0.0498.

The micro-particulate feed also recorded the highest amylase activity of 2.20 units/ min followed by 1.020 units/min in the case of the commercial feed. Shrimp meat recorded 0.803 units/min of amylase activity as against 0.278 units/min in *Artemia* nauplii. Specific activity for amylase ranged between 0.014 to 0.086 for the micro-particulate feed, commercial feed and shrimp meat, while a very low value of 0.006 was obtained in the case of *Artemia* nauplii. An esterase activity of 2.37 units/min was recorded in the commercial feed with a specific activity of 0.047. The micro-particulate feed recorded 1.933 units/min of esterase activity with a specific activity of 0.076. Shrimp meat exhibited comparatively low esterase activity of 0.066 units/min with a concomitantly low specific activity of 0.001. No esterase activity was detected in *Artemia* nauplii.

Artemia nauplii recorded the highest protein content of 55.6% followed by 47.0% in the micro-particulate feed, 45.5% in the commercial feed and 40.2% in shrimp meat (Table 4). Highest fat content of 15% was recorded in *Artemia* nauplii, while the other values were 6.5% in commercial feed, 5.9% in micro-particulate feed and 4.0% in shrimp meat. The commercial feed had a NFE content of 30.89% followed by 23.86% in the case of the micro-particulate feed. *Artemia* nauplii had 13.03% carbohydrate while shrimp meat recorded the lowest value of 4.80% for the NFE content.

Discussion

Conventional feeding regimes of *Penaeus monod* during the nursery stages have so far comprised of combination of live feeds, preferably zooplankt comprised chiefly of *Brachionus plicatilis* and *Arte* nauplii (Watanabe *et al.*, 1983). Micro-particulate fe have been used with some success recently along w artificial feeds. Artificial feeds are very important dur the weaning phases on the context of availability and c Munilla-Moran *et al.* (1990) determined a range of d dietary enzyme activities in common live diets compri of rotifers, *Artemia* sp. and copepods, and reported lo enzyme levels expressed as units per individual in roti They reported that enzyme contents in *Artemia* depe on the nutritional state and developmental stage, while copepods showed very high enzyme levels. *Moina* sp. in the experiments of Lauff and Hofer (1984) distinguished by very high tryptic activities whereas lower activities were found in *Artemia* sp. o zooplankton from lakes. However, it can be concluded enzymic content of the feed may vary depending upon source of the live diet, even under identical ha conditions, the nutritional state of the live feed and (Marco *et al.*, 1980; Samain *et al.*, 1980, 1985; Watan *al.*, 1983; Munilla-Moran *et al.*, 1990).

In the present study, contrary to expectation live feeds *Moina* and *Artemia* nauplii as well artificial feeds clam meat and shrimp meat in spite o high protein contents, reported lower proteolytic act in comparison to the micro-particulate and artificial Surprisingly the formulated and micro-particulate recorded high proteolytic activities which co attributed to the induction of enzyme activities on of the mild processing conditions accorded for pro of these feeds. Only severe processing conditions form of heat treatments are expected to cause irre denaturation of enzyme proteins which was not the the formulated feed as well as the micro-particulate

Though not reflected in the case of protea correlation between nutritional composition of the enzyme activity was evident in the case of a activity wherein highest amylase activity of 3.72 was detected in clam meat which incidentally reco highest NFE content of 31.76% amongst all similar finding was observed in the case of est *Moina* recording the highest fat content of amongst the feeds analyzed in the present study high esterase activity of 1.737 units/min. N activity was recorded in *Artemia* nauplii.

Apart from the few investigations on content of live feeds there is a dearth in literature

studies on the enzyme content of various aquaculture feeds and hence it is not possible to compare the findings of our study. The present study has generated enormous information on the enzyme content of some natural, live and artificial feeds, *hitherto* not investigated. Further, elaboration of this work on the probable induction of endogenous enzyme activity in *P. monodon* by these exogenous enzymes present in various feeds has also brought forth conclusive evidence with regard to the benefit of these exogenous enzymes in digestion.

References

AOAC, 1995. Official Methods of Analysis, 12th edition. Association of Official Agricultural Chemists, Washington, DC, 1015 pp.

Galgani, M. L. and Aquacop, 1988. Replacement of live unicellular algae by microparticulate diet during larval rearing of zoeal stages of some penaeid prawns. *Aquaculture*, **69**: 115-127.

Jancarik, A., 1964. Die Verdauung der Hauptnährstoffe beim Karpfen. Zt. *Fisch. deren Hilfswiss.*, **12**: 601-684.

Kamarudin, M. S., 1992. Studies on the digestive physiology of crustacean larvae. Ph. D. Thesis. University of Wales, Bangor, United Kingdom.

Kunitz, M. 1947. Crystalline soybean trypsin inhibitor, general properties. *J. Gen. Physiol.*, **30**: 291-310.

Kurmaly, K., D. A. Jones, A. B. Yule and J. East, 1989. Comparative analysis of the growth and survival of *Penaeus monodon* larvae from protozoea 1 to post-larvae 1 on live feeds, artificial.feeds and on combination on both. *Aquaculture*, **81**: 27-35.

Lauff, M. and R. Hofer, 1984. Proteolytic enzymes in fish development and the importance of dietary enzymes. *Aquaculture*, **37**: 335-346.

Lowry, O. H., N. J. Rosebrough, A. L. Farr and R. J. Randall, 1951. Protein measurement with the Folin-Phenol reagent. *J. Biol. Chem.*, **193**: 265-271.

Marco, R., C. G. Vallejo, R. Garesse and R. Perona, 1980. A hypothesis on the activation process of the proteolytic activities during Artemia early development. *In*: The Brine Shrimp *Artemia* (Eds. G. Persoone, P. Sorgeloos, O. Roels and E. Jaspers), Vol. 2, Universa Press, Wetteren, pp. 315-323.

Munilla-Moran, R., J. R. Stark and A. Barbour, 1990. The role of exogenous enzymes in digestion in cultured turbot larvae (*Scophthalmus maximus* L.). *Aquaculture*, **88**: 337-350.

Nachlas, M. M. and G. Seligman, 1949. Evidence for the specificity of esterase and lipase by the use of three chromogenic substrates. *J. Biol. Chem.*, **181**: 345-355.

Nelson, N., 1944. A photometric adaptation of the Somogyi method for the determination of glucose. *J. Biol. Chem.*, **153**: 375-380.

Samain, J. F., J. Moal, J. Y. Daniel, J. R. LeCoz and M. Jezequel, 1980. The digestive enzymes amylase and trypsin during the development of Artemia:effect of food conditions. *In*: The Brine Shrimp *Artemia*, Vol.2 (Eds. G. Persoone, P. Sorgeloos, O. Roels and E. Jaspers), Universa Press, Wetteren, pp. 239-255.

Samain, J. F., A. Hernandorana, J. Moal, J. Y. Daniel and J. R. Le Coz, 1985. Amylase and trypsin activities during Artemia development on artificial ascemoc media effect of starvation and specific deletions. *J. Exp. Mar. Biol. Ecol.*, **86**: 225-270.

Somogyi, M., 1952. Notes on sugar determination. *J. Biol. Chem.*, **195**: 19-23.

Uys, W., T. Hecht and M. Walters, 1987. Changes in digestive enzyme activities of *Clarias gariepinus* (Pisces: Clariidae) after feeding. *Aquaculture*, **63**: 243-250.

Watanabe, T., C. Kitajima and S. Fujita, 1983. Nutritional values of live organisms used in Japan for mass propagation of fish-A review. *Aquaculture*, **34**: 115-143.