Asian Fisheries Science 5(1992):73-81.
Asian Fisheries Society, Manila, Philippines

https://doi.org/10.33997/j.afs.1992.5.1.006

Effects of Different *Artemia* Diets on the Growth and Digestive Enzyme Activities of Early Postlarval *Penaeus monodon*

HOUNG-YUNG CHEN and HSIAN-FU LIN

Institute of Marine Biology
National Sun Yat-sen University
Kaohsiung, 804 Taiwan

Abstract

The growth performance and digestive enzymic responses of early postlarval *Penaeus monodon* to four dietary groups including live, acid-treated and frozen *Artemia salina* nauplii and a compounded diet with a chemical composition similar to that of *Artemia* were studied. The growth of postlarvae fed live or acidified *Artemia* which had the highest protease activities was significantly greater than with the other two diet groups. Levels of dietary amylase activities did not cause differential growth results. The growth of shrimp could not be related directly to the digestive enzyme activities of the shrimp.

Introduction

There has been much effort to develop compounded larval or postlarval feeds comparable to live food organisms in both nutrition and availability. Live organisms, nonetheless, are still the food of choice in most hatcheries. One major nutritional difference between live food organisms and compounded feeds is the digestive enzymes in live food organisms. These enzymes of dietary origin (exogenous enzymes) play an important role in promoting the digestion and even growth of fish (Dabrowski 1979), especially in stomachless fish larvae or in larvae initially without stomachs (Dabrowski 1982). Jancarik (1964) found that the extract of food organisms activated digestive enzymes (endogenous enzymes) and thus supported the digestive process in fish. The contribition of exogenous enzymes in digestion was more significant in larval and juvenile fish than in adult fish (Dabrowski and Glogowski 1977a). The completely developed digestive system in adult fish may explain the age (size)-related difference.

Growth enhancement effects of exogenous enzymes in crustaceans have also been studied. Maugle et al. (1983) successfully enhanced the growth of 0.6-g *Penaeus japonicus* with diets supplemented with various levels of microencapsulated amylase and bovine trypsin. Dietary inclusions of *P. monodon* hepatopancreas acetone powder significantly promoted the growth of early postlarval (PL 7) *P. monodon* (Chen and Lin 1990). The growth and survival of 3-4-g *P. japonicus* given live short-necked clam, frozen clam or a compounded diet with a chemical composition similar to that of the clam (Maugle et al. 1982) were not significantly different. These past research results seemed to indicate a size (age)-dependent response for the growth enhancement effects of exogenous enzyme supplementation.

The present study investigated the growth performance and digestive enzymic responses of early postlarval *P. monodon* fed live, acid-treated or frozen *Artemia* nauplii or a compounded feed with a chemical composition similar to that of *Artemia* nauplii. Growth, survival and midgut gland protease and amylase activities of the postlarvae fed the various test diets were compared.

Materials and Methods

P. monodon postlarvae from a single spawner were artificially reproduced in a commercial hatchery in Kaohsiung, Taiwan. After being acclimatized in 34 ppt and 30°C seawater and being fed live *Artemia* nauplii for five days, the PL7 postlarvae attained an average body length of 8.94 mm and an average body weight of 0.38 mg. Eight-hundred postlarvae were randomly transferred to each of twelve 36-l rectangular aquaria. Three aquaria were randomly assigned to one dietary treatment. Water temperature was maintained at 30±0.5°C.

Four dietary treatments included: live *Artemia*, acidified *Artemia*, frozen *Artemia*, and a compounded diet called FFO. *Artemia* cysts used in the study were from a single batch (Ocean Star International Inc., Snowville, UT, USA). Live *Artemia* and acidified *Artemia* were prepared daily to ensure their nutritive value. Acidified *Artemia* were prepared by the method of Lauff and Hofer (1984) by incubating newly hatched *Artemia* nauplii in 0.1 N HCl for 5 minutes. The acid-treated nauplii were then neutralized by adding 0.1 N NaOH for another 5 minutes, then washed 5 minutes with water. Acid-denaturation causes an irreversible

inactivation of amylase in the *Artemia*, while freezing inactivates the proteolytic enzymes (Lauff and Hofer 1984). Frozen *Artemia* were prepared by freezing the *Artemia* nauplii in ice cubes with fresh seawater at -20°C. The nauplii were washed with fresh seawater before being frozen and after thawing. Sedimentation of the nauplii during feeding was reduced by circulating seawater with slight aeration.

The compounded diet FFO was formulated to have a similar chemical composition to that of *Artemia* nauplii and was regarded as a control diet. It was composed of 60% casein (vitamin-free, Sigma Chemical, St. Louis, MO, USA), 12% white fishmeal, 5% whole shrimp meal, 5% soybean meal, 12.2% fish oil (all obtained from Hanaqua Feed Co., Kaohsiung, Taiwan), 1.8% vitamin mixture (based on a formula designed by Kanazawa et al. 1977) and 0.5% choline chloride as well as 2.5% sodium alginate (HV) and 1% sodium hexametaphosphate as binder. The ingredients were compounded into pellets by the method of Meyer and Zein-Eldin (1972). After being freeze-dried, the pellets were hammer-crushed and particles between 0.177 and 0.250 mm were sieved then preserved at -20°C until feeding. The proximate compositions of *Artemia* nauplii and FFO are listed in Table 1.

Table 1. Proximate composition (per cent) of *Artemia* nauplii and the compounded diet FFO used in growth experiments.

	Artemia nauplii		FFO	
Composition	Wet weight	Dry weight	Wet weight	Dry weight
Moisture	87.40	0	7.62	0
Protein	8.47	67.22	60.44	65.42
Lipid	1.93	15.32	12.18	13.18
Carbohydrates	1.34	10.63	13.34	14.44
Ash	0.86	6.83	6.42	6.95

From the first feeding (PL7) until the age of PL15, the postlarvae were fed four times daily at 000, 900, 1300 and 1800 hours, respectively. They were fed three times daily at 100, 900 and 1700 hours between PL16 and PL26, when the trial ended. The shrimp were fed to satiation during each feeding. After the end of the 20-day (PL7-PL26) feeding trial, the body weights of the shrimp

were individually measured and the survivals recorded. The midgut glands of the shrimp were removed, pooled, weighed and were homogenized in 0.85% NaCl solution for 3 minutes and centrifuged at 4°C and 10,000 rpm for 30 minutes. The supernatants of the homogenates were used as the enzyme solution and were preserved in liquid nitrogen prior to analyses. The compounded diet FFO was also examined for residual enzyme activities. Procedures of enzyme extraction and quantification were carried out in a 4°C cold room.

Amylase and protease activities of *Artemia* nauplii, FFO and shrimp postlarvae were studied. Amylase activity was assayed using the 3,5-dinitrosalicylic acid method (Rick and Stegbauer 1974) on soluble starch substrates. The reaction mixture consisted of 1 ml substrate solution in 0.02 M phosphate buffer at pH 6.9 and 0.05 ml enzyme solution. Digestion was stopped at different intervals between 3 and 60 minutes with 2 ml of 1%, 3,5-dinitrosalicylic acid. The mixture was heated in a boiling water bath for 5 minutes and measured at 546 nm. For the evaluation of data, a maltose calibration curve was used.

Proteolytic activity was assayed using 1.25% casein substrate (pH 7.6). The hydrolysis of the substrate was monitored at 280 nm. A tyrosine calibration curve was used to evaluate the data. Total protein was determined according to the method of Lowry et al. (1951) using bovine serum protein for calibration.

Table 2. Total amylase and protease activities in *Artemia* nauplii and the compounded test diet FFO (n=3).

Diet	Amylase[a]	Protease[b]
Live *Artemia*	0.419	228
Acidified *Artemia*	0.000	301
Frozen *Artemia*	0.352	0
FFO*	24.552	12

*on dry weight basis
[a]mg maltose produced per minute per g wet tissue
[b]µg tyrosine produced per minute per g wet tissue

The protease and amylase activities of *Artemia* nauplii and FFO are listed in Table 2. No trace of amylase was detected in acidified *Artemia*. Frozen *Artemia* contained no protease activity. There were amylase and protease activities in the compounded feed FFO.

Mean final body weight, survival and midgut gland amylase and protease activities of the test postlarvae were analyzed for statistical significance ($P<0.05$) by analysis of variance. Individual differences between treatments were determined by Duncan's new multiple-range test.

Results

The growth of postlarvae fed live or acidified *Artemia* was significantly ($P<0.05$) greater than those fed with frozen *Artemia* or FFO (Table 3). Shrimp fed FFO attained a significantly higher survival than those fed with any of the three *Artemia* diets. When growth results were compared with the types and levels of digestive enzymes in the foods, no correlation could be found for amylase activities and growth. The postlarvae attained better growth when their diets contained higher protease activities (Table 2).

Table 3. Mean final weight and survival after 20 days of postlarval *Penaeus monodon* fed various test diets. Values represent means ± standard deviation.[1]

Diet	Mean body weight (mg)	Survival (%)
Live *Artemia*	22.67±1.53[a]	28.6±6.8[a]
Acidified *Artemia*	20.10±1.73[a]	25.7±2.0[a]
Frozen *Artemia*	15.03±2.65[b]	25.4±9.4[a]
FFO	9.33±1.53[c]	37.3±4.8[b]

[1]Means with different superscripts are statistically different ($P<0.05$; Duncan's new multiple range test).

The total and specific activities of the midgut gland protease and amylase of the postlarvae are displayed in Table 4. Total activities and specific activities of the two enzymes showed consistent patterns in response to the dietary treatments. Protease activities of postlarvae fed frozen *Artemia* were significantly ($P<0.05$) higher than those of the other dietary groups. Frozen *Artemia* contained no proteolytic enzyme activities (Table 2). The postlarvae given diets containing the highest protease activities (live or acidified *Artemia*) had the lowest protease activities in their midgut gland. Postlarvae fed frozen *Artemia* or FFO exhibited amylase activities significantly ($P<0.05$) higher than those fed live or acidified *Artemia* (Table 4). On dry weight basis, FFO had the highest amylase activities (Table 2). The amylase activities of frozen *Artemia* were lower than those of live *Artemia*.

There was no obvious correlation between enzymic response and growth performance of the postlarvae. The postlarvae fed live

Table 4. Midgut gland amylase and protease activities (means ± standard deviation) after 20 days of postlarval *Penaeus monodon* fed various diets.[1]

Diet	Protease[2]		Amylase[3]	
	Total activity	Specific activity	Total activity	Specific activity
Live *Artemia*	122±34[a]	53±14[a]	3.08±0.70[a]	1.46±0.25[a]
Acidified *Artemia*	151±46[a]	72±15[a]	4.13±1.26[a]	2.10±0.54[a]
Frozen *Artemia*	230±72[b]	124±48[b]	6.33±1.95[b]	3.48±1.64[b]
FFO	167±66[a]	55±27[a]	6.72±1.80[b]	2.90±0.59[b]

[1]Activities in the same column with different superscripts are statistically different ($P<0.05$, Duncan's new multiple range test).
[2]μg tyrosine produced per minute per g wet tissue (n=12).
[3]mg maltose produced per minute per g wet tissue (n=12).

or acidified *Artemia* exhibited the lowest amylase and protease activities and attained the greatest growth (Tables 3-4). The postlarvae fed frozen *Artemia* had the highest amylase and protease activities in their midgut gland, but their growth was inferior to the other two *Artemia* diet groups. Shrimp fed FFO had the second highest midgut gland enzyme activities, the highest survival and the least growth (Tables 3-4).

Discussion

Reduction or elimination of digestive enzyme activities significantly affected the nutritive value of *Artemia* nauplii as shrimp food. Exogenous digestive enzymes, thus, are essential in presenting *Artemia* as a nutritive food organism. This implies that inclusion of some digestive enzymes in compounded diets for shrimp, especially for larval shrimp, is beneficial. In an intensive aquaculture system where natural foods are not readily available, the use of enzyme-supplemented compounded diets becomes more critical. Either protease or amylase seemed to yield some effective results, but the effectiveness is not conclusive. The use of food organism substitutes such as algae substitute and artificial plankton is becoming more popular. Effective supplementation of exogenous enzymes in these microparticulated diets would greatly enhance their nutritive values. In a preliminary study, we found that the

activities of unprotected exogenous enzymes in test diets reduced greatly (up to 75% in the case of protease) within 20 minutes after administration. Protective measures, such as microcoating or microencapsulation, are needed to ensure effective supplementation of digestive enzymes.

The superiority of live food organisms in larval nutrition over existing compounded diets is partly derived from exogenous enzymes in the live organisms. Exogenous enzymes in diets join the endogenous enzymes of the animal in achieving the enzymatic processes. Dabrowski and Glogowski (1977b) investigated the proteases of 25 species of invertebrates which constituted a major portion of the diet in fish and found that enzyme characteristics of the invertebrate proteases such as activity and optimal pH value were similar to those of fish. This result was consistent with a previous finding that the extract of food organisms could activate digestive enzymes of fish (Jancarik 1964). Lauff and Hofer (1984) fed 8-day-old carp fry with live or acidified (to inhibit enzyme activity) *Moina* sp. and found that exogenous trypsin represented a high portion of the total tryptic activity. Young animals with less developed digestive systems benefit more from exogenous enzymes than do adults. Supplements of rotifer and *Artemia* in the larval feeding provide the source of exogenous enzymes.

Although exogenous enzymes have been found to support digestion, supplementation of enzymes in diets of aquatic animals, especially crustaceans, has met with mixed results. Studies using larval (Maugle et al. 1983) and early postlarval penaeid shrimp (Chen and Lin 1990) showed positive growth enhancements with exogenous enzyme supplementation. The growth and survival of 3-4 g *P. japonicus*, on the other hand, did not show any difference when fed live short-necked clam (*Venerupis philippinarum*), frozen clam or a compounded diet having similar chemical composition to that of the clam (Maugle et al. 1982). In the present study, shrimp fed live or acidified *Artemia* (no amylase activities) showed higher growth than those fed frozen *Artemia* (no protease activities) or the compounded diet FFO. Living *Artemia* did not result in significantly better growth than the nonliving acidified *Artemia*. The difference in amylase activities also did not yield differential growth. Significant differences in growth enhancement between the two nonliving *Artemia* diets might be caused by differences in the enzyme compositions or in nutritive values or both. Frozen

zooplankton were found to have a higher leaching rate of nitrogenous compounds such as free amino acids (Grabner et al. 1981) than the live ones. Leaching decreases nutritive values.

The relationship between digestive enzyme activity and growth performance of fish or shrimp is unclear. In the study with *P. japonicus* (Maugle et al. 1982), shrimp given live short-necked clam (high in protease activity) showed a higher protease activity than those fed frozen clam or a compounded diet (both were low in protease activity). The shrimp fed those three diets, however, did not differ in growth. Recent studies with early postlarval *P. monodon* (Chen and Lin 1990) and *Coregonus lavaretus* larvae (Segner et al. 1989) also indicated that growth cannot be related directly to digestive enzyme activities of shrimp or fish.

The lack of correlation between growth and digestive enzyme activities in the present study also suggests that growth was affected by dietary variables other than digestive enzyme activities. Although digestive enzyme analysis is a useful tool to assess the catalytic abilities of marine shrimp, digestive enzyme activities alone do not forecast the growth potential of the shrimp. Enzyme activities appear to respond to dietary differences in less time than is required to express those differences in growth rate (Lee et al. 1984).

References

Chen, H.Y. and H.F. Lin. 1990. The effects of exogenous enzymes on the growth of early postlarval *Penaeus monodon*, p. 349-352. *In* R. Hirano and I. Hanyu (eds.) The Second Asian Fisheries Forum. Asian Fisheries Society, Manila, Philippines.

Dabrowski, K. 1979. The role of proteolytic enzymes in fish digestion, p. 107-126. *In* E. Styczynska-Jurewicz, T. Backiel, E. Jaspers and G. Persoone (eds.) Cultivation of fish fry and its live food. Eur. Maricult. Soc. Spec. Publ. 4, Bredene.

Dabrowski, K. 1982. Proteolytic enzyme activity decline in starving fish alevins and larvae. Env. Biol. Fish. 7(1):73-76.

Dabrowski, K. and J. Glogowski. 1977a. Studies on the proteolytic enzymes of invertebrates constituting fish food. Hydrobiologia 52:171-174.

Dabrowski, K. and J. Glogowski. 1977b. Studies on the role of exogenous proteolytic enzymes in digestion processes in fish. Hydrobiologia 54:129-134.

Grabner, M., W. Wieser and R. Lackner. 1981. The suitability of frozen and freeze-dried zooplankton as food for fish larvae: A biochemical test program. Aquaculture 26:85-94.

Jancarik, A. 1964. Die verdauung der hauptnahrstoffe beim karpfen. Zeit. F. Fishere Hilfswiss 12:601-684.

Kanazawa, A., S. Teshima and S. Tokiwa. 1977. Nutritional requirements of prawn-VI. Effects of dietary lipids on growth. Bull. Japan. Soc. Sci. Fish. 43:849-856.

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

Lee, P.G., L.L. Smith and A.L. Lawrence. 1984. Digestive proteases of *Penaeus vannamei* Boone: relationship between enzyme activity, size and diet. Aquaculture 42:225-239.

Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall. 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem. 193:265-275.

Maugle, P.D., O. Deshimaru, T. Katayama and K.L. Simpson. 1982. Effect of short-necked clam diets on shrimp growth and digestive enzyme activities. Bull. Japan. Soc. Sci. Fish. 48:1758-1764.

Maugle, P.D., O. Deshimaru, T. Katayama, T. Nagatani and K.L. Simpson. 1983. Effect of microencapsulated amylase and bovine trysin dietary supplements on growth and metabolism of shrimp. Bull. Japan. Soc. Sci. Fish. 49:1421-1427.

Meyer, S.P. and Z.P. Zein-Eldin. 1972. Binders and pellet stability in development of crustacean diet. Proc. World Maricult. Soc. 3:351-364.

Rick, W. and H.P. Stegbauer. 1974. Alpha-amylase, p. 885-894. *In* H.V. Bergmyer (ed.) Methods of enzymatic analysis. Vol. 2. Academic Press, New York.

Segner, H., R. Rosch, H. Schmidt and K.J. von Poeppinghausen. 1989. Digestive enzymes in larval *Coregonus lavaretus* L. J. Fish Biol. 35:249-263.

Manuscript received 22 February 1991; accepted 4 October 1991.