Effects of Planktonic Food Organisms on Fatty Acid Composition of *Penaeus monodon* Fabricius Larvae

Piyapong CHOTIPUNTU

School of Agricultural Technology, Walailak University, Thasala, Nakhon Si Thammarat 80160, Thailand.

ABSTRACT

Penaeus monodon larvae at the first mysis stage were reared to the first post larval stage (PL-1) in 1-L lmhoff cones on three different diets: rotifers enriched with *Pavlova lutheri* (Rp), rotifers enriched with a commercial micro-encapsulated diet (Re), and a mixture of *P. lutheri* and *Artemia salina* nauplii (PA). The 14 essential fatty acids in the larvae that received the three different diets were measured and compared. Profiles of the fatty acids in the larvae and diets were also described.

Fatty acid composition of the larvae resembled that of the diets. The total fatty acid per dry weight of larvae was higher in larvae fed PA than that of larvae fed Rp or Re ($p<0.01$). The highest levels of the four major essential fatty acids – 18:2(n-6), 18:3(n-3), 20:5(n-3), and 22:6(n-3) – were found in the larvae that received Re, PA, and Rp respectively. There was no significant difference in survival rates (86.7 - 92.5%) of the larvae receiving different diets ($p>0.05$). The fatty acid composition of the larvae fed a mixture of Pavlova and Artemia resembled that found in Artemia but not in Pavlova.

Key words: *Penaeus monodon* – Larvae - Fatty acid

INTRODUCTION

Fatty acids are an essential energy source in shrimp diets (1). They enhance growth of the organisms and facilitate absorption of other fat-soluble nutrients such as sterols and vitamins. Polyunsaturated fatty acids (n-3 and n-6 PUFA) are the most important ingredient constituting the larval diets (2,3,4). The polyunsaturated fatty acids permit a great degree of unsaturation which is necessary to maintain flexibility and permeability of shrimp tissue (4). Since *Penaeus monodon* larvae do not exhibit *de novo* synthesis of linoleic acid [18:2(n-6)], linolenic acid [18:3(n-3)], eicosapentaenoic acid [20:5(n-3)], and docosahexaenoic acid [22:6(n-3)] (5), they require these fatty acids in a variety of forms depending upon their elongation-desaturation abilities (6). It is essential that the selected food organisms for shrimp include n-3 and n-6 fatty acids (7).

The most common live foods for penaeid shrimp larvae are microalgae, rotifers, and Artemia nauplii (8). Microalgae such as Chaetoceros, Skeletonema, and Tetraselmis are fed to zoea larvae – the first

feeding larvae. Rotifers are used as a transition food organism when the mysis larvae are transforming into the post larvae (9). Artemia nauplii are fed to the last mysis and post larvae. However, shrimp larvae are also reported to survive solely on microalgae (10,11). The quality of the live prey depends on their source and the diet they consume. Rotifers fed on some types of artificial diets such as cake baker's yeast are not suitable as larval food. It has been suggested that the quality of rotifers could be enhanced with diets rich in n-3 PUFA, such as some species of microalgae (12,13,14) or microparticulated diets rich in essential fatty acids prior to being fed to shrimp larvae (15).

This study investigated the fatty acid composition, growth and survival of the post larvae of tiger prawn (*Penaeus monodon*) fed microalgae – *Pavlova lutheri*, rotifers (*Brachionus plicatilis*) or nauplii of *Artemia salina*.

MATERIALS AND METHODS

Preparation of Food Organisms

Three different feeds were used as testing diets: rotifers (*Brachionus plicatilis*) enriched with prymnesiophyte algae (*Pavlova lutheri*), namely Rp; rotifers enriched with a commercial micro-encapsulated diet, namely Re; and a mixture of *P. lutheri* culture and the instar-I brine shrimp nauplii (*Artemia salina*), namely PA.

Rotifers were collected from a mass culture maintained by using baker's yeast and occasionally fed with some green algae, flagellates or diatoms. The prymnesiophyte algae (*P. lutheri*) was obtained from a 200-L continuous culture. The micro-encapsulated diet used was a commercial product named Frippak Booster, France. Nauplii of brine shrimp were obtained from cysts of Artemia from Great Salt Lake, USA.

Prior to being fed to the tiger prawn larvae, the rotifers were boosted with *P. lutheri* at a density of 5.9×10^6 cells/100 rotifers/ml (16) for 24 hours or with 1.0 g of the Frippak booster per million rotifers (twice the amount recommended by the manufacturer). The enriched rotifers were rinsed several times with clean sea water. Artemia nauplii were hatched from the partially decapsulated cysts and rinsed with clean sea water.

Experimental Organism

Nauplii of *Penaeus monodon* (Fabricius) were obtained from an eyestalk ablated female held in a closed culture tank. The nauplii were initially reared to the first mysis stage using *Isochrysis galbana* at a density of 5×10^4 cells/ml. Water temperature and salinity were kept at 28.1±0.6°C and 32.0±0.0 ppt. Prior to use, sea water was conditioned with Na-EDTA at a concentration of 10 ppm and filtered through a series of cartridge filters – 10, 5 and 1 μm.

Experimental Design

A total of 1,200 of the first mysis larvae were divided into 3 groups of 400 larvae – each group received one of the three different diets. Each diet was separated into 4 replicates of 100 larvae per replicate. The mysis were reared in the 1-L lmhoff cones at a stock density of 100 larvae/L and fed with the tested diets – Rp, Re or PA. Temperature of the rearing vessels was controlled by using a water bath unit (17). Water in the cones was gently aerated using the pasteur pipettes to prevent settling of the larvae and diet particles.

Water quality was monitored in terms of ammonia concentration, pH, temperature, and salinity as shown in **Table 1**. Total ammonia was determined by a HACH DR/2000 spectrophotometer using the Salicylate Method range of 0.00-0.05 mg NH_3-N/L. pH was measured using HI-8424 microcomputer pH meter (HANNA Instruments). Water temperature was measured using a calibrated N2-filled thermometer (-10 to 50°C), and salinity was measured using a calibrated optical salinometer (Shibuya' S-10).

Table 1. Salinity, temperature, pH and total ammonia concentrations in *Penaeus monodon* rearing vessels.

Diets	Salinity (ppt) n=5	Temperature (°C) n=20	pH n=4	Total NN_3 ($mgNH_3$-N/L) n=4
Rp	32 ± 0.0	29.6 ± 0.2	8.0 ± 0.1	0.23 ± 0.10
Re	32 ± 0.0	29.6 ± 0.2	8.0 ± 0.1	0.24 ± 0.10
PA	32 ± 0.0	29.6 ± 0.2	8.1 ± 0.1	0.02 ± 0.01

Feeding Protocol

The mysis were fed to satiation once a day with enriched rotifers or a mixture of Artemia and *P. lutheri*, at a density of 30 rotifers/ml (18,19), 3.0-5.5 Artemia/ml (3.0/ml at mysis-1, 4.0/ml at mysis-2 and 5.5/ml at mysis-3) and $1x10^5$ algal cells/ml (17) respectively. Water and the uneaten food in the cones were completely removed daily. Mysis were reared to the first post larval stage when the experiment was terminated. Growth and survival rates were measured. All post larvae were immediately frozen and preserved for fatty acid analyses.

Analyses of Fatty Acids

The post larvae and the tested diets were rinsed three times with 0.4 M ammonium formate solution to remove the excess NaCl (20). The clean samples were preserved at -70°C in glass tubes for further analysis.

Fatty acid composition was analyzed by a Hewlett-Packard 5890-A gas chromatography unit using a hydrogen carrying on-column injection system. FAME samples were injected at 45°C, then incubated for 2 minutes.

The temperature was then increased by 30°C /min up to 120°C, then by 3°C /min up to 260°C and maintained at that temperature until all peaks eluted. The column type was a BPX 70 high temperature polar column (fused silica 50 mm long, 0.32 mm internal diameter, film thickness of 0.25 µm: SGE, Australia). Identification and quantification of fatty acids were verified with a HP-1 non-polar column (Hewlett-Packard). Peaks were recorded on a Shimadzu C-R3A chromatopac recording integrator and also recorded on the computer (Microbits) using DAPA software. The peaks were further identified by comparing with the known FAME standards (Nu Chek Prep, Elysain Mn).

Data Analysis

The data expressed as a percentage were transformed using square root arcsine (21). Fatty acid composition, survival rates, and dry weight of larvae were analyzed using one-factor analysis of variance. When the significant differences occurred ($p<0.05$) the pairwise comparisons using a Fisher's LSD method were made.

RESULTS

Fatty Acid Composition

Fatty acid composition of the tested larvae varied among the diets, reflecting both content levels and composition of their diets **(Table 2).** Of the 14 major fatty acids measured in the larvae, all except for the 16:0 and 18:0, varied among the three different groups ($p<0.05$). Except for the 18:3(n-3) and 18:4(n-3), larvae that received two different rotifers (Rp and Re) differed in fatty acid contents. The dominant fatty acids found in the larvae were 16:0, 18:0, 20:5(n-3) and 22:6(n-3). Total fatty acid contents per dry weight of the larvae fed Artemia and Pavlova were much higher than those in the larvae fed enriched rotifers ($p<0.05$). The total fatty acid contents in the Artemia and Pavlova were also higher than those found in the enriched rotifers.

Larval Development and Survival Rate

Larvae survived and developed well in all tested diets **(Table 3)**. Growth in terms of total biomass as dry weight marginally differed among the tested diets. However, the differences were not significant ($P>0.05$). The survival rates and percentage of PL-1 at day-5 were not significantly different among the tested diets ($P>0.05$). The larvae survived well through the mysis stages (86.7-92.5%) and most of the larvae (90.0-91.1%) developed to PL- 1 within 5 days.

Table 2. Fatty acid composition (mean±SD, n=4) in *Penaeus monodon* (PL-1) and the tested diets, a, b and c followed each figure in the same row indicated significance of the differences among the diets

Fatty acids (%)	Larvae received			Diets			
	Rp	Re	PA	Rp	Re	P	A
14:0	1.3 ± 0.1 a	1.0 ± 0.1 b	0.4 ± 0.1 c	6.9	3.9	9.6	0.6
16:0	14.2 ± 0.4 a	14.4 ± 0.4 a	13.5 ± 1.3 a	10.1	9.7	12.7	9.6
16:1(n-7)	4.6 ± 0.2 a	3.9 ± 0.1 b	1.6 ± 0.1 c	19.4	17.3	17.8	2.1
18:0	11.9 ± 0.3 a	11.7 ± 0.1 a	11.9 ± 0.5 a	2.7	4.5	1.0	5.4
18:1(n-7)	7.4 ± 0.2 a	5.8 ± 0.1 b	6.3 ± 0.1 c	5.0	4.4	2.6	5.1
18:1(n-9)	5.1 ± 0.2 a	7.8 ± 0.3 b	11.5 ± 0.3 c	4.1	15.4	0.4	15.0
18:2(n-6)	3.4 ± 0.1 a	5.6 ± 0.3 b	3.5 ± 0.0 a	1.5	5.8	0.3	5.0
18:3(n-3)	0.9 ± 0.1 a	1.1 ± 0.2 a	14.7 ± 0.5 b	1.0	1.3	1.0	34.5
18:4(n-3)	0.8 ± 0.1 a	0.7 ± 0.2 a	1.7 ± 0.4 b	1.4	0.8	3.6	7.7
20:4(n-6)	3.7 ± 0.1 a	5.9 ± 0.3 b	2.0 ± 0.1 c	0.8	1.9	0.5	0.4
20:4(n-3)	1.2 ± 0.1 a	1.4 ± 0.1 b	1.0 ± 0.0 c	1.3	1.6	0.3	1.5
20:5(n-3)	18.7 ± 0.4 a	13.8 ± 0.5 b	11.5 ± 0.2 c	19.3	7.3	24.1	1.8
22:5(n-3)	2.8 ± 0.1 a	2.3 ± 0.1 b	0.1 ± 0.1 c	1.7	1.4	1.2	0.0
22:6(n-3)	9.9 ± 0.2 a	9.3 ± 0.2 b	6.8 ± 0.2 c	4.9	3.2	6.4	0.0
Others	14.2 ±1.4 a	15.1 ±1.3 a	13.1 ± 0.5 a	19.7	21.4	18.5	11.5
Total fatty acid mg/g.dry weight	19.0 ± 5.6 a	18.0 ± 1.0 a	30.9 ± 1.5 b	53.1	45.5	103.4	74.7

Table 3. Survival rates, percentage of the first post larvae and dry weight (mean ± SD, n=4) of *Penaeus monodon* larvae fed with three different diets.

Larvae received	Survival rate (%)	PL-1 (%)	Dry weight (mg/larva)
Rp	88.3 ± 6.3 a	90.4 ± 0.6 a	104.9 ± 31.9 a
Re	92.5 ± 2.4 a	91.1 ± 3.5 a	107.8 ± 4.0 a
PA	86.7 ± 6.4 a	90.0 ± 3.7 a	87.5 ± 8.6 a

DISCUSSION

Although the total fatty acids contents of Artemia and Pavlova were higher than those found in the enriched rotifers, this level did not enhance the growth and survival rates of *P. monodon* larvae. Kanazawa et al (22) found that the addition of n-3 did not increase survival rates of *P. japonicus* larvae when the diet contained adequate nutritional fatty acids. Fenucci et al (23) also found no correlation between weight gain in juvenile *P. stylirostris* and the percentage of fatty acids of n-3 series or the amount of 20:5(n-3) and 22:6(n-3). However, Volkman (14) pointed out that high weight gain in larvae was only achieved when significant amounts of 20:5 and 22:6 fatty acids were part of the diets.

This study suggested that fatty acids found in *P. monodon* larvae were likely to be obtained through the two following processes: accumulation and chain elongation. When the essential components of the diets were inadequate, larvae performed chain elongation-desaturation from the short chain components. Though the process was observed to be extremely inefficient in other larvae (5), this study showed that larvae of *P. monodon* exhibited chain elongation-desaturation of 20:4(n-6) from 18:2(n-6), and 20:5(n-3) and 22:6(n-3) from 18:3(n-3).

This study also showed that accumulation of fatty acids in *P. monodon* was limited to a certain level. Some fatty acids may accumulate in the larvae at levels much greater than those found in their diets. For example, larvae fed Rp contained twice as much 18:2(n-6) as Rp. In contrast, Rp contained 19.4% of 16:1 (n-7), whereas the larvae fed Rp contained only 4.6% of this fatty acid. This suggests that shrimp larvae do not accumulate fatty acids indefinitely, as there are probably other important fatty acids to be incorporated into their tissue. Jones et al (3) found that the larvae of *P. japonicus* incorporated only 6.4% of 18:2(n-6). Clarke and Wickins (24) also found 1.4 -10.6% of 18:2(n-6) in juvenile of Penaeus and Metapenaeus.

CONCLUSION

Rotifers enriched with *Pavlova lutheri* or a microparticulated diet rich in fatty acids were likely a better food organism for *P. monodon* larvae compared to the Artemia nauplius. Artemia was found to lack 22:5(n-3) and 22:6(n-3) and contained a very low content of 20:5(n-3) which are very important fatty acids for shrimp larvae development. However, larvae exhibited the ability to synthesize those fatty acids from 18:3(n-3) which is abundant in Artemia. Those processes caused excessive energy expenditures which retarded the growth of *P. monodon* larvae.

REFERENCES

1) Bottino NR Gennity J Lilly ML Simons E Finne G. Seasonal and nutritional effects on the fatty acids of three species of shrimp *Penaeus setiferus, Penaeus azatecus* and *Penaeus duorarum*. *Aquaculture 1980; 19: 139-48.*
2) Millamena MO Quinitio ET. Lipids and essential fatty acids in the nutrition of *Penaeus monodon* larvae. *In*: Taki Y Primavera JH Llobrera JA (eds). Proceedings of the First International Conference on the Culture of Penaeid Larvae/Shrimps. SEAFDEC Aquaculture Department, Philippines, 1985, p. 181.
3) Jones DA Kanazawa A Ono K. Studies on the nutritional requirements of the larval stages *Penaeus japonicus* using microencapsulated diets. *Marine Biol 1979; 54: 261-7.*
4) Bautista MN Parado-Estepa F Millamena OM Borlongan EL. Large scale hatchery production of *Penaeus monodon* using natural food and artificial diets. *The lsraeli of Aquaculture Bamidgeh 1991; 43 (4): 137-44.*
5) Kanazawa A. Nutrition of penaeid larvae and shrimps. *In*: Taki Y Primavera JH Llobrera JA (eds). Proceedings of the First International Conference on the Culture of Penaeid Larvae/Shrimps. SEAFDEC Aquaculture Department. Philippines, 1985, p. 123-30.
6) Castell JD Conklin DE Carigie JS Norman-Bourdeau K. Aquaculture nutrition. *In*: Bilio M Rosenthal H Sinderman CJ (eds). Realism in Aquaculture, Achievements, Constrains, Perspectives. Europe Aquaculture Society. Belgium, 1986, p. 251-308.
7) Catacutan MR Kanazawa A. Effect of the dietary fatty acids on the fatty acid composition of *Penaeus monodon* juveniles. *In*: Taki Y Primavera JH Llobrera JA (eds). Proceedings of the First International Conference on the Culture of Penaeid Larvae/Shrimps. SEAFDEC Aquaculture Department, Philippines, 1985. p. 181.
8) Leger P Sorgeloos P. Optimized feeding regimes in shrimp hatcheries. *In*: Fast AW Lester LJ (eds). Marine Shrimp Culture: Principles and Practices. Elsevier, Amsterdam, 1992, p. 225-44.
9) Scura ED Vunker MP. Recent advances in *Penaeus monodon* hatchery technology. *In*: Sloane P (ed). Larva Farming for Profit, Potential for Success. SCP Fisheries Consultants Aust, Greenwich NEW, 1987. p.1-9.
10) Sanchez MR. Rearing of mysis stages of *Penaeus vannamei* fed cultured algae of three species. *Aquaculture 1986 58: 139-44.*
11) Loya-Javellana GN. Ingestion saturation and growth responses of *Penaeus monodon* larvae to food density. *Aquaculture 1989; 81: 329-36.*
12) Brown MR Jeffrey SW Garlard CD. Nutritional aspect of microalgae used in mariculture, a Literature Review. Report 205, CSIRO Marine Laboratory, 1989, p.44.
13) Nichols PD Holdsworth DG Volkman JK Daintish M Allanson S. High incorporation of essential fatty acids by the rotifer *Brachionus plicatilis* fed on prymnesiophyte alga *Pavlova lutheri. Aust J Mar Freshwater Res 1989; 40: 645-55.*
14) Volkman JK. Fatty acid of microalgae used as feedstock's in aquaculture. *In*: Cambie R (ed). Fast for the Future, Ellis Horwood, Chichester, 1989, p. 263-83.

15) Ferreiro MJ Fernandez-Reiriz MJ Planas M Labarta U Garrido JL. Biochemical composition of enriched and starved rotifers. *In*: Lavens P Sorgeloos P Jaspers E Ollevier F (eds). Lavi'91, Fish and Crustacean Larviculture Symposium. Belgium, 1991, p. 36-8.
16) Daintith M. Live feeds for aquaculture. *In*: Multi-Skilling in Aquaculture, a Hands-on Training Workshop. National Key Centre for Teachings and Research in Aquaculture, University of Tasmania at Launceston, 1992, p.32.
17) Smith LL Samocha TM Biedenbach JM Lawrence AL. Use of one-liter lmhoff cones in larviculture production. *In*: Fast AW Lester LJ (eds). Marine Shrimp Culture: Principles and Practices. Elsevier, Amsterdam, 1992, p. 287-300.
18) Emmerson WD. Predation and energetics of *Penaeus indicus* (Decapoda: Penaeidae) larvae feeding on *Brachionus plicatilis* and *Artemia salina* nauplii. *Aquaculture 1842; 38: 201-9.*
19) Samocha TM Uziel N Browdy CL. The effect of feeding two prey organisms, nauplii of Artemia and rotifers, *Brachionus plicatilis* (Muller), upon survival and growth of larval marine shrimp *Penaeus semisulcatus* (De Haan). *Aquaculture 1989; 77: 11-9.*
20) Hodgson PA Henderson RJ Sargent JR Leftley JW. Patterns of variation in the lipid class and fatty acid composition of *Nannochloropsis oculata* (Eustigmatophyceae) during batch culture. *J Appl Phycol 1991; 3: 169-81.*
21) Zar JH. Biostatistical Analysis. 2nd ed. Prentice-Hall, New Jersy, 1984, p. 718.
22) Kanazawa A Teshima SL Sakamoto M. Effects of dietary lipids, fatty acids, and phospholipids on growth and survival of larvae *(Penaeus monodon). Aquaculture 1985; 50: 39-49.*
23) Fenucci JL Lawrence AL Zein-Eldin ZP. The effect of fatty acid and shrimp meal composition of prepared diets on growth. *J World Maricul Soc1 1981; 2(1): 315-24.*
24) Clarke A Wickins JF. Lipid content and composition of the cultured *Penaeus merguiensis* fed with animal food. *Aquaculture 1980; 20: 17-27.*

บทคัดย่อ

ปิยะพงค์ โชติพันธุ์[1]

ผลของแพลงค์ตอนอาหารต่อชนิดและปริมาณกรดไขมันในลูกกุ้งกุลาดำ (*Penaeus monodon* Fabricius)

ทำการเปรียบเทียบปริมาณกรดไขมันที่จำเป็นจำนวน 14 ชนิด ต่อการเจริญเติบโตของลูกกุ้งกุลาดำ (*Penaeus monodon*) ที่เลี้ยงด้วยอาหารต่างชนิด และเปรียบเทียบปริมาณของกรดไขมันที่พบในลูกกุ้งและในอาหารที่ใช้เลี้ยง ศึกษาโดยการเลี้ยงกุ้งกุลาดำจากระยะไมซิส 1 จนถึงระยะโพสลาวา 1 ในภาชนะรูปกรวยขนาด 1 ลิตร ให้อาหารมีชีวิต 3 ชนิดคือ โรติเฟอร์ที่เสริมคุณค่าทางอาหารด้วยการให้กินสาหร่ายเซลล์เดียวชนิด *Pavlova lutheri* ใช้ชื่อย่อว่า “อาหารชนิด Rp” โรติเฟอร์ที่เสริมคุณค่าทางอาหารด้วยการให้กินอาหารผง ใช้ชื่อย่อว่า “อาหารชนิด Re” และตัวอ่อนของไรน้ำเค็ม (*Artemia salina*) ผสมกับสาหร่าย Pavlova ใช้ชื่อย่อว่า “อาหารชนิด PA”

ชนิดของกรดไขมันที่พบในลูกกุ้งเหมือนกับที่พบในอาหารที่ใช้เลี้ยง ปริมาณกรดไขมัน-รวมต่อน้ำหนักแห้งในลูกกุ้งที่ได้รับอาหารชนิด PA มีมากกว่าในลูกกุ้งที่ไดรับอาหารชนิด Rp และ Re อย่างมีนัยสำคัญ ($p<0.01$) แต่ปริมาณกรดไขมัน 4 ชนิดที่จำเป็นต่อการเจริญเติบโตของลูกกุ้งมากที่สุดคือ 18:2(n-6), 18:3(n-3), 20:5(n-3) และ 22:6(n-3) พบมากที่สุดในลูกกุ้งที่เลี้ยงด้วยอาหารชนิด Re, PA และ Rp ตามลำดับ กรดไขมันส่วนใหญ่ที่พบในลูกกุ้งที่เลี้ยงด้วยอาหารชนิด PA จะมีปริมาณใกล้เคียงกับที่พบในตัวอ่อนไรน้ำเค็ม มากกว่าปริมาณกรดไขมันที่พบในสาหร่าย Pavlova อัตราการรอดของลูกกุ้งจากระยะไมซิส 1 ถึงระยะโพสลาวา 1 (86.7 – 92.5%) ที่เลี้ยงด้วยอาหารทั้งสามชนิดไม่มีความแตกต่างกันทางสถิติ ($p>0.05$)

[1] สำนักวิชาเทคโนโลยีการเกษตร มหาวิทยาลัยวลัยลักษณ์ อำเภอท่าศาลา จังหวัดนครศรีธรรมราช 80160