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SOME ASPECTS OF LARVAL NUTRITION OF THE FRESHWATER PRAWN, MACROBRACHIUM ROSENBERGII (DE MAN) WITH AN EMPHASIS ON LIPID METABOLISM

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BY

PAYMON ROUSTAIAN

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To my parents

for their true love, constant trust and principles that guide my life

To my wife

for her devotion, understanding and support during all difficulties

To my children

for making everything worthwhile

and

to many researchers whose works have not given due recognition for the many hours spent in the laboratory and fields to provide humanity with solutions to better life

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Changes in biochemical composition and fatty acid metabolism during larval development of the freshwater prawn, *Macrobrachium rosenbergii* (de Man) were studied to enhance the current understanding of the physiology and nutritional requirements of the growing larvae. Protein was always the major organic constituent followed by lipids and then carbohydrates. Protein concentration increased during development of both fed and starved larvae, suggesting its important role in morphogenesis. The decline of lipid during the larval development was more rapid for starved larvae which suggests a probable utilisation of lipid as metabolic source of energy. Low levels of carbohydrates in both fed and starved larvae may indicate their limited role in larval metabolism. The amino acid composition appeared to be relatively unchanged during the larval growth, suggesting that the amino acids requirement of the freshwater prawn is relatively constant during larval life. Unlike amino acids, fatty acids

revealed much wider range of variation during larval development. Although a decrease in total saturated fatty acid and monounsaturated fatty acids was observed, polyunsaturated fatty acids tended to increase during larval development. *M. rosenbergii* larvae appeared to have capability to sufficiently convert palmitic (16:0), linoleic (18:2*n*-6) and linolenic (18:3*n*-3) acids to stearic (18:0), arachidonic (20:4*n*-6) and ecosapentaenoic (20:5*n*-3) acids, respectively. Findings revealed that dietary lipid level of >15% (dry weight) has detrimental effects on the larval growth and survival. The best performance in terms of postlarval production, dry weight, protein and lipid depositions were observed for the diet containing 12% lipid. The lack of suitability of diets rich in saturated fatty acids for larval freshwater prawn was noted. Despite a wide variation in the dietary saturated to polyunsaturated fatty acid ratio, larvae revealed a narrow range indicative of the importance of such ratio in the larval metabolism. Moreover, regardless of the significant dietary variations in *n*-3 to *n*-6 ratios, the larval *n*-3 to *n*-6 ratios were remarkably similar, indicating the importance of such ratio in the larval fatty acid metabolism. The incorporation of linseed oil (as a source of linolenic (18:3*n*-3) acid) at 25 to 75 % of supplemented oil increased postlarval production, while larvae fed with linseed oil at 100% revealed significantly lower postlarval production. Although larval tissue level of arachidonic (20:4*n*-6) acid was not significantly affected by the dietary level of *n*-3 or *n*-6 fatty acids, biosynthesis of eicosapentaenoic (20:5*n*-3) acid is most likely affected.

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BEBERAPA ASPEK PEMAKANAN LARVA UDANG GALAH, *MACROBRACHIUM ROSENBERGII* (DE MAN) DENGAN PENEKANAN KE ATAS METABOLISME LIPID

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Perubahan dalam komposisi biokimia dan metabolisme asid lemak semasa perkembangan larva udang galah, *Macrobrachium rosenbergii* (de Man) telah dikaji untuk meningkatkan kefahaman terkini terhadap fisiologi dan keperluan pemakanan larva yang sedang membesar. Protein sentiasa menjadi komposisi organik yang utama diikuti dengan lipid dan karbohidrat. Kepekatan protein meningkat semasa perkembangan kedua-dua larva yang diberi makan dan yang dilaparkan. Ini mencadangkan peranan penting protien dalam morfogenesis. Pengurangan lipid semasa perkembangan larva yang lebih cepat untuk larva yang dilaparkan mencadangkan kemungkinan penggunaan lipid sebagai sumber tenaga. Takat karbohidrat yang rendah di dalam kedua-dua larva yang diberi makan dan yang dilaparkan mungkin menunjukkan peranannya yang terhad dalam metabolisme larva. Komposisi asid amino kelihatan secara relatifnya tidak berubah semasa pertumbuhan larva. Ini mencadangkan bahawa keperluan asid amino udang galah adalah secara relatifnya tetap di sepanjang

peringkat larva. Tidak seperti asid amino, asid lemak menunjukkan variasi yang besar semasa perkembangan larva. Walaupun terdapat pengurangan dalam jumlah asid lemak tepu dan asid lemak mono tak tepu, asid lemak poli tak tepu didapati meningkat semasa perkembangan larva. Larva *M. rosenbergii* kelihatan mempunyai kebolehan untuk menukarkan asid-asid palmetik (16:0), linoleik (18:2*n*-6) dan linolenik (18:3*n*-3), masing-masingnya, kepada asid-asid stearik (18:0), arakidonik (20:4*n*-6) and ekosapentaenoik (20:5*n*-3). Keputusan menunjukkan bahawa takat lipid diet melebihi 15% (berat kering) mempunyai kesan yang merencatkan pertumbuhan dan kemandirian larva. Keputusan yang terbaik dari segi pengeluaran pascalarva, berat kering, penghasilan protein dan lipid adalah didapati pada diet yang mengandungi 12% lipid. Secara amnya, didapati bahawa diet yang kaya dengan asid lemak tepu adalah kurang sesuai untuk larva udang galah. Di sebalik variasi yang besar dalam nisbah asid lemak tepu kepada poli tak tepu di dalam diet, tisu larva menunjukkan julat nisbah yang kecil yang mencadangkan kepentingan nisbah tersebut di dalam metabolisme larva. Walaupun terdapat nisbah-nisbah *n*-3 : *n*-6 dalam diet, nisbah-nisbah *n*-3 : *n*-6 dalam larva mempunyai persamaan yang menakjubkan. Ini menunjukkan kepentingan nisbah tersebut di dalam metabolisme asid lemak dalam larva. Penambahan minyak "linseed" (sebagai sumber asid linolenik, 18:3*n*-3) pada 25 hingga 75% minyak tambahan didapati meningkatkan pengeluaran pascalarva, sementara larva yang diberi 100% minyak "linseed" sebagai minyak tambahan menunjukkan pengurangan yang ketara dalam pengeluaran pascalarva. Walaupun paras asid arakidonik (20:4*n*-6) dalam tisu larva tidak terjejas dengan ketaranya dengan takat asid-asid lemak *n*-3 atau *n*-6 dalam diet, biosintesis asid eikosapentaenoik (20:5*n*-3) adalah yang paling berkemungkinan terjejas.

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A/E = Essential amino acid ratio

ANOVA = Analysis of variance

CGAF = Calories per gram ash-free dry weight

CLO = Cod liver oil

DW = Dry weight

EPL= Early postlarvae

FAME = Fatty acid methyl esters

HUFA = Highly unsaturated fatty acids

LL= Late larvae

LSD = Least significant difference

MDS = Mean developmental stage

MUFA = Monounsaturated fatty acids

PL = Postlarval

ppm = Part per million

ppt = Part per thousand

PUFA = Polyunsaturated fatty acids

sd = Standard deviation

SFA = Saturated fatty acids

CHAPTER I

INTRODUCTION

Background of the Study

As capture fishery approaches its maximum sustainable yield, the significance of aquaculture to provide nutritious food of high protein value is gaining more appreciation at global spectrum. Moreover, economic restriction such as increasing operational cost of fishing fleets as well as diminishing and unpredictable yields of oceans will most probably contribute to aquaculture growth and expansion in years to come.

Information compiled by tremendous research efforts and its application at various aspects of aquatic farming both in freshwater and marine environment have profoundly altered farming of aquatic organisms during the latter part of 20^{th} century. The extent of this advancement has been as such that nowadays it is referred to as the "blue revolution" (Lovell, 1989). Aquaculture is now recognized as a viable and a profitable practice that has attracted commercial investment at a large scale.

Farming of aquatic crustaceans has become increasingly significant due to their superior taste, high nutritious values and excellent market (New, 1995). The global production of cultured crustaceans in late 80's/early 1990's was approximately 982,000 metric tones annually, which contributed to about 20-25% of world crustacean supplies (Lee and Wickins, 1992; New, 1995). Out of this figure, shrimps

as referred to *Penaeus* and *Metapenaeus* spp. according to FAO terminology (Fast, 1992) provided 90% while prawns (*Macrobrachium* spp. according to FAO terminology) contributed 3.2%. Accordingly, crayfish and other crustaceans contributed to 6.8% of total farmed crustacean production (New, 1995).

Despite the apparent dominance of penaeid farming at global scope, an increasing trend in freshwater prawn production and its recent exports from some Southeast Asian countries have demonstrated an ample culture potential of this commodity. Farmed prawn production globally expanded by a factor of 3.5 in the seven years period from 1984 (~10500 metric tonnes) to 1991 (~ 37000 metric tonnes) (New, 1995). Prawn production in Malaysia also revealed an increasing trend from 68 metric tonnes in 1984, and 78.5 tonnes in 1995 to 95.6 tones in 1996 (Anon, 1985, 1996, 1998). Nearly all records of farmed prawn are attributed to *Macrobrachium rosenbergii* which is one of the largest species of the *Macrobrachium* genus (New, 1995). Although the genus possesses some other large species of economic value, culture of *M. rosenbergii* is favored due to its good survival and rapid growth (New, 1990), resistance to common disease problems (Johnson, 1982), wide range of salinity and temperature tolerance of postlarvae and juveniles (Smith *et al.*, 1982), ability to become mature, mate and spawn repeatedly in captivity (Wickins, 1976) and omnivorous feeding regime (Lee *et al.*, 1980; Weidenback, 1982). *M. rosenbergii* is also suitable to culture in inland freshwater bodies which may reduce pressure on limited coastal resources and increase income generating activities. These factors plus high market price at global scale (Table 1.1) render the *M. rosenbergii* culture a viable practice (New, 1995). However, large-scale production

Table 1.1: Examples of value of farmed freshwater prawns adapted from New (1995).

Location	Date	Price (US$ / kg)	Note
Brazil	1991	9-12	Whole; on ice, semi-wholesale to restaurants
	1993	5-12	Whole; domestic
France	1987	10	Imported from south-east Asia
		16	Imported from French Caribbean
	1989	20	Imported from French Caribbean
Martinique	1993	23-30	(20-30/kg) Whole; fresh or live
		19-23	(30-40/kg) Whole; fresh or live
Nepal	1994	6-7	Whole
Taiwan	1993	12.6	Domestic; Whole
United Kingdom	1994	18.2	Heads-off, shell on (supermarket)
USA	1994	22-27	Live (farm-gate)
Malaysia	1999	7-8	Whole; domestic (personal observation)

of *M. rosenbergii* has yet to be refined in terms of seed production and appropriate culture technology for rural farmers.

Statement of the problem

The high market value and the great demand of *M. rosenbergii* in Malaysia provide a strong stimulus for its culture. Although the larval rearing of *M. rosenbergii* was first reported in Malaysia, its culture industry has not developed as expected. Shortage of seeds, absence of a suitable feed, absence of an appropriate culture system and poor management are recognized as the major bottlenecks in development of culture industry in this country (Ang *et al.*, 1990).

To insure a stable local or international market, a reliable, constant and reasonable quantities of high quality cultured prawn is a prerequisite. This, in turn, is ultimately linked to status of juvenile production which is the corner stone in culture of any aquatic species. Freshwater prawn juveniles can either be obtained from wild or through hatcheries. Collection of juveniles from natural sources for stocking in ponds is faced with many problems such as difficulties in obtaining individuals of the same uniform sizes, limited and unreliable supply, laborious identification, difficulties and high cost associated with transport of juveniles from collection sites to growing farms (Bian and Pang, 1982; Suharto *et al.*, 1982). A well-operated prawn hatchery is thus the best alternative as the source of seed supply.

Hatchery larviculture and production of postlarvae of *M. rosenbergii*, in turn, are far from perfect and require much improvement. In a typical *M. rosenbergii* larviculture, mortality is caused either by poor water quality, problems at first

feeding, disease or any combination of such factors (New, 1982). These setbacks, in general, stem out from a prolonged larval life of *M. rosenbergii* (Wickin, 1976; New, 1990).

Among the problems associated with a long larviculture period as well as the expenses concerning physical maintenance and labor, the dependence of larvae upon *Artemia* nauplii as indispensable food (Murai and Andrews, 1978; Aniello and Singh, 1982; Lovett and Felder, 1988; Alam *et al.*, 1993a) is considered a major parameter that restricts the development of this industry in developing nations. Despite the ease of use and preparation as well as its nutritional qualities, the main disadvantage of *Artemia* based diet in larviculture of freshwater prawn is its high cost and occasional scarcity especially in developing countries. Cost of feeding may well reach to as high as 60% of the total production costs in *M. rosenbergii* hatcheries (Hagood and Willis, 1976). Variations in hatchability and nutritional value of *Artemia* from different geographic sources and even from one batch to another within the same strain, are also of great concern in the hatcheries operation (Daniels *et al.*, 1992; Sorgeloos and Leger, 1992). Another disadvantage of *Artemia* based diet is that brine shrimp exuvia and shed cyst capsules accumulates in larval culture vessels. Bacterial degradation of these materials fouls the water while accumulated debris entangles larvae and increases larval mortality (Lovett and Felder, 1988). Of parallel importance is the nutritional value of *Artemia* nauplii that apparently decreases rapidly after hatching due to reduced yolk material (Maddox and Manzi, 1976).

Substitution by any relevantly non-expensive live food could to some extent alleviate the problem. However, the number of undesirable side effects, such as man

power, required skill, nutritional variability and high risk of pathogenic infection associated with use of live food in general, are limiting parameters in application of such feed. The development and application of high quality artificial diet that can reduce the technical difficulties and operational costs associated with live feed preparation would be highly advantageous.

Problems associated with digestibility, acceptability, stability and nutritional status of the formulated diets may single-handedly or in combination contribute to lack of success in total replacement with artificial diet in caridean shrimps larval culture including *M. rosenbergii* (Jones *et al.* 1993; New, 1995). The most promising results for *M. rosenbergii* larvae were obtained by Deru (1990) who reared the larvae using Frippak microencapsulated diets exclusively from day 16 (larval stage of 6-7) to metamorphosis. However, high cost of this diet in developing countries would still prevent the growth of freshwater prawn aquaculture industry. An easily available, cheap, and nutritionally sound artificial diet is therefore desired to reduce the dependency of *Artemia* in the larval rearing of *M. rosenbergii* in developing nations. Understanding of biochemical and physiological processes pertaining to larval *M. rosenbergii* life cycle would provide valuable information in formulating such diet.

Significance of the study

As it was indicated earlier, the major challenge in the development of culture system for larval *M. rosenbergii* is to reduce larval dependency upon supplies of live food organisms which are both costly and nutritionally inconsistent. The development of high-quality artificial diet could reduce the high cost of live food as well as ameliorate water quality and disease problems (Bengtson, 1993). Knowledge