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DIET EVALUATION AND ESSENTIAL FATTY ACIDS INCORPORATION IN KURUMA PRAWN, *Penaeus japonicus*

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ABSTRAK

Penelitian ini bertujuan untuk mengetahui pengaruh komposisi asam lemak (fatty acid) dalam makanan terhadap pertumbuhan dan kelangsungan hidup post larva (PL) udang kuruma, *Penaeus japonicus*. Disamping itu, komposisi dan mekanisme transfer asam lemak diuji sesuai urutan rantai makanan: algae, *Artemia* dan udang. Hasil penelitian menunjukkan bahwa bobot badan (Body Weight=BW) tertinggi dicapai oleh udang yang mengkonsumsi *Artemia* pemakan algae, *Isochrysis galbana* (T.*Iso*) (P<0,05). Sedangkan untuk panjang total (Total Lenght=TL) tertinggi ditunjukkan oleh udang-udang yang mengkonsumsi *Artemia* pemakan algae, T.*Iso* dan yang mengkonsumsi *Artemia* pemakan algae, *Tetraselmis suecica*. Makanan alami *Artemia* lebih baik untuk pertumbuhan larva udang dibanding makanan buatan. *Artemia* dapat mentransfer zat-zat gizi khususnya asam lemak yang terdapat dalam algae ke udang. Dalam keadaan lapar, katabolisme asam lemak terjadi dalam tubuh udang, dan asam lemak ganda tak jenuh omega 3 lebih efektif untuk mendukung pertumbuhan udang dibanding omega 6. Keseimbangan komposisi antar berbagai asam lemak ganda tak jenuh kemungkinan sangat penting untuk pertumbuhan udang.

Kata kunci : Udang kuruma, algae, *Artemia*, asam lemak, pertumbuhan.

ABSTRACT

A 4-weeks experiment was undertaken to study effects of dietary fatty acid composition of post larval growth and survival of *Penaeus japonicus*. Fatty acid composition and transfer mechanism were also examined along the following food chain: algae – *Artemia* – prawn. Results indicated that the highest body weight gain was observed for the P. japonicus fed on *Artemia* consuming algae *Tetraselmis iso*, while the highest total length was indicated by the prawn fed on *Artemia* consuming both *T. iso* and *T. suecica*. It was also shown that the live feed was superior for the post larval growth of *P. japonicus* compared to commercial feed, and that the *Artemia* was good in transferring important nutrients from algae to the prawn. The body fatty acid was shown to be catabolized when the prawn was in starved condition. Polyunsaturated fatty acids (PUFAs) of the n3 series appeared to be more effective in supporting the post larval growth of *P. japonicus* which may indicate that the n3 series FA is more important than the n6 series.

Key words : Penaeus japonicus, algae, *Artemia*, fatty acids, growth

I. INTRODUCTION

The potential and economic importance of kuruma prawn, *Penaeus japonicus* has attracted interest of this species in aquaculture industry. This penaeid species has unique requirements for cholesterols and phospholipids with regard to the growth and development, and these are indicative of the importance of dietary lipid to penaeid growth and development (D'Abramo, 1997).

The nutritional value of dietary lipids is particularly determined by their fatty acid composition in relation to metabolic processes such as catabolism and storage of energy. It is well documented that most of marine animals require specific dietary **fatty acids (FAs)**, such as eicosapentaenoic acid (EPA, 20:5n3) and docosahexaenoic acid (DHA, 22:6n3) for good growth and survival during larval and juvenile stages. Unfortunetaly, the penaeid prawns have only a limited capability for *de novo* synthesis of certain **essential fatty acids**

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(EFAs) and so must obtain them from the diet to achieve good growth (Kanazawa, 1985; Merican and Shim, 1997). D'Souza and Loneragan (1999) concluded that the survival and development of prawn larvae was influenced by the distinctly different FAs profile in each species of algae used. Brown *et al.* (1989) indicated that FAs of most algae comprise up to 40 % of the total lipid (weight basis) and these FAs are suitable to meet prawn larvae lipid's requirements.

Following the earlier studies on *P. monodon* (Rosyida, 2002), in the present study, the growth and the survival rate of *P. japonicus* were also examined based on various diets *i.e* Tetraselmis suecica, *Isochrysis galbana* (the Tahitian strain, referred to as *T.Iso*), *Dunaliella tertiolecta,* Brine shrimp (*Artemia*) and a commercial diet. On the other hand, the capability of *Artemia* to transfer some valuable FAs from different species of microalgae into *P. japonicus* post larvae (PL) were assessed. A commercial prawn diet was used as a control and its FA composition was also assessed for comparison with the *Artemia* diets.

II. MATERIALS AND METHODS

2.1 Culture of algae and *Artemia*

The three species of algae used : *Dunaliella tertiolecta*, *Isochrysis galbana* (Tahitian clone) and *Tetraselmis suecica* were cultured under a semi continuous batch culturing system (as described in O'Melley and Daintith ,1993) in the 'Key Center', School of Aquaculture, Univ. of Tasmania.

Artemia used in these trials were AS (*Artemia* Systems, Belgium), INVE Aquaculture. The cysts were incubated in filtered seawater using conical containers at 25 – 26 ^{o}C under continuous aeration and light using the methods of Daintith (1996).

Freshly hatched *Artemia* nauplii (36 hours old) were raised on the 3 different species of algae for 6 or 8 days. Nauplii were suspended at a density of 10/ml in aerated tanks (100 L volume) and fed algae twice daily at a density of at least 1 x 10^5 cells/ml. After 6 or 8 days, the *Artemia* were harvested onto a 150 μm screen, thoroughly rinsed with sterile filtered seawater, and counted by microscopic examination using a Sedgwick Rafter slide and poured into ice cube trays before being frozen.

2.2 Experimental animals and design

P. japonicus PL used in this experiment were obtained from the Rocky Point hatchery, Woongoolba, Queensland. The mean initial weights (W) of individual larvae was 1.20 mg and the mean total length (TL) was 3.10 mm.

The experiments were conducted in a recirculating system for 4 weeks. Fifteen 52 L dark tanks were used, each with a flow rate of 300 ml/min. Aeration was provided by one airstone in each tank, and water quality was measured and checked every week. Water temperature was maintained at 26 to 27° C by a heater chiller unit (Acktron 7.9 kW) and salinity of 33±1 $^{0}/_{00}$ was measured and adjusted daily when saltwater was added in the sump.

Five experimental treatments run in triplicate : three *Artemia* diets raised on different alga species, one control treatment where prawns were fed commercial diet (Fry No.T_1 with a protein and fat content of 40 % and 4.5 %, respectively), and one treatment where the animals were unfed.

The prawns were stocked at 50 PL in each tank (1 PL/L), and were fed three times daily, at 0800, 1200 and 1700 h. *Artemia* were given in the amount of 100 *Artemia*/PL/feed, while the commercial diet was provided at 0.1 g/tank/feed. Uneaten diet from each tank was gently siphoned through a small airline fitted with a Pasteur pipette at the siphon end that contacted the water to ensure that no prawns were sucked out.

2.3 Sampling for fatty acid determinations

The experimental procedures showing the mechanisms of transferring diets to the prawns is presented in the following diagram.

As algae fed to *Artemia* everyday, the samples were taken for several times to estimate the average. Samples of algae were collected by filtering approximately 250 ml of each species onto Whatman glassfibre filters (47 mm) which had been precombusted for 4 hours @ 450°C. Samples and filters were then folded in half and placed into aluminium foil pockets. *Artemia* were sampled at each age fed to prawns (6 and 8 days old) and the necessary amount was placed into aluminium pockets. Seven to ten prawns from each treatment were randomly sampled at the beginning and at the end of each experiment and then placed into foil pockets. All samples were labelled and sealed in plastic bags filled with nitrogen gas, and stored in a - 80°C freezer until further analysis. Fatty acid methyl esters (FAMEs) were prepared from the samples by saponification, then followed by methylation as described in Whyte (1988). At the end, the extraction results stored in a freezer until further analysis.

2.4 Sampling for growth determinations

Thirty PLs were randomly selected from the stock population for initial weight (W) and total length (TL) determinations, while ten to twenty prawns were sampled from each treatment for the same measurements at the end of experiment. Total survival was also calculated at the end.

2.5 Fatty acid analysis and Statistical analysis

Analysis of fatty acids (FA) was undertaken at CSIRO-Marine Laboratories, Hobart, Tasmania. Gas chromatograph (GC) analysis of FAMEs were performed and details of this GC are provided by Nichols *et al.* (1989).

The effect of diet on growth and survival were determined by one way analysis of variance (ANOVA) using SPSS version 9.0. Post hoc comparisons were undertaken with Tukey HSD, and the results were considered statistically significant at the $P \leq 0.05$ level. Fatty acids of phytoplankton, *Artemia* and prawns were analysed statistically using ANOVA and followed with a Kruskal Wallace ANOVA on ranks.

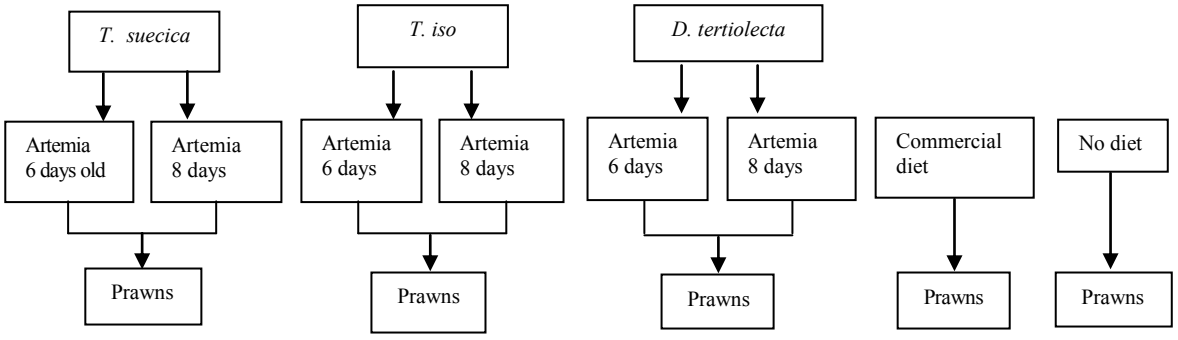

Figure 1. The Diagram of Experimental Procedures Showing The Mechanisms of Transferring Diets to The Prawns, *P. japonicus*.

III. RESULTS AND DISCUSSION

3.1 Growth and survival

There was no significant difference in the survival of amongst Kuruma prawn fed different diets (P>0.05) (Fig. 2a). Weight gain was greatest when the prawns were fed on *Artemia* raised on *T. iso* (P<0.05) (Fig. 2b). On the other hand, there was no significant difference amongst prawns fed on the other 3 diets (P>0.05). When the prawns were left unfed, however, they grew significantly slower compared to any other treatments (P< 0.05).

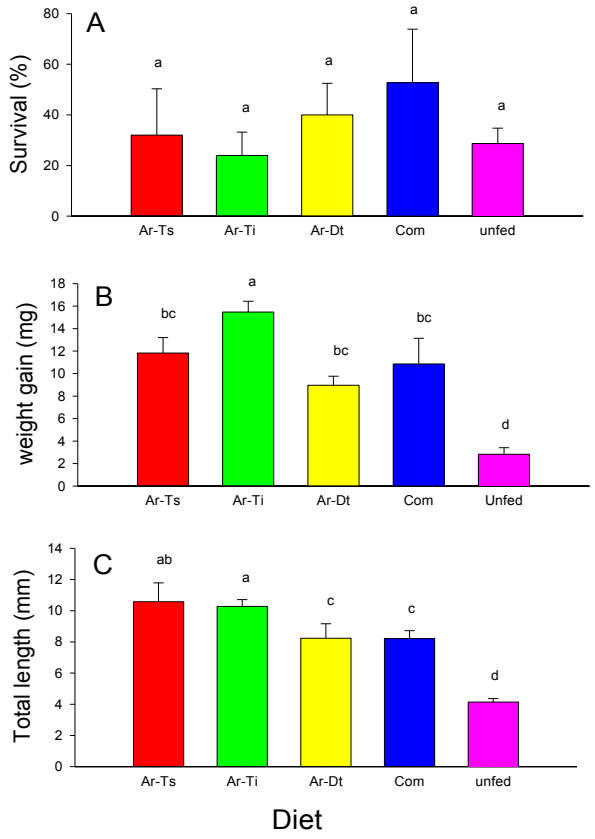

Figure 2. Survival (A), Weight gain (B) and Total Length (C) of *P.japonicus* fed on various diets: Ar-Ts = *Artemia* raised on *T.suecica*, Ar-Ti = *Artemia* raised on T.Iso, Ar-Dt = *Artemia* raised on *D.tertiolecta* and com=commercial diet. Vertical bars represent standard deviation. Values that share the same superscript are not significant different.

The prawns fed on *Artemia* raised on *T. suecica* and *T. Iso* obtained the longest tot 309 length (Fig. 2c), which increased almost threefold over the experimental period. The total length between prawns fed on *Artemia* raised on *D. tertiolecta* and commercial diet were similar (P>0.05), while unfed prawns increased only 1 mm of the total length which was significantly less compared to all fed prawn treatments (P<0.05).

3.2 Fatty Acid Composition of Algae, *Artemia*, commercial diet and prawn, *P. japonicus*

FA composition of algae, *Artemia,* commercial diet and prawn, *P.japonocus* used in this trial varied considerably as shown in Fig.3 dan Fig.4.

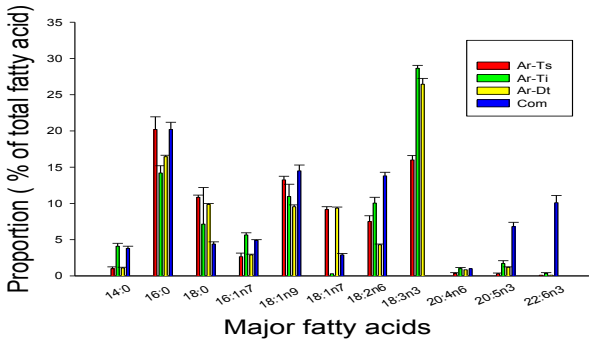

Figure 3. The proportion of major Fatty Acids in *P.japonicus* diets. Ar-Ts=*Artemia* raised on *T.suecica*, Ar-Ti=*Artemia* raised on *T.Iso*, Ar-Dt=*Artemia* raised on *D.tertiolecta* and com= commercial diet.Vertical bars

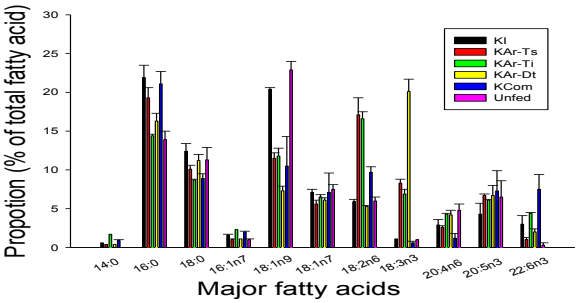

Figure 4. The proportion of major fatty acids in the kuruma prawns, *P .japonicus*. KI=prawns initial, KAr-Ts=prawns fed on *Artemia* raised on *T.Suecica*, KAr-Ti=prawns fed on *Artemia* raised on *T.Iso,*KAr-Dt= prawns fed on *Artemia* raised on *D.tertiolecta* and KCom=prawns fed on *Artemia* raised on commercial diet. Vertical bars represent standard deviation.

The present study showed that the lack of linolenic acid, 18:3n3 may effect the growth of *P. japonicus* PLs. Even though the linoleic acid (18:2n6); EPA (20:5n3) and DHA (22:6n3) were greater in the tissue of the prawns fed on the commercial diet compared to the other fed prawns, the near absence of linolenic acid may have resulted in the slowest growth of these prawns. The essentiality of these four FAs in larva and juvenile *P. japonicus* diets has been well defined (Kanazawa, 1985).

The ratio of linolenic and linoleic acids in prawn diets may also be important. In the present study, the linolenic : linoleic ratio of 2-3 : 1 seemed to support the best prawn growth. The greater proportion of linoleic to linolenic acid as shown in the prawns fed on the commercial diet was less effective in increasing the prawn's weight gain, suggesting that the former is inferior to that of the latter FA. This finding agrees well with Kanazawa *et al.* (1979) who noted that both linolenic and linoleic were essential,but linolenic was superior for satisfactory juvenile *P. japonicus* growth.

When the proportion of linolenic acid was relative high (more than 6 % of total FA), however, the growth response of the prawns did not improve. The present study showed that length was significantly lower ($P<0.05$) in the prawns fed on Ar-Dt which contained a relatively greater proportion of 18:3n3 than those prawns fed on Ar-Ti and Ar-Ts. These data suggest the excessive proportion of linolenic acid in the diet may not significantly increase the prawn weight gain as indicated earlier in another penaeid species (Rees *et al*., 1994; Merichan and Shim, 1997).

Regardless of the FAs composition, earlier studies on the complete replacement of live feed by an artificial diet reported slower growth rate in some penaeid species larvae, although the survival was similar to the prawns fed on live feed (Jones *et al*., 1987; Kumarly *et al*., 1989). More studies have confirmed that the preference of live feed in larval diets was related to trypsin activity, the dominant digestive enzyme during larval development (Le Vay *et al*., 1993). *Artemia* may provide readily or more easily digestible nutrients which improve the ability of prawns to meet their nutritional requirements.

T. Iso contained greater proportion of DHA compared to the other microalgae used. However, when this microalgae was fed to *Artemia*, the content of DHA in *Artemia* was relatively low, suggesting a retroconversion of DHA to lower chain PUFA (Estevez *et al.,* 1998) or this EFA is not incorporated into the lipid of *Artemia*. Similar findings were reported by Volkman *et al*. (1993). When microalgae rich in DHA were fed to rotifers and *Artemia*, they found the content of DHA in rotifers increased, but not in *Artemia*. Nevertheless, in the present study, there was an increased in the proportions of DHA when *Artemia* were fed to the prawn. This FA, is possibly derived from 18:3n3 which was abundant in the *Artemia*. Several studies have shown the ability of larval and juvenile *P. japonicus* to convert the linolenic acid, 18:3n3 into EFA, EPA and DHA (Jones *et al*., 1979; Teshima *et al*., 1992). However, the conversion may be very slow, as indicated in the other *Artemia* fed prawns. The proportion of DHA was less in the prawns fed on Ar-Ts and the prawns fed on Ar-Dt than in the initial prawn compared to the prawns fed on Ar-Ti. Kayama *et al*. (1980) reported the capacity of bioconversion of linoleic and linolenic acids to n-6 and n-3 HUFA were less active in juvenile *P. japonicus*. The results of the present study suggest that the contribution of 18:3n3 seems to be insufficient to supply DHA via elongation and desaturation so that supplementation of dietary DHA is essential in the prawn diet.

This study found a similar pattern of FA in unfed prawn and the initial prawn, which may lead to an assumption that the prawns had attacked and eaten the other prawns in the tank to sustain life under starved conditions. High mortality in the unfed treatments coupled with some growth in weight suggests cannibalism occurred in the present experiments. Some FA, such as 18:1n9, 18:1n7, 18:2n6, 18:3n3, 20:5n3 in unfed prawns were shown to follow the similar patterns as those seen in the prawns at the beginning of the experiment. However, the DHA was detected in low proportion in unfed prawn.

These results may be due to poor or slow incorporation of this FA that was obtained through cannibalism (as in *Artemia*, discussed above). Another possibility was that the unfed prawns have catabolised this DHA to sustain their life during starvation. Some crustaceans have been found to selectively catabolise this EFA during starvation (Olsen, 1998). Another FA that may decrease during starvation is palmitic acid (16: 0) as occurred in the present study. This fatty acid may also be utilised by the prawns under critical condition, such as starvation. Guary *et al*. (1975) in Bottino *et al.* (1980) has indicated the decreases of palmitic acid level in prawn lipids when prawns were left starved.

IV. CONCLUSIONS

There was no signicant difference in survival between fed treatments. The FA composition of prawns, *P. japonicus* reflect their dietary lipid composition. The greatest weight gain rates appeared in the prawns fed on *Artemia* raised on *T. iso*. And overall, the use of live feed produced a greater response in terms of prawn weight gain compared to the commercial diet. The polyunsaturated fatty acid of n3 series appeared to be more effective for supporting penned prawn growth.

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