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# Fatty Acid Exchanges and *De novo* Synthesize in the White Leg Shrimp (*Litopenaeus vannamei*) Tissues Fed Different Levels of Dietary Highly Unsaturated Fatty Acid (HUFA) During Maturation

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**Abstract:** Changes in total fatty acids were studied in ovaries and mid-gut glands of cultured *Litopenaeus vannnamei* female broodstock during maturation. The shrimp were obtained from Bandrargah Shrimp Research Station (Southeast Iran) in 2010. Hepatopancreatic n-6 HUFAs (20:4n-6) and ovarian n-3 HUFAs (5n-3), increased during ovarian development. The most important differences of fatty acids were shown between total n-3/n-6 ratios of ovaries and mid-gut glands. Ovarian fatty acids were dominated by Total MUFAs (Monounsaturated fatty acids), foloowed by saturated fatty acids (SAFA) and mid-gut glands showed a similar trend as well. The major fatty acids 15:1, 16:0, 16:1n7, 18:0, 18:1n9, 18:1n7, 18:2n6, 20:4n6, 20:5n3 and 22:6n3, had some fluctuations between ovaries and mid-gut glands. The Higher levels of the EPA and DHA in most ovaries and all mid-gut glands, respectively. Certain FA such as the case of AA, EPA and DHA appeared to be *de novo* synthesized and/or retained.

**Key words:** Fatty Acids · *de novo* · White Leg Shrimp · HUFA · Maturation

## INTRODUCTION

Some marine organisms including decapod shrimp require certain essential polyunsaturated fatty acids (PUFA) to be supplied in their diet [1]. These compounds have crucial roles in the structure and functioning of cell membranes and the animals require substantial amounts during periods of growth and reproduction [2-4].

For shrimp, highly unsaturated fatty acids (HUFA) such as arachidonic (20:4n-6, AA), eicosapentaenoic (20:5n-3, EPA) and docosahexaenoic (22:6n-3) are considered as essential fatty acids (EFA), because of these organism's limited ability to elongate and desaturate shorter-chain PUFA to HUFA [5]. Preliminary studies stated that crustaceans all have a limited ability to synthesize *de novo* the n-3 and n-6 families of fatty acids (FA) [1, 6]. They have been shown that the maturation and spawning of penaeid shrimp is affected by the types of fatty acids in the diet, such as in *P. setiferus* [7, 8] and *P. japonicus* [9-11]. In other words, Lipids totally, are indispensable sources of energy and may also be essential nutrients that can only be synthesized *de novo* by shrimp to a very limited extent, e.g. HUFA, or can not be synthesized *de novo*, e.g. sterols [12, 13].

In a previous study [14] with the Pacific white shrimp *L. vannamei* changes that occurred in fatty acid composition of the body tissues during ovarian maturation were described, showing a high increase in total lipid levels of HUFA immature ovaries. Preliminary researches on other shrimp species revealed similar findings; *L. setiferus* [15], *Marsopenaeus japonicus* [9], *Pleoticus muelleri* [16], *P. kerrathurus* [17].

The previous studies reported that the fatty acid pattern of shrimp tissue reflects that of dietary lipids (*P. monodon* [17, 18]; *M. japonicus* [19, 20] and *F. indicus* [21].

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Polyunsaturated and Highly unsaturated fatty acids are essential for the growth of crustaceans. They have been reported to improve the growth performance of freshwater prawns (*Macrobrachium resenbergii* [22] and various other shrimps, such as *P. japonicus* [1]; (*P. chinensis*: [23]; *P. indicus* [24] and *P. duodarum* [25]. In a previous study [26] on juvenile *Litopenaeus vannamei*, dietary n-3 fatty acid concentrations were the highest in the fish oil diet, followed by the diets containing soy oil or poultry fat. These fatty acid profiles were reflected in shrimp body fatty acid profiles, with shrimp fed the fish oil diet having the highest concentration of n-3 fatty acids, followed by those fed the diets containing soy oil or poultry fat.

Information on the effect of dietary EFA on fatty acid composition of shrimp broodstock is still very limited. The aim of this study was to investigate the effect of various dietary highly unsaturated fatty acids on quantitative amounts and *de novo* synthesize of FAs in mid-gut gland and ovary tissues of female broodstock *L. vannamei* and survey on fatty acid exchanges between these organs during the last maturation development stage.

## MATERIALS AND METHODS

**Shrimp Culture Design:** Pacific white leg female pre-adult were obtained from a captive stock located in Bandargah Shrimp Research Station (Boushehr province, Iran), with average weight were 31±1 g [27]. Four sub-adult shrimps were reserved per 300 liter tank. Treated with formalin upon arrival and acclimated to laboratory conditions at the research laboratory of the Bandargah Research Station systems for a period of approximately 2 weeks while they were fed fresh food (cuttlefish, *Melalis* bivalve and *Perinereis* worm) twice a day. All animals were collected and ablated at the end of the acclimation phase. For examinant weeks they were fed the trial diets. Ovarian maturity staging was carried out by visual examination [28].

**Diet Preparation:** Three experimental diets were formulated using as a base the same commercial food (Havourash-4006 meal) as the diet given to the broodsock females reared in outdoor ponds. Amount of dietary HUFA level was enhanced in the using Havourash-4006 meal dough, with 3% HUFA of diet, 2% HUFA of diet and 1% HUFA of diet. HUFA levels were altered by varying the relative portions of HUFA rich oil (*Spiraselco*, Inve Aquaculture, Iran). From the mixture, stiff dough was obtained and passed through a meat mincer. The resulting spaghetti-like strings were died and kept in a modified version of the method described by [28]. All diets were prepared and dried at the Havourash aquatic feed factory (Kazeroun road, Boushehr province, Iran) according to a modified version of the method described by Wouters *et al* [14]. As such, after ablation, a fresh food replacement level of 100% was selected as control diet (CD) for the present study. Frozen (Cuttlefish and *Melalis* bivalve) and live fresh (Worm) items were in 1:1:1 ratio. During acclimation a total feeding rate of 4% $day^{-1}$ of the total shrimp biomass was applied, after ablation the feeding rate was increased to 6% $day^{-1}$. However fresh food ingestion rate in CD shrimps was approximately 10% $day^{-1}$. All diet samples were then transported to University of Urmia (Urmia city, Western Azerbaijan province, Iran). Chemical analyzed HUFA values of trial diets were close to expected values.

**Culture Conditions and Monitoring:** The following culture conditions were observed in whole trial period. 0.55 $m^2$ plastic tanks, 4 sub-adult female *L. vannamei* per tank, 2 air stone diffuse per tank, 250 liter sand-filtered and treated sea water per tank, 50% daily water exchange, 32 g $L^{-1}$ average salinity, 6.5 $mgL^{-1}$ average dissolved oxygen, 28.8°C average temperature and 8.2 average pH. Automatic timers allowed a controlled photoperiod (14-h light: 10-h dark).

Ablation was applied to all animals by cutting one eyestalk and cauterizing the wound immediately. The experiment fined when the majority of females had some degrees of maturation, which this was 40 days post-ablation for all treatments.

**Shrimp Tissue Sampling Procedure:** Finally, the ovaries and mid-gut glands (hepatopancreas) were then dissected-out. Samples were immediately frozen in liquid nitrogen and stored in small plastic tubes which were identified by tank number for each treatment and replicate number at -80°C until analysis. They were then transported by air in a Styrofoam box with dry ice to the Urmia University (Iran) for fatty acid analysis. Only maturated (stage 4) ovaries were analyzed to allow easier interpretation of the comparative results.

FA composition was chemically analyzed in a modified version of the method described by Lochmann and Gatlin [29].

**Statistical Analysis:** In this experiment four treatments were tested with three replications each. Each tank was calculated as a trial unit for replication. Four animals were stocked in each tank at the beginning of the experiment. One-way ANOVA and subsequent Duncan's multiple-range test for detection of treatment differences [30] were used for statistical analysis.

## RESULTS

Total fatty acid content in ovary, mid-gut gland, trial and fresh food mix diets: In an attempt to identify the fatty acid factors responsible for differences in reproductive success, the fatty acid composition analysis of the three experimental diets and control diet are shown in Tables 1 to 3.

Table 1: FA composition (% of total FA) of the experimental artificial broodstock diets and fresh food mix (cuttlefish/ worm/ bivalve 1: 1: 1) used in the present experiment.

| | Diets | | | |
|---|---|---|---|---|
| Fatty acids | 1% TDH | 2% TDH | 3% TDH | Fresh food mix(CD) (cuttlefish/worm/ bivalve) |
| Saturated (SAFA) | | | | |
| 14:0 | 95/2 [a] | 71/1 [ab] | 84/1 [ab] | 0/84 [b] |
| 15:0 | 93/0 [ab] | 49/0 [b] | 45/0 [b] | 1/65 [a] |
| 16:0 | 87/29 [a] | 99/24 [ab] | 72/24 [ab] | 11/70 [b] |
| 17:0 | 16/1 [ab] | 66/0 [b] | 71/0 [b] | 2/98 [a] |
| 18:0 | 29/8 [a] | 42/7 [ab] | 48/7 [ab] | 3/11 [b] |
| 20:0 | 00/0 | 42/0[b] | 45/0[b] | 2/97 [a] |
| Monounsaturated (MUFA) | | | | |
| 14:1n-5 | 00/0 | 00/0 | 00/0 | 00/0 |
| 15:1 | 00/0 | 00/0 | 00/0 | 0/23[a] |
| 16:1n-7 | 83/2 [ab] | 66/4 [a] | 4/96 [a] | 1/45 [b] |
| 17:1n-7 | 25/0 [b] | 34/0 [ab] | 37/0 [ab] | 1/13 [a] |
| 18:1n-9 | 44/29 [a] | 22/31 [a] | 69/30 [a] | 1/57 [b] |
| 18:1n-7 | 55/1 [a] | 83/0 [ab] | 03/1 [ab] | 0/29 [b] |
| 20:1n-9 | 00/0 | 04/0[b] | 00/0 | 3/02[a] |
| PUFA and HUFA(n-6) | | | | |
| 18:2n-6 | 36/12 [ab] | 11/18 [a] | 70/16 [ab] | 0/79 [b] |
| 20:2n-6 | 0/20[b] | 0/31[ab] | 0/44[ab] | 1/78[a] |
| 20:4n-6 | 19/0 [b] | 35/0 [ab] | 38/0 [ab] | 0/56 [a] |
| PUFA and HUFA(n-3) | | | | |
| 18:3n-3 | 00/0 | 26/1 [a] | 19/1 [a] | 0/41 [b] |
| 20:3n-3 | 0/09 [b] | 0/11 [b] | /014 [b] | 1/97[a] |
| 20:5n-3 | 40/0 [b] | 88/0 [b] | 06/1 [a] | 0/58 [ab] |
| 22: 6n-3 | 49/0 [b] | 46/1 [a] | 68/1 [a] | 0/82 [ab] |
| Total SAFA | 43/20 [a] | 41/63 [a] | 35/65 [ab] | 23/25 [b] |
| Total MUFA | 34/07 [a] | 37/09 [a] | 37/05 [a] | 6/91 [b] |
| Total PUFA and HUFA(n-3) | 0/98 [b] | 3/71 [a] | 4/07 [a] | 3/78 [a] |
| Total PUFA and HUFA(n-6) | 12/75 [ab] | 18/77 [a] | 17/52 [a] | 3/13[b] |
| n-3/n-6 | 0.07 | 0.19 | 0.23 | 1.20 |

Table 2: Variation in total ovarian fatty acid content (% FA/dry weight) of *Litopenaeus vannamei* female broodstock at different dietary HUFA treatments

| | Diets | | | |
|---|---|---|---|---|
| Fatty acids | 1% TDH (T1) | 2% TDH (T2) | 3% TDH (T3) | Fresh food mix(CD) (cuttlefish/worm/ bivalve) |
| Saturated (SAFA) | | | | |
| 14:0 | 95/0 [b] | 97/0 [b] | 84/0 [b] | [a] 85/ 1 |
| 15:0 | 1/04[c] | 1/67[bc] | 2/97 [b] | 4/89 [a] |
| 16:0 | 88/6 [c] | 32/15 [b] | 80/14 [b] | [a] 46/24 |
| 17:0 | 1/12[b] | 1/37[b] | 1/45[b] | 6/78[a] |
| 18:0 | 55/6 [b] | 35/9 [a] | 33/9 [a] | 0 [a]7/8 |
| 20:0 | 33/0 [ab] | 44/0 [a] | 42/0 [a] | 04/0 [b] |
| Monounsaturated (MUFA) | | | | |
| 14:1n-5 | 33/0 [ab] | 38/0 [ab] | 43/0 [a] | 9 [b]1/0 |
| 15:1 | 3/09[c] | 4/22[c] | 9/67 [b] | 11/07 [a] |
| 16:1n-7 | 69/5 [ab] | 05/6 [a] | 81/6 [a] | [b] 55/0 |
| 17:1n-7 | 0/00 | 0/00 | 0/00 | 4/56[a] |
| 18:1n-9 | 64/6 [b] | 98/19 [ab] | 61/19 [ab] | 06/29 [a] |
| 18:1n-7 | 66/6 [ab] | 99/6 [ab] | 47/7 [b] | 84/17 [a] |
| 20:1n-9 | 27/0 [b] | 35/0 [ab] | 34/0 [ab] | 44/2 [a] |
| PUFA and HUFA(n-6) | | | | |
| 18:2n-6 | 84/6 [ab] | 43/7 [a] | 55/6 [ab] | 99/1 [c] |
| 20:2n-6 | 88/0 [a] | 74/0 [ab] | 59/0 [b] | 26/0 [b] |
| 20:4n-6 | 37/1 [d] | [a] 14/6 | [b] 91/4 | 41/3 [c] |
| PUFA and HUFA(n-3) | | | | |
| 18:3n-3 | 59/1 [b] | 49/1 [b] | 67/1 [b] | 69/11 [a] |
| 20:3n-3 | 00/0 | 48/0 [b] | 48/0 [b] | 42/6 [a] |
| 20:5n-3 | 80/3 [c] | 88/6 [b] | 15/9 [a] | 17/10 [a] |
| 22: 6n-3 | 94/4 [b] | 47/5 [ab] | 20/6 [a] | 63/5 [ab] |
| Total SAFA | 16/87 [b] | 29/12 [ab] | 29/81 [ab] | 64/72 [a] |
| Total MUFA | 22/88 [b] | 37/97 [ab] | 44/33 [ab] | 65/71 [a] |
| Total PUFA and HUFA(n-3) | 10/33 [b] | 14/32 [ab] | 17/50 [ab] | 33/91 [a] |
| Total PUFA and HUFA(n-6) | 9/09 [ab] | 14/31 [a] | 12/05 [ab] | 5/66 [b] |
| n-3/n-6 | 1.13 | 1.00 | 1.45 | 5.99 |

Data are means of three samples (one sample per replicate of every treatments. standard deviation omitted for clarity).

Tables 1 and 3 show that with the expectation of 18: 2n-6, 18: 3n-3, 20: 5n-3 and 22: 6n-3 fatty acid ratio, As well with the expectation of 18: 1n-9 and 18: 2n-6 of fatty acid amounts, the fatty acid composition of shrimp ovaries was not strongly affected by the percent of dietary HUFA in the broodstock diets, totally.

The major fatty acids were 15:1, 16:0, 16:1n7, 18:0, 18:1n9, 18:1n7, 18:2n6, 20:4n6, 20:5n3 and 22:6n3. Total saturated fatty acids 16:0 and 18:0 were the most deposited MUFA in ovaries but 14:0 was in the least amount. Monounsaturated fatty acids were higher than MUFAs and PUFAs in both ovaries and mid-gut glands. On the other hand, total saturated fatty acid concentrations of the mid gut gland of was lesser than ovaries.

Table 3: Variation in total fatty acid content (% FA/dry weight) in mid-gut gland of *Litopenaeus vannamei* female broodstock at different dietary HUFA treatments

| | Diets | | | |
|---|---|---|---|---|
| Fatty acids | 1% TDH (T1) | 2% TDH (T2) | 3% TDH (t3) | Fresh food mix(CD) (cuttlefish/worm/ bivalve) |
| Saturated (SAFA) | | | | |
| 14:0 | 96/0 [ab] | 90/1 [ab] | 19/2 [a] | 68/0 [b] |
| 15:0 | 0/89 [b] | 1/13[ab] | 1/02[ab] | 2/67 [a] |
| 16:0 | 86/16 [ab] | 19/15 [b] | 73/17 [ab] | 55/28 [a] |
| 17:0 | 1/01[b] | 1/23[ab] | 1/11[ab] | 3/34[a] |
| 18:0 | 96/5 [b] | 34/5 [b] | 05/5 [b] | 52/9 [a] |
| 20:0 | 32/0 [b] | 29/0 [b] | 30/0 [b] | [a] 31/3 |
| Monounsaturated (MUFA) | | | | |
| 14:1n-5 | 35/0 [b] | 28/0 [b] | 39/1 [a] | 21/0 [b] |
| 15:1 | 1/59[b] | 2/11[ab] | 3/99[ab] | 4/45[a] |
| 16:1n-7 | 79/5 [a] | 47/5 [a] | 46/4 [ab] | 40/2 [b] |
| 17:1n-7 | 0/00 | 0/00 | 0/00 | 4/89[a] |
| 18:1n-9 | 19/24 [a] | 27/25 [a] | 90/25 [a] | 70/15 [b] |
| 18:1n-7 | 13/5 [ab] | 89/4 [ab] | 95/2 [b] | 53/15 [a] |
| 20:1n-9 | 39/0 [b] | 34/0 [b] | 33/0 [b] | 79/0 [a] |
| PUFA and HUFA(n-6) | | | | |
| 18:2n-6 | 90/12 [b] | 20/16 [ab] | 83/16 [ab] | 12/26 [a] |
| 20:2n-6 | 00/1 [b] | 12/1 [ab] | 08/1 [ab] | 24/3 [a] |
| 20:4n-6 | 3.77[a] | 2.15[b] | 09/2 [b] | 02/2 [b] |
| PUFA and HUFA(n-3) | | | | |
| 18:3n-3 | 51/1 [ab] | [ab] 32/1 | 11/1 [b] | 24/6 [a] |
| 20:3n-3 | 90/0 [b] | 10/0 [ab] | 16/0 [ab] | 19/1 [a] |
| 20:5n-3 | 98/5 [a] | 66/2[b] | 69/4 [ab] | 16/4 [ab] |
| 22: 6n-3 | 01/6[a] | 78/3 [b] | 14/5 [ab] | 47/5 [ab] |
| Total SAFA | 26/00 [b] | 25/08 [b] | 27/40 [b] | 47/89 [a] |
| Total MUFA | 37/44[b] | 38/36 [b] | 39/02 [b] | 46/42 [b] |
| Total PUFA and HUFA(n-3) | 14/40[ab] | 7/77 [b] | 11/10 [ab] | 17/06 [a] |
| Total PUFA and HUFA(n-6) | 17/67 [b] | 19/47 [ab] | 20/00 [ab] | 31/38 [a] |
| n-3/n-6 | 0.81 | 0.39 | 0.55 | 0.54 |

Data are means of three samples (one sample per replicate of every treatments. standard deviation omitted for clarity).

The females seemed to exert considerable control over the total saturated fatty acids incorporated into the mid-gut glands and ovaries, as the differences in saturated fatty acid content were much greater among in shrimp ovaries and mid-gut glands fed the different dietary HUFA treatments than the among dietary HUFA diets.

The ovary and mid-gut gland's fatty acids of shrimp fed fresh food diets were contained higher amount of PUFAs (n-3 and n-6) and HUFAs (n-3 and n-6) (mid-gut gland and ovary respectively) than those of the other HUFA supplement treatments, as such higher amount of n-9 fatty acid in both ovary and mid-gut gland.

The ovaries from the shrimp fed the 1% dietary HUFA (T1) had the lowest amounts of both n-3 and n-6 HUFA and PUFAs. Eggs of prawn fed the 3% dietary HUFA (T1) and fresh food mix diets had a higher content of 22: 6n-3 while lower 20: 4n-6 (Table 2) compared ovaries of shrimp from all other treatments.

The mid-gut gland and ovary tissues of shrimp fed by fresh food mix diet stored largest amounts of saturated, monounsaturated, poly unsaturated and highly unsaturated fatty acids subsequently followed by 3% dietary HUFA (T3) diet. Although individual fatty acids such as 14: 0, 14: 1n-5, 17: 1n-7 and 20: 3n-3 also displayed large decreases.

The variation of HUFA contents of ovaries and mid-gut gland are given in Tables 2 and 3. According to the fact that all ovary and mid-gut gland were dissected from mature (stage 4) females, it is suggested that HUFAs totally, decreased in concentration between mid-gut gland and ovary with the exception of 20:5n-3 which is increased by transferring from mid-gut gland to ovary during sexual maturation. In other word the major HUFAs in the FAs of the ovary and hepatopancreas tissues were AA, EPA and DHA. The fatty acid composition of the test diets was not reflected to a certain extent in the fatty acid composition of hepatopancreas and ovary tissues of shrimp. For instance, EPA was significantly higher in ovary tissues and DHA was significantly higher in hepatopancreas tissues of all shrimps apart from diets were fed them. DHA and EPA were significantly higher in T3 diets rather than CD fresh foods, however, the ovaries, showing the highest EPA and DHA absorption in T3 and CD females jointly ($P<0.05$). The most EFA of the experimental artificial diets and fresh food mix were shown in hepatopancreas of T1 females, however in those that were fed the rest of the dietary treatments, it did not change significantly ($P>0.05$).Table 2, is showing a rapid increase in EPA absorption in T1 ovaries rather than DHA and AA ($P<0.05$).

Totally, the FA compositions of ovaries and mid-gut gland were similar to those of the Females fed by 2% and 3% dietary HUFA, However, n-3/n-6 ratios in the ovaries were higher than those of the mid-gut gland.

## DISCUSSION

An increase in the lipid concentration of the ovaries with a concurrent decrease in the hepatopancreatic lipids has been noted by Teshima and Kanazawa [9] for wild *Penaeus japonicus* females during sexual maturation. Also according to the finding reported by Millamena and

Pascual [31], during gonadal development, total lipid content of the ovaries and mid-gut gland of *P. kerathurus* females were higher than the lipid contents of those organs of wild-caught females of *P. monodon*. In totally, an inverted pattern was exhibited by mature *L. vannamei* females in the present study. The exception of females fed by 1% dietary HUFA (T1) in some fatty acids could be during differences between species (*L. vannamei* vs. *P. japonicus, P. kerathurus and P. monodon*) or Lipid sources (HUFA n-3/n/6 oil vs. free fatty acid, triacylglycerol, phosphatidylcholine and sterol esters).

Gonads displayed a higher *n*-3/*n*-6 ratio than mid-gut glands, i.e. 2:1 trial diets, 10.9:1 natural diets. It Might be due to *n*-3 FA was preferentially transferred to the gonads for offspring development. This suggests that *n*-3 FA have a higher value than *n*-6 FA for *L.* v*annamei* broodstock, in agreement with the observations made on the FA requirements of wild *L. vannamei* nauplies [14] and *Fen. chinensis* juveniles [32, 33].

In *P. kerathurus*, 65% of fatty acids of the total ovarian lipids are conveyed to eggs and embryos during spawning process; the transferred concentrations of 20: 5n-3and 22:6n-3 comprises 4 and 4.5% of the total ovarian lipids [34]. Mourente and Rodriguez [17] reported the lipids of mid-gut gland of *P. kerathurus* contained very high levels of 16:0 MUFAs same as 16:1n-7 and18: 1n-9 and HUFAs same as 20:4n-6, 20: 5n-3 and 22: 6n-3. This is in agreement with the finding of present study. However, with the combined results of present and previous studies, it seems to 16:0 MUFAs containing in mid-gut gland has a constant amount in mature females of penaeid shrimp. In other hand, it may be indicated that the majority of the neutral lipids ingested by *L. vannamei* females are converted to polar lipids (HUFAs and PUFAs) and transported to the developing ovaries for use as constituent and reserve lipids during sexual maturation.

Decreases in most of the fatty acids profile of the mid-gut gland before maturation may be in result of transferring of the fatty acids to in ovaries. This result is in line with the theory proposed by Chang and O'Connor [12], who suggested a similar relation between fatty acids contents of the mid-gut gland and gonadal development. Dall [34] suggested that these fatty acids which stored as triacylglycerolin the mid-gut gland may also play an important role in the case of prolonged starvation before or during maturation.

Amounts of *n*-3 HUFA in current study were comparatively higher in all ovaries and mid-gut glands rather than the trial artificial diets and natural diet fed to *L. vannamei* females. HUFA either can not be synthesized *de novo* [12; 35; 36], or may only be synthesized to a very limited extent through the conversion of 18:2*n*-6 and 18:3*n*-3 to C20 and C22 HUFA [37]. Wouters *et al*. [28] demonstrated that diet is the origin resource of the HUFA in the mid-gut gland and ovaries of the female broodstock of *L. vannamei.*

Increasing the concentration of hepatopancreatic n-6 HUFAs (20:4n-6) and ovarian n-3 HUFAs (5n-3) would seem to reinforce the theory that long-chain fatty acids are necessary for vitellogenesis of penaeid shrimp. These qualitative exchanges of fatty acids agree the results reported by previous authors [8, 17].

The Essential fatty acid (HUFA and PUFA) ratio of the experimental diets was not reflected in the EFA amount of hepatopancreas and ovary tissues. In other words, certain FA appeared to be *de novo* synthesized and/or retained, because they were presented in small amounts in diets but in relatively higher amounts in hepatopancreas and ovary tissue, as the case of AA, EPA and DHA. The previous studies on ovarian lipids displayed the fatty acid pattern of shrimp tissue reflected thae dietary lipid's profile (*P. monodon*: [18, 19]; *F. indicus*: [21]; *M. japonicus*: [6, 20]. This could be due to differences between the Penaeid species.

Noteworthy are the higher levels of the EPA and DHA in most ovaries and all mid-gut glands, respectively.

AA as a precursor in the synthesis of prostaglandins, which plays a key role in the reproduction of mammals and certain fish and insect species [37], has some non-significant fluctuations between different treatments. However increasing this FA in ovaries rather than mid-gut glands during maturation development, demonstrates that this FA is deposited preferentially into the ovaries. This finding is contradicting the theory that AA is necessary for shrimp maturation [8, 14].

SAFA and MUFA are abundant in detritus and in natural food organisms that are consumed by shrimp [16]. In addition, these FA are synthesized by crustaceans during maturation process [12, 36]. This activity could explain the increase in 16:0 and MUFA levels in both ovary and mid-gut gland of those *L.* v*annamei* females fed by natural food during early ovarian maturation in present study.

Although the data presented in this study may help to elucidate the role of different fatty acid groups in female broodstock *Litopenaeus vannamei* during maturation, but further studies seem are necessary to elaborate the understanding of FA metabolism during ovarian development in this species.

## REFERENCES


1. Kanazawa, A., S. Teshirna and K. Ono, 1979. Relationship between essential fatty acid requirements of aquatic animals and the capacity for bioconversion of linolenic acid to highly unsaturated fatty acids. Comp. Biochem. Physiol., B 63: 295-298.
2. Jonasdottir, J.H., 1994. Effects of food quality on the reproductive success of *Acartia tonsa* and *Acartia hudsonica*: laboratory observations. Mar. Biol., 121: 67-81.
3. Sargent, J.R., M.V. Bell, Beil, J.G.R.G. Henderson and D.R. Tocher, 1995. Origins and functions of n-3 polyunsaturated fatty acids in marine organisms. In: Ceve G. Paltauf F (eds) Phospholipids characterization, metabolism and novel biological applications. American Oil Society Press, Champaign, IL, pp: 248-259.
4. Pond, D.W., R.P. Harns, R. Head and D. Harbour, 1996. Environmental and nutritional factors determining the seasonal variability in the fecundity and egg viability of *Calanus helgolandicus* in coastal waters off Plyrnouth, UK. Mar. Ecol. Prog. Ser., 143: 45-63.
5. Gonzalez-Felix, M.L., A.L. Lawrence, D.M. Gatlin and M. Perez-Velazquez, 2001. Growth, survival and fatty acid composition of juvenile *Litopenaeus vannamei* fed different oils in the presence and absence of phospholipids. Aquaculture, 205: 325- 343.
6. Kayama, M., M. Hirata, A. Kanazawa, S. Tokiwa and M. Saito, 1980. Essential fatty acids in the diet of prawn: III. Lipid metabolism and fatty acid composition. Bull. Jpn. Soc. Sci. Fish. 46: 483-488.
7. Lawrence, A.L., D. Ward, S. Missler, A. Brown, J. McVey and B.S. Middleditch, 1979. Organ indices and biochemical levels of ova from penaeid shrimp maintained in captivity versus those captured in the wild. Proc. World Maricult. Sot., 10: 453-463.
8. Middleditch, B.S., S.R. Missler, H.B. Hines, J.P. McVey, A. Brown, D.G. Ward and Lawrence and A.L. 1980. Metabolic profiles of penaeid shrimp: Dietary lipids and ovarian maturation. J. Chromatogr. 195: 359-368.
9. Teshima, S. and A. Kanazawa, 1983. Variation in lipid composition during the ovarian maturation of the prawn. Bull. Jpn. Soc. Sci. Fish., 49(6): 957-962.
10. Teshima, S., A. Kanazawa, K. Horinouchi and S. Koshio, 1988. Lipid metabolism in destalked prawn *P. japonicus:* induced maturation and transfer of lipid reserves to the ovaries. Nippon Suisan Gakkaishi, 54: 1115-1122.
11. Teshima, S.I., A. Kanazawa, S. Koshio and K. Horinouchi, 1989. Lipid metabolism of the prawn, *Penaeus japonicus* during maturation: Variation in lipid profiles of the ovary and hepatopancreas. Comp. Biochem. Physiol., 92B: 45-50.
12. Chang, E. and J. O'Connor, 1983. Metabolism and tranzsport of carbohydrates and lipids. In: Mantel, H. (Eds). The Biology of Crustacean, vol. 5, Academic Press, New York, pp: 263-287.
13. D'Abramo, L.R., 1997. Triacylglycerol and fatty acids. In: D'Abramo, L.R. Conklin, D.E. Akiyama, D.M. (Eds), Crustacean Nutrition, vol. 6, World Aquaculture Society, Baton Rouge, LA, pp: 71-84.
14. Wouters, R., C. Molina, P. Lavens and J. Calderón, 2001a. Lipid composition and vitamin content of wild female *Litopenaeus* v*annamei* in different stages of sexual maturation. Aquaculture, 198: 307-323
15. Middleditch, B.S., S.R. Missler, D.G. Ward, J.P. McVey, A. Brown and A.L. Lawrence, 1979. Maturation of penaeid shrimp: dietary fatty acids. Proc. World Maricult. Sot., 10: 472-476.
16. Jeckel, W.H., J.E.A. de Moreno and V.J. Moreno, 1989. Biochemical composition, lipid classes and fatty acids in the ovary of the shrimp *Pleoticus muelleri* Bate. Comp. Biochem. Physiol., 92B: 271-276.
17. Mourente, G. and A. Rodriguez, 1991. Variation in the lipid content of wild-caught females of the marine shrimp *Penaeus kerathurus* during sexual maturation. Mar. Biol., 110: 21-28.
18. Deering, M.J., D.R. Fielder and D.R. Hewitt, 1997. Growth and fatty acid composition of juvenile leader prawns, *Penaeus monodon*, fed different lipids. Aquaculture, 151: 131- 141.
19. Millamena, O.M., 1989. Effect of fatty acid composition of broodstock diet on tissue fatty acid patterns and egg fertilization and hatching in pond reared *Penaeus monodon*. Asian Fish. Sci., 2: 127-134.
20. Guary, J.C., M. Kayama, Y. Murakami and H.J. Ceccaldi, 1976. The effects of a fat-free diet and compounded diets supplemented with various oils on molt, growth and fatty acid composition of prawn, *Penaues japonicus* Bate. Aquaculture, 7: 245- 254.
21. Colvin, P.M., 1976. The effect of selected seed oils on the fatty acid composition and growth of *Penaeus indicus*. Aquaculture, 8: 81-89.
22. D'Abramo, L.R. and S.S. Sheen, 1993. Polyunsaturated fatty acid nutrition in juvenile freshwater prawn *Macrobrachium rosenbergii*. Aquaculture, 115: 63- 86.

23. Xu, X., J. Wenjuan, J.D. Castell and R. O'Dor, 1993. The nutritional value of dietary n-3 and n-6 fatty acids for the Chinese prawn (*Penaeus chinensis*). Aquaculture, 118: 277-285.
24. Read, G.H.L,. 1981. The response of *Penaeus indicus* (Crustacea: Penaeidea) to purified and compounded diets of varying fatty acid composition. Aquaculture, 24: 245-256.
25. Sick, L.V. and J.W. Andrews, 1973. The effect of selected dietary lipids, carbohydrate and protein on the growth, survival and body composition of *P. duorarum.* Proc. World Maricult. Sot., 4: 263-276.
26. Cheng, Z.J. and R.W. Hardy, 2004. Protein and lipid sources affect cholesterol concentrations of juvenile Pacific white shrimp, *Litopenaeus vannamei* (Boone). J. Anim. Sci., 82:1136-1145.
27. Broke, J.A. and K.L. Main, 1994. A guide book to the common problems and diseases of cultured *Penaeus vannamei*. The Oceanic Institute Publication. pp: 241.
28. Wouters, R., X. Piguave, L. Bastidas, J. Calderón and P. Sorgeloos, 2001b. Overian maturation and haemolymphatic vitellogenin concentration of Pacific white shrimp *Litopenaeus vannamei* (Boone) fed increasing levels of total dietary lipids and HUFA. Aquacult. Res., 32: 573-582.
29. Lochmann, R.T. and D.M. Gatlin III, 1993. Essential fatty acid requirement of juvenile red drum (*Sciaenops ocellatus*). Fish Physiology Biochemistry, 12: 221- 235.
30. Mead, R., R.N. Curnow and A.M. Hasted, 1993. Statistical Methods in Agriqulture and Experimental Biology. London, Champen and Hall. pp: 227-235.
31. Millamena, O.M. and F.P. Pascual, 1990. Tissue lipid content and fatty acid composition of *Penaeus monodon* Fabricius broodstock from the wild. J. World Aquacult. Soc., 21: 116-121.
32. Xu, X., J. Wenjuan, J.D. Castell and R. O'Dor, 1994. Essential fatty acid requirement of the Chinese prawn, *Penaeus chinensis*. Aquaculture, 127: 29- 40.
33. Mourente, G. M.P. Pereiro and A. Rodriguez, 1990. Contenido en acidos grasos de los lipidos totales, polares neutros en musclo, hepatopancreas y ovario del crustaceo *Penaeus kerathurus* Forskal, antes y despues de la puesta. In: Mourente, G. (Eds). In vitro metabolism of C-14-polyunsaturated fatty acids in mid-gut gland and ovary cells from *Penaeus kerathurus* Forskal at the beginning of sexual maturation. Comp. Biochem. Physiol., 115B: 255-266.
34. Dall, W., 1981. Lipid absorption in the Norwegian lobster, *Neohrops norvegicus* (L.) J. exp. mar. Biol. Ecol., 50: 33-45.
35. Kanazawa, A., S.I. Teshima and S. Tokiwa, 1977. Nutritional requirements of prawn: VII. Effect of dietary lipids on growth. Bull. Jpn. Soc. Sci. Fish., 43: 849-856.
36. Clarke, A., 1982. Lipid synthesis and reproduction in the polar shrimp *Chorismus antarticus*. Mar. Ecol. Prog. Ser., 9: 81-90.
37. Mourente, G., 1996. In vitro metabolism of C-14-polyunsaturated fatty acids in mid-gut gland and ovary cells from *Penaeus kerathurus* Forskal at the beginning of sexual maturation. Comp. Biochem. Physiol., 115B: 255-266.