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Effect of Fatty Acid Composition of Broodstock Diet on Tissue Fatty Acid Patterns and Egg Fertilization and Hatching in Pond-Reared *Penaeus monodon*

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Abstract

The effect of dietary fatty acid composition on tissue fatty acid patterns and spawn quality of pond-raised *Penaeus monodon* was evaluated with the use of three practical diets. Diets were formulated to contain the same basal components but with various lipid supplementation: Diet B1, 6% cod liver oil; Diet B2, 3% cod liver oil and 3% soybean lecithin; and Diet B3, 6% soybean lecithin.

Three flow-through maturation tanks were each stocked with 50 broodstock at a sex ratio of 1.5 female to 1 male, with the females ablated on one eyestalk. Broodstock performance measured as total number and nature of spawnings, egg fertility and average hatching rate of eggs of each dietary treatment was assessed.

Results showed that the fatty acid composition of broodstock diet affected the tissue fatty acid patterns and hatchability of eggs from pond-reared *P. monodon*. Females fed a diet high in 20:4n-6, 20:5n-3 and 22:6n-3 polyunsaturated fatty acids (PUFA) and n-3/n-6 fatty acid ratio gave higher percentages of fertilized eggs and higher hatching rates than did other diets.

Introduction

Intensive research effort has been directed towards controlled reproduction of *P. monodon* in order to fully close the life cycle of this species in captivity. Current research has placed emphasis on pond-reared broodstock as an alternative to wild, the supply of which is both unreliable and costly. So far, satisfactory maturation and spawning of viable larvae have been obtained; however, gaps such as low fecundity and erratic hatching rate of eggs remain unresolved (Aquacop 1983).

Broodstock nutrition has been shown to play a prominent role in shrimp reproduction and influences larval viability. Several researchers have compared diets for effects on reproductive performance. Primavera et al. (1979) compared various combinations of artificial (pellets) and natural food and concluded that the combination of frozen mussel meat and pellets comprised the best maturation diet for *P. monodon*. On the other hand, ablated *P. monodon* fed solely on pellets with no fresh food supplement spawned eggs which were completely unfertilized (Aquacop 1979). Cahu et al. (1986) reported on the effect of fatty acid composition in food on egg quality of *P. vannamei* broodstock. More recently, Millamena et al. (1986) obtained highest larval production and egg hatching rates from ablated *P. monodon* females fed high protein diets with n-3 highly unsaturated fatty acid supplementation, in combination with natural food. However, no attempt was made to correlate broodstock diet and tissue fatty acid composition with spawn quality.

The objective of this study was to examine further the effect of dietary fatty acid composition on tissue fatty acid patterns and egg fertility and hatchability in pond-reared *P. monodon* broodstock.

Materials and Methods

Experiments were conducted at the SEAFDEC Aquaculture Department, Philippines. *P. monodon* broodstock obtained from brackishwater fishponds in Villa, Iloilo, were stocked in 12-m^3 flow-through maturation tanks at a female to male ratio of 1.5:1 with 50 broodstock per tank. They were acclimated to the diets prior to unilateral ablation of females.

Three practical diets were formulated to contain the same basal ingredients (Table 1). Protein sources which were also sources of lipids consisted of squid meal, shrimp head meal and fish meal. The diets were supplemented with various sources of lipids: 6% cod liver oil (CLO) for Diet B1, 3% cod liver oil and 3% soybean lecithin for Diet B2, and 6% soybean lecithin (SBL) for Diet B3. Feeding consisted mainly of the three formulated diets given in the morning (0800 hours) and natural food: frozen squid and marine annelids (*Nereis* sp.) in the afternooon (1700 hours). Daily feeding rates were approximately 1.5% wet weight of total broodstock for pellets and 5% for natural food based upon average daily feed consumption and adjusted for mortalities. Water temperature ranged from 26 to 32°C while salinity varied from 30 to 32.5 ppt.

Table 1. Per cent composition of formulated diets for *P. monodon* broodstock.

Ingredients	%
Squid meal	30.0
Shrimp head meal	20.0
Fish meal	20.0
Wheat flour	5.5
Gulaman (seaweed)	4.0
Rice bran	5.2
Vitamin mix	2.7
Mineral mix	6.0
Cholesterol	0.5
Supplemental lipid[a]	6.0
Butylated hydroxy toluene	0.1

[a]Diet B_1 - 6% cod liver oil
B_2 - 3% cod liver oil + 3% soybean lecithin
B_3 - 6% soybean lecithin

Ovarian maturation was monitored by external examination of the dorsal exoskeleton. Females ready to spawn (Stages III and IV ovaries) were transferred to individual 300-l tanks for spawning. Estimates of total number of eggs and nauplii produced were made by taking five 250-ml subsamples from the spawning tank. Nauplii from each treatment were reared to metamorphosis to the zoea stage.

Broodstock performance was evaluated based on total number of spawnings, nature of spawnings and average hatching rate of eggs. Culture period was 90 days from ablation. Upon termination of the experiment, the shrimp were dissected, the hepatopancreas and gonad were excised and held at -20°C then lyophilized for lipid analysis.

The diets and shrimp tissues were analyzed for fatty acid composition using lipid extraction by the Bligh and Dyer (1959) technique as modified by Kates (1972) and subsequent methylation with 14% (w/v) boron triflouride-methanol. Fatty acid methyl esters (FAME) were analyzed by gas liquid chromatography in a Shimadzu GC-4C gas chromatograph equipped with flame ionization detector and 10% di-ethylene glycol succinate (DEGS) column. FAME were identified using authentic fatty acid standards and literature values for published oils (Ackman and Burgher 1965) and quantified by an electronic integrator (Hewlett Packard 3390A).

Results and Discussion

The response of *P. monodon* broodstock to the diets in terms of number and nature of spawnings, egg fertility and hatching rates, is shown in Table 2. Among the formulated diets, B1 gave the highest number of spawns, complete spawnings, proportion of fertilized eggs and egg hatching rate. Diets B2 and B3 had more partial spawnings and infertile eggs with correspondingly low egg hatching rates; egg hatchability was lowest with Diet B3. However, eggs that were hatched from all dietary treatments successfully metamorphosed into the zoea stage.

Table 2. Number and nature of spawnings, egg types and average hatching rate of eggs in *P. monodon* females fed the three formulated diets.

Diet	No. of spawnings	Nature of spawnings Complete (%)	Partial (%)	Percentage eggs fertilized	Egg hatching rate (%)
B_1	35^a	79.41	20.59	67.10^a	36.67^a
B_2	20^b	70.00	30.00	63.09^a	27.27^a
B_3	29^{ab}	72.41	27.59	61.45^a	19.53^a

Treatment means with the same superscripts in each column are not significantly different at $P < 0.05$.

The lipid content and fatty acid composition of the diets are presented in Table 3. Total lipid levels were similar for the three diets at 12.08 to 12.15%. The main difference among the diets tested was in their fatty acid compositions, particularly the content of n-3 and n-6 polyunsaturated fatty acid. Diet B1 (CLO supplemented) contained more n-3 PUFA with high n-3/n-6 fatty acid ratio of 1.81; Diet B3 (SBL supplemented) had relatively more n-6 PUFA with low n-3/n-6 fatty acid ratio of 0.72; while Diet B2 had a fatty acid pattern and n-3/n-6 ratio that was intermediate between Diets B1 and B3.

The resulting fatty acid spectra of the female hepatopancreas and mature ovaries are shown in Tables 4 and 5. The fatty acid profile of the hepatopancreas which acts as storage organ for dietary lipids prior to its mobilization and incorporation in the specific tissues was influenced by the diet. The n-3/n-6 ratios were 1.46, 1.05 and 0.83, respectively, for females fed diets B1, B2 and B3. There was

Table 3. Fatty acid composition (%) of broodstock diets.

Fatty acid	Diet B1	Diet B2	Diet B3
C 14:0	1.2	1.0	0.4
15:0	0.1	0.1	0.1
16:0	7.5	9.5	8.8
16:1n-7	6.4	8.1	7.5
18:0	6.4	6.4	5.9
18:1n-9	14.3	16.0	14.9
18:2n-6	7.2	13.2	20.5
18:3n-3	3.8	4.4	9.3
18:4n-3	5.2	4.1	-
20:1n-9	12.5	10.0	7.8
20:4n-6	6.5	5.1	5.1
22:1	7.2	6.9	5.6
20:5n-3	11.8	8.4	5.4
22:4n-6	2.1	1.7	2.8
22:5n-3	1.6	1.0	0.9
22:6n-3	6.2	5.1	4.9
Total lipid (% of diet)	12.10	12.08	12.15
n-3/n-6	1.81	1.15	0.72

Table 4. Fatty acid composition (%) of hepatopancreas of *P. monodon* spawners.

Fatty acid	Hepatopancreas Diet B1	Diet B2	Diet B3
C 14:0	1.1	0.6	0.5
15:0	0.3	0.7	0.4
16:0	10.8	10.3	13.1
16:1n-7	9.2	8.8	11.1
18:0	4.8	6.8	0.4
18:1n-9	16.3	14.9	14.9
18:2n-6	9.02	13.0	15.6
18:3n-3	3.6	8.3	9.7
18:4n-3	2.9	1.0	-
20:1n-9	8.1	5.8	4.4
20:2	4.2	4.1	3.6
20:4n-6	5.9	5.6	5.5
22:1	5.0	5.8	6.3
20:5n-3	10.6	7.3	4.4
22:4n-6	1.9	2.7	2.9
22:5n-3	2.3	0.8	1.0
22:6n-3	5.1	4.9	5.5
n-3/n-6	1.46	1.05	0.83

Table 5. Fatty acid composition (%) of ripe *P. monodon* ovary.

Fatty acid	Diet B_1	Ovary Diet B_2	Diet B_3
C14:0	0.5	0.4	0.8
15:0	0.1	0.1	0.3
16:0	11.9	8.5	12.3
16:1n-7	7.0	5.0	6.0
18:0	6.0	6.2	3.6
18:1n-9	15.7	12.8	15.6
18:2n-6	8.7	13.0	15.0
18:3n-3	5.6	6.5	7.0
20:1n-9	5.7	5.3	3.1
20:2	4.0	3.6	2.8
20:4n-6	6.8	6.8	6.1
22:1	5.9	9.9	6.5
20:5n-3	6.7	5.7	2.6
22:4n-6	1.6	3.8	4.1
22:4n-3	1.6	1.1	1.0
22:5n-3	1.1	1.0	0.6
22:6n-3	12.1	11.5	9.5
n-3/n-6	1.58	1.09	0.86

biosynthesis of C20:2 fatty acid presumably by chain elongation and desaturation of C20:1. The variations in levels of n-3 and n-6 fatty acids reflected those of the diet.

In the ovarian lipid, there was a drop in 20:5n-3 with a concomitant rise in 22:6n-3 to double the values found in the hepatopancreas. This implies the importance of this fatty acid during egg development. Castell (1981) and Chapelle (1966) stated that the ovarian fatty acid pattern reflects a combination of dietary influence and specific requirement of the tissue. Shima et al. (1977) and Watanabe et al. (1984) reported on improved egg hatchability with an increase in 22:6n-3 fatty acid content of the egg lipid.

Overall, the fatty acid pattern of female ovaries showed lower percentages of total monoenes but higher n-3 and n-6 PUFA than those found in the hepatopancreas (Table 6). A comparison of female ovaries fed the different diets showed high n-3 PUFA for those fed Diet B1 and conversely, high n-6 PUFA for those fed Diet B3. The n-3/n-6 ratios were similar to those of the hepatopancreas at 1.58, 1.09 and 0.86, respectively, for females fed diets B1, B2 and B3. High fatty acid ratios were consistent with high fertilized egg and hatching rates.

Table 6. Fatty acid classes of broodstock diet, *P. monodon* hepatopancreas and ripe ovary.

	Diet			Hepatopancreas			Ovary		
	B1	B2	B3	B1	B2	B3	B1	B2	B3
Fatty acids (% of total fatty acids)									
Total monoenes	40.3	41.0	35.7	38.7	35.2	36.7	34.3	32.9	31.2
Total saturates	15.1	17.0	15.2	17.0	18.6	14.2	17.6	15.2	17.0
Total n-3	28.6	23.0	20.5	24.5	22.3	20.6	27.1	25.8	21.7
Total n-6	15.8	20.0	28.4	16.8	21.3	24.9	17.1	23.6	25.2
n-3/n-6 ratio	1.81	1.15	0.72	1.46	1.05	0.83	1.58	1.09	0.86

The observed differences among the diets in stimulating reproductive ability and enhancing egg fertility in pond-reared *P. monodon* are related to the dietary fatty acid composition, particularly the n-3 and n-6 PUFA content and n-3/n-6 fatty acid ratios. However, other nutrients present in cod liver oil and soybean lecithin such as vitamins, sterols, carotenoids and phospholipids that are important components of eggs may contribute as well to the differences in broodstock response. Moreover, nutrients present in the natural food that forms part of the diet also played a role in overall broodstock response. Our previous work, however, showed poor reproductive performance in *P. monodon* fed natural food alone. This was manifested by resorption of mature female ovaries and low survival rates. Feeding broodstock with a combination of artificial and natural food markedly improved reproductive success.

This study shows an important relationship between the fatty acid composition of broodstock diet, *P. monodon* tissues and quality of spawned eggs. Several authors have pointed out a correlation in lipid composition of the ripe ovary and spawned eggs with broodstock diet (Middleditch et al. 1980; Cahu et al., in press). This correlation is also evident in our data which showed an improvement in egg quality and hatching efficiency with n-3 PUFA supplementation and higher n-3/n-6 fatty acid ratio in broodstock diet.

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