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The effect of lipid-enriched *Artemia franciscana* on reproductive performance of broodstock and larval quality of Pacific white shrimp *Litopenaeus vannamei*

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Abstract

Broodstock maturation diets are an important component of shrimp hatchery management, since the nutritional status of spawners can impact on gonadal maturation, egg fecundity, embryo hatchability and overall larval quality. The ability to manipulate the biochemical composition of *Artemia* to deliver key nutrients to cultured animals has rendered their inclusion in broodstock feeds increasingly common. Lipid enrichment of *Artemia franciscana* to boost their unsaturated fatty acid content is a standard procedure. During this study, frozen, lipid-enriched adult *Artemia* biomass, squid and mollusc were fed to *Litopenaeus vannamei* broodstock to investigate the suitability of enriched *Artemia* as a maturation diet for the species and elucidate the role of essential fatty acids in the reproductive performance of the shrimp. Four lipid enrichment levels: PUFA (P group), MUFA (M group), HUFA (H1and H2 groups), un-enriched (NE group), No-*Artemia* (NA group), were fed to *L. vannamei* over 45 days. Significant differences in weight gain and specific growth rate were observed. Greatest mean SGR was recorded for shrimp of P-group as well as greatest mean final weight on day 45, while the lowest mean SGR was found for shrimp fed Diet NA. The fecundity of the broodstock shrimp fed diets either H1 or H2 increased compared with that of the shrimps fed NA (p<0.05). Significant differences in the hatching rate were observed. Changes in gonado-somatic index (GSI) of broodstock during ovarian maturation were very pronounced, but changes in hepato-somatic index (HSI) were not significantly different between the broodstock groups.

Keywords: *Artemia* biomass, HUFA, MUFA, PUFA, *Litopenaeus vannamei*

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Introduction

According to the Food and Agriculture Organization of the United Nations, the world's production of captured and farmed shrimp is approximately 6 million tones (FAO, 2016). The exceptional increment in production over the past four decades has been primarily attributed to increased production from shrimp farming activities, since capture fisheries have been assumed to be at maximum sustainable yield for many years. In fact, more than 43% (2.6 million tons) of the world's total shrimp production was from farming (FAO, 2016). The Pacific white shrimp *Litopenaeus vannamei* is the most important cultivated shrimp species and has presented the highest value of all traded crustacean products (FAO, 2016). The fast rise in *L. vannamei* culture has increased demand for reproduction optimization in commercial hatcheries (Palacios *et al.*, 1999) and yielded improvements on feeding practices (Browdy, 1998; Wouters *et al.*, 2001).

In penaeid shrimp, nutritional factors play a critical role in the stimulation of sexual maturation and mating, the enhancement of fertility, and the viability and quality of offspring (Browdy, 1992; Browdy, 1998; Harrison, 1990). Although considerable interest lies in rearing and maintaining broodstock in captivity for seedling production, nutritional bottlenecks remain unsolved (Wouters *et al.*, 2001).

During maturation, which can take from 3 days to 1 month, the weight of the ovaries increases by four to eight folds (Jeckel *et al.*, 1989; Mourente and Rodriguez, 1991; Ravid *et al.*, 1999). Within that time span, sufficient nutrients must be accumulated in the egg yolk to allow normal development of the embryos and pre-feeding larvae. In several studies, poor reproductive performance or low offspring quality was observed when suboptimal broodstock diets were used. (Chamberlain and Lawrence, 1981; Bray *et al.*, 1990; Cahu *et al.*, 1995). The influence of broodstock diet on the quality of the eggs and early larval stages has been well documented for crustaceans (Millamena, 1989; Bray *et al.*, 1990; Alava *et al.*, 1993; Xu *et al.*, 1994; Cahu *et al.*, 1995; Marsden *et al.*, 1997; Cavalli *et al.*, 1999; Wouters *et al.*, 1999).

Crustaceans require dietary lipids as a source of essential fatty acids (EFA) and other lipid classes like phospholipids (PL), sterols and carotenoids. In the case of marine organisms, polyunsaturated and especially highly unsaturated fatty acids (PUFA and HUFA, respectively) are important and essential because these animals possess a limited ability to synthesize them (Kanazawa *et al.*, 1979).

The effects of dietary lipid level on the growth, survival, food conversion and production had been studied in several species, such as *Panaeus japonicus* (Deshimaru *et al.*, 1979), *Cherax tenuimanus* (Fotedar *et al.*, 1997), *Macrobrachium reosenbergii* (Felix and Jeyaseelan, 2005; Hien *et al*., 2005), *Panaeus monodon* (Briggs *et al.*, 1994; Sheen and Wu, 2002), *Penaeus indicus* (Shivaram and Raj, 1997;

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Immanuel *et al.,* 2003), *Panulirus cygnus* (Glencross *et al.,* 2001) and *L. vannamei* (Gonzalez-Felix and Gatlin, 2003; Tzeng *et al.,* 2004).

In 2006 aquaculture production relied on fish oil for feeding species which consumed almost 22.7 million tons of aquafeeds containing about 835,000 tons of fish oil (Tacon and Metian, 2008). Since global fish oil production in 2006 amounted for 943,000 tons, it represents 88.5% of the total reported fish oil production for that year, at that time priced at more than US$750 per ton (Decision News Media, 2006).

Continued expansion of aquaculture will be possible only if cost-effective alternative sources of high quality oils are available to be used in aquafeeds. The use of vegetable oil use in aquafeeds without marine fish oil is often limited by the potential problems associated with insufficient levels of essential fatty acids (FA), anti-nutritional factors and poor palatability (Francis *et al.,* 2001).

However, several authors have reported that partial or total replacement of fish meal and fish oil with soybean meal and soy oil had no adverse effect on growth performance (Davis and Arnold, 2000; Cheng and Hardy, 2004; Samocha *et al.,* 2004).

The purpose of this study was to evaluate the effects of replacement of fish oil with plant oils, in *Artemia* enrichment on offspring quality and fatty acid composition of *L. vannamei* broodstock.

Materials and methods

Animals and experimental design

The shrimp, *L. vannamei*, used in this study were obtained from a commercial shrimp hatchery (Tiab, Minab, Hormozgan, Iran). The shrimp were acclimated for 4 weeks before experimentation. After acclimation, 216 shrimp were selected, and grouped in 18 circular maturation tanks (180cm in diameter) for further experimental work.

There were six dietary treatments, each with three replicates, each replicate consisting of 6 pairs of similar-sized shrimp. The shrimp were in the range of 151.45±4.08 mm total length and average weight of 36.88±3.64 g and were allocated to the treatments randomly.

After filtration, the water was heated to 28°C and distributed to the tanks at an exchange rate of 200% day^{-1}. During the experiment, water temperature was 26.9±0.4, pH was 7.55±0.32, dissolved oxygen was 3.48±0.23 and salinity was 34.30±0.27 g L^{-1} with constant aeration. Uneaten food and excreta were removed daily. Shrimp were fed 3 times daily at 6:00, 12:00 and 18:00 hours for 60 days. The tanks were submitted to a 13:11 h light: dark photoperiod.

Artemia enrichment and experimental diets

The enrichment procedure consisted of acclimation of adult *Artemia franciscan*a to normal sea water (35 ppt) for 8 h, with overnight storage in holding tanks of 1000 liter capacity at densities of about 1000 *Artemia* per liter. Enrichment was performed in 100

[DOR: 20.1001.1.15622916.2020.19.4.13.1]

1 conical tanks at densities of 5000 *Artemia* per liter using experimental oil emulsions. Following enrichment the brine shrimp were harvested, rinsed in sea water and fresh water, and frozen in 0.5-cm thick blocks of 300 g at -22°C.

Different feeding regimes, based on a mixture of fresh-frozen *Artemia* biomass, squid (*Photololigo* sp.) and mollusc *(Solen vagina)*, divided into four equal daily rations (at 9:00; 13:00; 17:00 and 21:00, respectively) accounting for a total daily supply of 16% of wet weight biomass, and was adjusted daily. The diets were as follows:

Mixture of mollusc at 8% and squid at 8% (NA group),

Mixture of un-enriched *Artemia* biomass at 8%, mollusc at 4% and squid at 4% (NE group). Mixture of 80% HUFA enriched *Artemia* biomass at 8%, mollusc at 4% and squid at 4% (H1 group).

Mixture of 100% HUFA enriched *Artemia* biomass at 8%, mollusc at 4% and squid at 4% (H2 group).

Mixture of PUFA enriched *Artemia* biomass at 8%, mollusc at 4% and squid at 4% (P group). Mixture of MUFA enriched *Artemia* biomass at 8%, mollusc at 4% and squid at 4% (M group).

The fatty acid compositions of the experimental oils for *Artemia* enrichment are listed in Table 1.

Egg and larval quality

During the first two hours after dusk the tanks were inspected and mated female *L. vannamei* were transferred to spawning tanks of 300 1 capacity filled with sand-filtered sea water kept at 28°C. Upon spawning, the animals were returned to their respective maturation tanks. The number of eggs per female (fecundity) was recorded from three 5 ml subsamples after stirring the spawning tank. Egg hatchability was determined in vitro. 100 eggs from first broodstock subgroup were placed in a 200 ml conical plastic tube to hatch, which was triplicated for each female. Moderate aeration provided oxygenation. The eggs hatched within 14–16 h after fertilization at the temperature of 30 °C and salinity of 34 ppt. Hatching rate was calculated from the number of live larvae and dead eggs, 8 h after hatching. The number of viable nauplii per spawn was estimated after positive phototropism selection.

All larvae were cultured under identical conditions. From each spawn, triplicate groups of several hundred larvae were reared to the postlarval stage under the following conditions: 10 L buckets, water temperature 30±0.6°C, 100 nauplii L^{-1}, salinity 30±0.4 g L^{-1}. During larval culture, estimations of survival percentage at each larval substage were made. The diet was composed of *Artemia* sp. (from zoea III) and *Chaetoceros muelleri* algae (from nauplii V). Algae (from nauplii V to zoea III) were administered with a continuous feeding system combined with a daily water exchange of 30%. The amount of diet was adjusted for each larval stage. Feeding from PL6 (6 days old postlarvae) onwards, consisted of *Artemia sp.* and dry pellets with 600 g kg^{-1} protein.

[DOR: 20.1001.1.15622916.2020.19.4.13.1]

The larval quality was determined with temperature and salinity stress test at PL15 (15 days old postlarvae). The temperature stress test consisted of transferring the larvae at PL15 to cold water (15°C) for 30 min, then back to water with normal temperature for another 30min, and assessing the percentage of survival in three replicates averaging 50 individuals each. The salinity stress test consisted of transferring the larvae at PL15 to fresh water for 30 min, then back to salinity 30.5±0.8 g L^{-1} for another 30 min, and assessing the percentage of survival in three replicates averaging 50 individuals each (Mengqing *et al.*, 2004).

Biochemical composition and fatty acids profile

Changes in fatty acids profile in hepatopancreas and gonads were used as indicators of nutritional condition of female broodstock. At the end of the experiment, animals (three mature females from each treatment) were dissected on ice and the midgut gland and ovaries were removed carefully, with a paper towel weighed and placed in a 1.5-ml Eppendorf tube, pooled per treatment and preserved/stored at -80°C for further lipid analysis. Hepatosomatic index (HSI=100×hepatopancras weight/animal weight) and gonadosomatic index (GSI=100×ovary weight/animal weight) were calculated as the percentage of the ovary and hepatopancreas weight relative to their body weight (Tahara and Yano, 2004).

Statistical analyses

Differences in final mean weights, growth rate, larval quality, fecundity and hatching rate were analyzed using one-way ANOVA to determine if significant (p<0.05) differences existed among treatment means. Duncan's multiple range test was used as the mean separation procedure. Statistical analyses were conducted using SPSS statistical software version 15.

Table 1: Composition and fatty acids profiles of experimental diets.

Fat source	Treatment				
	H 1	**H 2**	**M**	**P**	**Un-ENRICH**
Kilka fish oil	80	100	0	0	0
Canola oil	0	0	18	30	0
Linseed oil	2	0	1.5	6.3	0
Corn oil	18	0	0	0	0
Olive oil	0	0	52.75	5.5	0
Sunflower oil	0	0	4.75	28.2	0
Coconut oil	0	0	23	30	0
HUFA	15.90	20.09	0.00	0.00	0.81
MUFA	41.23	40.67	57.63	41.18	0.79
PUFA	13.98	4.73	13.85	30.15	0.78
SFA	23.70	29.51	23.52	23.73	0.53
C18:2n6	11.31	2.81	11.22	24.45	8.85

[DOR: 20.1001.1.15622916.2020.19.4.13.1]

Table 1 continued:

C18:3n3	2.67	1.92	2.63	5.69	1.16
C20:4n6	0.56	0.72	0.00	0.00	3.05
C20:5n3	4.50	5.62	0.00	0.00	5.75
C22:6n3	10.85	13.55	0.00	0.00	6.82
C18:1n9	27.27	33.34	53.30	27.83	5.58
HUFA/PUFA	1.14	8.61	0.00	0.00	1.03
HUFA/MUFA	0.39	0.49	0.00	0.00	1.02
HUFA/SFA	0.67	0.68	0.00	0.00	1.52
18:2n6/18:3n3	4.24	1.46	4.27	4.29	7.63
DHA/EPA	2.41	2.41	0	0	1.19

Results

On day 45 post-feeding significant differences in weight gain (WG) and specific growth rate (SGR) were observed (Fig. 1). Greatest mean SGR was recorded for shrimp of diet P-group as well as greatest mean final weight on day 45, while the lowest mean SGR was found for shrimp fed diet NA. The broodstock shrimp fed with diet H1 and H2 exhibited significantly (p<0.05) higher fecundity, which was slightly less than the NA group fed with fresh clam (Table 2). The fecundity of the broodstock shrimp fed with either diets H1 or H2 significantly increased compared with that in the NA group (p<0.05) (Table 2).

After spawning, significant differences in hatching rate and fecundity were observed (Fig. 1). Greatest mean was recorded for H2-group, while the lowest mean of hatching rate and fecundity was found for shrimp fed with diet NA (Table 2).

Changes in gonado-somatic index (GSI) of broodstock during ovarian maturation were very pronounced, but changes in hepato-somatic index (HSI) were not significantly different between the broodstock groups (Table 2).

The fatty acid composition of the test diets was reflected to a certain extent in the fatty acid composition of hepatopancreas and gonad tissue of shrimp (Tables 3, 4). For instance, DHA, EPA and AA were always significantly higher in tissues of shrimp fed with diets H1 and H2. Oleic acid was significantly higher in hepatopancreas and gonad tissue of shrimp fed with diet M. Linolenic acid was significantly higher in hepatopancreas (Table 3) and gonad tissue (Table 4) of shrimp fed with diet P.

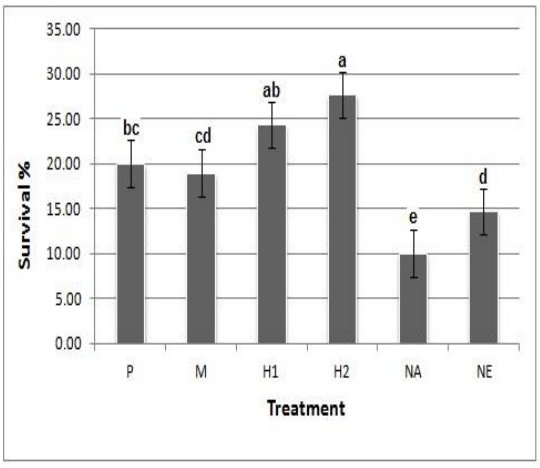

Figure 1: Survival rates of 15 days old postlarvae from broodstock fed with diets P, M, H1, H2, NA and NE. The bars with different letters are significantly different (p<0.05).

[DOR: 20.1001.1.15622916.2020.19.4.13.1]

Table 2: Effect of different diets on growth rate, HSI, GSI, hatching rate, percentage of larval survival from post larvae and result of stress test.

	Treatment					
	P	**M**	**H1**	**H2**	**NA**	**NE**
WG	30.17±5.46^{b}	19.94±2.32^{ab}	16.05±4.32^{a}	20.99±8.17^{ab}	15.47±3.69^{a}	22.15±6.27^{ab}
SGR	0.75±0.15^{b}	0.61±0.18^{ab}	0.42±0.13^{ab}	0.54±0.24^{ab}	0.41±0.11^{a}	0.57±0.18^{ab}
HIS	2.77±0.83^{a}	2.37±0.22^{a}	2.53±0.17^{a}	2.54±0.31^{a}	2.2±0.08^{a}	2.33±0.03^{a}
GSI	5.34±1.6^{ab}	5.34±1.6^{ab}	6.45±1.3^{ab}	6.80±0.25^{b}	3.87±0.89^{a}	4.67±0.55^{ab}
Hatching Rate	43±5.57^{ab}	38.67±2.08^{ab}	49.33±3.06^{b}	51.33±3.51^{b}	27±2.65^{a}	34.33±6.81^{ab}
Fecundity Rate	5936±978.75^{ab}	6131.33±708.99^{b}	7108.67±318.05^{b}	7033±658.69^{b}	4174±1807.01^{a}	5612±706.59^{ab}
Stress test (% survival)						
Temperature stress	78.67±6.11^{a}	75.33±11.02^{a}	82±11.14^{a}	83.33±15.14^{a}	64.67±16.29^{a}	81.33±5.03^{a}
Salinity stress	98.67±2.31^{ab}	96.67±4.16^{ab}	99.33±.15^{ab}	100^{b}	97.33±1.15^{ab}	94.67±4.16^{a}

*Mean±SD of three replicates. Means in the same row with different superscripts are significantly different (p<0.05). WG: Weight gain, SGR: Specific growth rate, GSI: gonadosomatic index, HSI: hepatosomatic index. P: Broodstock fed *Artemia* biomass enriched with PUFA, M: Broodstock fed *Artemia* biomass enriched with MUFA, H1: Broodstock fed *Artemia* biomass enriched with HUFA, H1: Broodstock fed *Artemia* biomass enriched with HUFA, NA: Broodstock fed Mixture of mollusc and squid, NE: Broodstock fed non-enriched *Artemia* biomass

Table 3: Fatty acid (FA) composition (in % of total FA and mg g^{-1} dry weight) in midgut glands of *Litopenaeus vannamei* after sexual maturation.

Fatty acids	**Treatment**					
	NA	**NE**	**H1**	**H2**	**P**	**M**
C14:0	1.857	3.61	1.53	1.78	2.54	2.79
C14:1n5	0.29	0.12	0.05	0.07	0.04	0.11
C15:0	0.3716	0.23	0.26	0.37	0.2	0.22
C15:1n5	0.39	0.15	0.81	0.47	0.35	0.3
C16:0	19.73	14.81	14.95	19.16	17.05	15.07
C16:1n7	5.52	5.05	2.2	5.55	2.92	7.3
C18:0	7.48	5.45	9.37	7.21	6.54	5.83
C18:1n7	20.76	27.4	22.54	19.53	27.41	32.05
C18:1n9	2.83	6.22	3.72	5.78	3.78	8.98
C18:2n6Cis	8.19	14.7	10.92	6.61	13.37	9.25
C18:2n6trans	2.32	3.69	4.48	2.77	1.41	0.27
C18:3n3	0.87	3.51	1.82	1.07	2.37	2.73
C20:0	0.85	0.29	0.95	0.73	0.6	0.26
C20:1n9	2.12	1.7	2.08	0.43	1.22	0.55
C20:2n6	0.78	0.19	0.8	0.83	1.18	0
C20:4n6	0.47	1.05	3.61	2.8	3.04	1
C20:3n3	0.08	0.07	0.15	0.2	0.2	0
C20:5n3	5.16	5.04	8.22	8.13	4.77	7.07
C22:0	0.24	0.27	0.49	0.27	0.26	0.47
C22:1n9	0	0.1	0	0	0.04	0
C22:6n3	6.16	3.02	8.74	10.29	6.82	0.13
% total fatty acids identified	92.4686	96.67	97.69	94.05	96.11	94.38
Others	7.5314	3.33	2.31	5.95	3.89	5.62

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Table 4: Fatty acid (FA) composition (in % of total FA and mg g^{-1} dry weight) in gonads of *Litopenaeus vannamei* after sexual maturation.

Fatty acids	Treatment					
	NA	NE	H1	H2	P	M
C14:0	5.16	1.36	2.75	1.52	2.39	0.84
C14:1n5	0.1	0	0.16	0.05	0.12	0.24
C15:0	0	0.26	0.28	0.3	0.2	0.3
C15:1n5	0	0.32	0.28	0.42	0.46	1.19
C16:0	8.57	17.18	15.32	17.53	18.05	16.95
C16:1n7	0.2	2.65	5.66	4.29	7	2.34
C18:0	4.14	6.44	6.5	6.52	6.93	11.22
C18:1n7	39.94	28.18	32.23	28.11	22.61	20.78
C18:1n9	1.43	5.66	7.9	5.14	8.23	4.58
C18:2n6Cis	31.6	11.68	10.37	6.93	8.83	7.98
C18:2n6trans	0	0.66	4.17	1.49	3.3	0
C18:3n3	6.96	1.51	2.38	1.22	2.02	0.79
C20:0	0.39	0.44	0.22	0.63	0.23	0.57
C20:1n9	0.68	3.09	1.62	1.35	1.27	0.92
C20:2n6	0.07	2.01	0.26	0.73	0.28	1.14
C20:4n6	0	2.58	1.31	2.89	1.52	4.77
C20:3n3	0	0.32	0	0.11	0	0.22
C20:5n3	0	2.73	4.9	11.98	6.82	7.58
C22:0	0.41	0.22	0	0.19	0.4	0.73
C22:1n9	0.33	0.23	0	0.05	0	0
C22:6n3	0	4.59	0.8	7.75	4.23	5.35
% total fatty acids identified	99.98	92.11	97.11	94.8	94.89	96.89
Others	0.02	7.89	2.89	5.2	5.11	3.11

Discussion

In general, broodstock nutrition studies range from supplementation of specific nutrients, comparison of food with different levels of essential nutrients and the use of artificial vs. fresh food. The variation in tissue levels of several biochemical components during gonad development also represents a useful approach for understanding broodstock nutritional requirements.

HUFAs are essential components in biological membranes. Since crustaceans have little ability to elongate and desaturate the fatty acid chains (Kanazawa *et al.*, 1979), these components have to be accumulated in gonads and eggs to sustain the intense cellular multiplication that occurs during embryogenesis. In general, the studies on broodstock nutrition evaluate the resulting reproductive performance (gonad maturation, number of spawns per female, fecundity) and early offspring quality (fertilization and hatching rate, number of nauplii, egg and nauplius biochemical composition). Burr and Burr (1930) first demonstrated that a specific unsaturated fatty acid configuration, which could not be synthesized by an animal, was essential in the diet. Since then, much research has been done on the essential fatty acid requirements of both land and aquatic

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animals. Most work done with aquatic animals has concentrated on fatty acid requirements of larvae and juveniles (Castell *et al.,* 1972; Sick and Andrews, 1973; Fujii and Yone, 1976; Guary *et al.,* 1976; Castell and Covey, 1976; Kanazawa *et al.,* 1979, 1991; Yu and Sinnhuber, 1979; Kayama *et al.,* 1980; Watanabe *et al.,* 1983; Kanazawa, 1985; D'Abramo, 1990; Sargent *et al.,* 1989). Although our knowledge of the nutrient requirements of prawn broodstock is very limited (Harrison, 1990) it has 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* (Lawrence *et al.,* 1979; Middleditch *et al.,* 1980) and *P. japonicus* (Teshima and Kanazawa, 1983; Teshima *et al.* 1988). In our 45-days feeding trial with white shrimp, the poor fecundity and egg hatchability of the shrimp fed with the mollusc and squid was probably due to the deficiency of essential fatty acids.

The results of the present study demonstrated that feeding with HUFA enriched *Artemia* biomass improved fecundity and it is possible that the n-3 HUFAs of these diets partially met the essential fatty acid requirements of broodstock of white shrimp for egg development.

When broodstock were fed the *Artemia* enriched with HUFA groups (H1 and H2), which are rich in n-3 HUFAs, both fecundity and hatchability were significantly improved (p<0.05). Penaeids have limited ability to elongate and desaturate 18-carbon fatty acids to 20:5n-3 and 22:6n-3 (Kanazawa *et al.,* 1979; Teshima *et al.,* 1992). In the present study, improvement in fecundity and hatchability of *L. vannamei* broodstock fed the *Artemia* biomass enriched with fish oil indicated that these HUFAs must be provided in their diet.

The long-chain n-3 fatty acid (docosahexaenoic acid) also has been shown to play an important role in enhancing egg hatchability in fish. Shimma *et al.,* (1977) found that carp (*Cyprinus carpio*) eggs with lipid levels of 22:6n-3 less than 10% exhibited poor hatchability, which is very similar to the results of the present study. Middleditch *et al.* (1980) demonstrated that *P. setiferus* would not produce eggs unless the diet contained 20:5n-3 and 22:6n-3. They suggested that long-chain unsaturated fatty acids are essential for vitellogenesis of penaeid shrimp.

Recently, several studies which document the fatty acid composition of the organs of different shrimp species have demonstrated that, throughout ovarian maturation, ovarian lipids contained higher proportions of 20:5n-3 and 22:6n-3 than the hepatopancreas (Teshima and Kanazawa, 1983; Jeckel *et al.,* 1989; Ji and Xu, 1992). Mourente *et al.* (1990) showed that 65% of the fatty acids of the total ovarian lipids are incorporated into eggs and embryos during ovarian maturation of *P. kerathurus.* Our results showed that the content of 20:5n-3 in the gonad correlates well with the egg production of the broodstock of *L. vannamei*, whereas the levels of 22:6n-3 in the egg exhibited a close correlation with the hatching rate of the eggs of *L. vannamei* (Table 2).

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These relationships suggest that 20:5n-3 may play some specific roles in the ovarian development process relating to fecundity, whereas 22:6n-3 may play some other roles in early embryogenesis which is related to egg hatchability of larvae in *L. vannamei.* Suzuki *et al.* (1992) have isolated and identified two molecular species of phosphatidylcholine from rainbow trout embryos which induce cell differentiation. These two molecular species of phosphatidylcholine contain 16:0 plus 22:6n-3 and 18:ln-9 plus 22:6n-3. These authors suggested that lipids containing docosahexaenoic acid or the fatty acid itself might play an important role in differentiation and development. The growth and development of the central nervous system is a critical component of the embryonic development of all animals. The n-3 HUFAs, especially 22:6n-3, have also been shown to be critical in brain and neurological development during embryogenesis of fish (Mourente and Tocher, 1992; Tocher *et al.,* 1992) as well as mammals (Neuringer *et al.,* 1986; Kanazawa *et al.,* 1991; Bourre *et al*., 1992) and could be a critical factor in the embryonic development of crustaceans. These two aspects of the role of 22:6n-3 in embryonic development may account for the apparent relationship between 22:6n-3 content in the eggs and hatchability.

The increase in GSI and HSI during sexual maturation of female white shrimp broodstock indicates nutrient accumulation. Lipid accumulation contributes significantly to this increase in ovarian mass during the early stage of maturation. The higher GSI for H2-fed females further confirms that the number of oocytes competing for a limited amount of yolk was probably higher for these females. This result is in accordance with one of the general patterns of the genus *Macrobrachium*, in which changes in egg size are met with a proportional variation in the number of eggs (Jalihal *et al*., 1993). Also the positive correlation between lipid levels in the ovary and GSI in H1 and H2 groups are in agreement with Galois, (1984), who found a correlation between lipid accumulation in the hepatopancras and ovary.

The term larval quality is widely used to refer to the physiological condition, performance during culture (survival and growth) and resistance to stress tests (e.g., manipulation stress and changes in environmental conditions). The temperature and formalin stress test with different modifications is routinely applied by hatcheries to test the resistance of PL (Bray and Lawrence, 1992; Rees *et al*., 1994). In our experiment, the temperature and formalin stress tests were applied at the same age (PL15) for the five groups. Higher survival observed in H2 ($p<0.05$) is probably an effect of increased development and good larval quality. Survival rate to temperature and salinity stress tests depends principally on the osmoregulation capabilities of the larvae, and this in turn is associated with the development of postlarvae (Charmantier *et al*., 1988). Our findings confirm that the offspring

[DOR: 20.1001.1.15622916.2020.19.4.13.1]

quality of shrimp is dependent on the quality of broodstock diet and these facts may indicate that the survival in stress test is associated with the use of fish oil enriched *Artemia* for shrimp broodstock. The results of the test may further confirm that highly unsaturated fatty acids (HUFA), are essential for improvement of egg quality and larval quality in *L. vannamei*.

The results of the present study showed that *Artemia* biomass is a potential candidate for supplementation or replacement of squid and mollusc in maturation diets for *L. vannamei.* PUFA (P group) enriched *Artemia* biomass administered to *L. vannamei* supported adequate growth, and fish oil (H2 group) enriched *Artemia* biomass provided the best results in terms of embryo hatchability and subsequent larval production.

It is suggested that egg development in this species may be only partly related to EPA and DHA, with an elemental role for other HUFAs. So significantly higher essential fatty acids in gonad and hepatopancras were found with the increased dietary essential fatty acids levels. Higher dietary levels of EPA and DHA may have positive effects on the improvement in fecundity and larval quality in *L. vannamei*.

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