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Evaluation of Marine Algal Fatty Acids Supplementation Broodstock Diets on *Macrobrachium rosenbergii* (De Man)

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Abstract

The present study was evaluating the marine algal fatty acids supplementation broodstock diets on *Macrobrachium rosenbergii*. Four broodstock diets were formulated with 3% (diet I), 5% (diet II), 7% (diet III), and 9% (diet IV) inclusion of algal fatty acids to quantify the fatty acid requirement of the brooders for three consecutive spawning cycles. The reproductive parameters such as fecundity, Midgut gland Somatic Index (MSI), and Gonado Somatic Index (GSI) were measured. The fatty acid level in the midgut gland, ovary, and eggs were also measured. The increasing concentration of algal fatty acids in the four broodstock diets have improved the Midgut gland Somatic Index (MSI), Gonado Somatic Index (GSI), and fecundity of the brooders among three spawning cycle. The n-6 and n-3 series of fatty acids level in the midgut gland, ovary, and eggs varied among three spawning cycles. Among the four broodstock diets, diet III performance was higher than diets I, II, and IV. Maximum fatty acids in diet IV showed comparatively less performance than diet III The present study suggested that algal fatty acids supplemented broodstock diets have improved the broodstock performance in fecundity of farm reared *M. rosenbergii* brooders.

Keywords: *Macrobrachium rosenbergii*; Broodstock diet; Algal fatty acids; N-6 and n-3 fatty acids

Introduction

Research on the formulation of nutritionally complete maturation diets commercially important for crustacean is limited [1,2]. A thorough knowledge on the nutritional requirement of crustacean species is important to formulate broodstock diet to augment seed production. Moreover, research on broodstock diet for most commercially cultured shrimps such as *M. rosenbergii* is lacking [3]. However, few studies have been carried out the formulated broodstock diet on *M. rosenbergii* in various aspects of amino acid profile of eggs [4], different levels of phospholipids [1], and polyunsaturated fatty acids [1,5,6]. Although these studies have not considered the blend of essential fatty acids required to optimize the quality of the brood. Further in these studies, the source of n-6PUFA and n-3HUFA is exclusively derived from fish oil [7].

For the past two decades the formulation of aquaculture feeds using fishmeal and fish oil are main ingredients [8-11]. Aquaculture 2010 demonstrated that fish oil to the tune of 0.96 million t or around 75% of the potential supply [12]. However the escalating demand and diminishing availability of fishery byproducts have shown concern over its quality and sustainability to investigate alternative nutrient source [13]. The substitution of fish oil an alternative oil sources, is therefore imperative for the successful expansion of the industry. In the recent years, fish oil replacement has gained considerable attention [14-17].

Various alternative sources have been identified and investigated to reduce the dependency on fish oil [18-21]. The unicellular organisms such as yeast, molds, bacteria, micro algae, and fungi have been used as additives to aquaculture feed formulation. Few studies have been demonstrated that partial replacement with yeast and bacteria. However, there feeds did not yield better survival, growth nor increased the resistance to disease [22,23].

In this regard micro algae constitute a better choice in replacement of fish oil in aquaculture feed industry. The advantages of unicellular organisms in feed is the availability of perfected technical know how for mass production under controlled and environmentally safe conditions. Moreover, the composition of many microorganisms can be manipulated to ensure higher levels of protein and lipid, by enriching with specific essential amino acids or fatty acids [24-27]. In fact, oil extracts from unicellular algae containing Long-Chain Polyunsaturated Fatty Acids (LCPUFA) are already used as nutritional supplements in human infant feeds [28]. Therefore in the present study aims to find out the marine algal fatty acids as a source of n-6 and n-3 series of fatty acids to replacement of fish oil in broodstock diets formulation to *M. rosenbergii* brooders.

Material and Methods

Brooder collection

Pond reared *M. rosenbergii* females were collected from Srikandapuram (10° 55' N, 78° 34' E) Nagapattinum District, Tamilnadu, India and were transported into laboratory in polyethylene bag filled with oxygenated freshwater. The total length (from tip of the rostrum to the end of the telson) and total weight of the females (after blotting them dry) and male were 15.9±0.66cm and

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Table 1: Morphometric parameters of *Macrobrachium rosenbergii* (n = 10; 8 blue claw females: 2 males) fed with broodstock diets (mean ± SD).

Experimental diets	Total length (cm)	Carapace length (cm)	Post orbital Length (cm)	Total weight (g)
I	17.0±1.41*	8.45±0.78*	4.7±0.14*	60.9±0.49*
	15.9±0.66*	7.9±0.50*	3.9±0.20*	39.61±0.98*
II	17.0±0*	8.95±0.64*	4.65±0.07*	60.3±0.28*
	15.97±0.51*	8.14±0.43*	4.03±0.18*	40.21±1.83*
III	17.1±0.14*	8.75±0.21*	4.5±0.14*	60.9±0.49*
	15.93±0.69*	8.03±0.33*	3.97±0.15*	40.43±0.63*
IV	16.9±0.35*	8.65±0.07*	4.5±0.14*	61.2±1.55*
	15.95±0.53*	8.06±0.47*	3.91±0.12*	40.3±0.49*

Within row the asterisks represent significant differences (P<0.05).

Table 2: Composition (% dry weight) of broodstock experimental diet (mean ± SD; n = 3).

Ingredients	Diets			
	I	II	III	IV
Fish meal I (*Stolephorus commersonii*)	16.0	16.0	16.0	16.0
Fish meal II (*Sardinella fimbriata*)	16.0	16.0	16.0	16.0
Prawn meal	15.0	15.0	15.0	15.0
Casein	10.0	10.0	10.0	10.0
Soybean meal	10.0	10.0	10.0	10.0
Wheat flour	10.0	10.0	10.0	10.0
Starch	10.0	8.0	6.0	4.0
Algal mix[a]	3.0	5.0	7.0	9.0
Cholesterol	1.0	1.0	1.0	1.0
Astaxanthin	1.0	1.0	1.0	1.0
Vitamin C	0.5	0.5	0.5	0.5
Vitamin E	0.5	0.5	0.5	0.5
Vitamin mix[b]	2.0	2.0	2.0	2.0
Mineral mix[c]	3.0	3.0	3.0	3.0
Choline chloride	1.0	1.0	1.0	1.0
BHT[d]	1.0	1.0	1.0	1.0
Analyzed proximate composition				
Crude protein	45.4±0.12*	45.4±0.20*	45.5±0.30*	45.8±0.25*
Crude fibre	0.5± 0.1*	0.6±0.1*	0.6±0.1*	0.6±0.1*
Total Ash	11.7±0.1*	11.8±0.1*	12.1±0.1*	12.3±0.1*
Lipid	8.76±0.15*	8.86±0.05*	8.93±0.23*	9.0±0.26*
Moisture	12.5±0.1*	13.2±0.1*	12.9±0.1*	13.4±0.1*

Within row the asterisks represent significant differences (P < 0.05).

[a]Algal mix comprises of Algamac 2000 and Algamac 3050 derivatives of spray dried cells of *Schizochytrium*; Aquagrow DHA and Aquagrow ARA a derivative of spray dried *Crypthecodinium*.

[b]Vitamin mix (Nicholas Piramal India Ltd) mix formulated to provide the following per kg of feed: Vit A, 1,00,000 IU; Vit D^3 10,000 IU; Thiamin, 100mg; Riboflavin, 100 mg; Pyridoxine hydrochloride, 30 mg; Cyanocobalamin, 150 mg; Nicotinamide, 1000 mg; Calcium pantothenate, 160.3 mg; Ascorbic acid, 1500 mg; α-Tocopheryl acetate, 250 mg; Biotin, 2.50 mg.

[c]Mineral mix (Nicholas Piramal India Ltd) formulated to provide the following per kg of feed: Calcium phosphate 1290 mg; Magnesium oxide, 600 mg; Dried ferrous sulphate, 320.04 mg; Manganese sulphate, 20.3 mg; Total phosphorus in the preparation, 250.80 mg; Copper sulphate 33.9 mg; Zinc Sulphate, 22.0 mg; Sodium molybdate, 2.5 mg and Sodium borate 8.8 mg.

[d]BHT - Butylated hydroxy toluene

40.21±1.83g and 17.0±1.41cm and 60.9±0.49g. *M. rosenbergii* mature precociously in farms between size of 6-10g [29] whereas the wild first maturity between 20-40g [30]. Therefore in the present study was used females weighing around 40g to ensure a good quality on par with size of the wild brooders at first maturity.

Experimental design

Each brooder was tagged individually with different color threads on the telson and transferred into four experimental cement tanks (capacity 3500 L) (Table 1). Each tank was supplied aeration. Prawns were fed with commercial grow out diet during the acclimation period. Country made tiles (size 18cm) were provided as shelter to minimize the interaction of the animals [31,32]. There is no significant difference (P<0.05) exist among the stocked prawns in terms of morphometric parameters such as total length, carapace length, postorbital length, and total weight among females at the beginning of the experiment. The experimental tanks were maintained under the ambient environmental conditions of the temperature 28±2 °C, photo period 12L: 12D cycle, ammonia 0.028mg L^{-1}, and nitrate 1.61mg L^{-1}, respectively recommended in a previous study [33]. Experiments were conducted in four groups of 8 females and 2 males in each at the ratio of 1 male: 4 female based on previous study [34]. These were used to replace the experimental animals periodically to quantify the fecundity, gonado-somatic, and midgut gland somatic index measurement.

Experimental diets, feeding and analytical procedures

Four isolipidic (9%) and isonitrogenous (45%) broodstock experimental diets were formulated (Table 2). The n-6 and n-3 polyunsaturated fatty acids were derived from heterotrophically grown spray dried cells of *Scizochytrium* and *Crypthecodinium* sp. of algal mix (Algamac 2000, Algamac 3050, Aquagrow DHA, and Aquagrow ARA). The four broodstock diets were formulated the increasing percentage of 3% (diet I), 5% (diet II), 7% (diet III), and 9% (diet IV) inclusion of algal mix containing increasing concentration of Linoleic (18:2n-6), Linolenic (18:3n-3), Arachidonic (20:4n-6), Eicosapentaenoic (20:5n-3), and Docosahexaenoic acids (22:6n-3) with other dietary ingredients. The brooders were fed to satiation at 3% body weight twice a day at 09:30 and 18:00 h for a period of 90 days. During the experiment 20% of water was exchanged after siphoning out the feces and uneaten feed. Moulting, gonad development, spawning, and mortality were recorded daily. The proximate composition of crude protein, fat, fiber, total ash, and moisture content of the formulated broodstock diets were determined

Table 3: Fatty acid composition (mg g^{-1} dry weight) of the experimental diets incorporated with algal.

Fatty acids	Diet I	Diet II	Diet III	Diet IV
	-3%	-5%	-7%	-9%
14:00	7.19	11.504	16.54	20.851
16:00	4.98	7.968	11.45	14.442
18:00	6.68	10.688	15.36	19.372
18:1n-9	5.74	9.184	13.2	16.646
18:2n-6	13.09	20.944	30.11	37.961
18:3n-3	4.41	7.056	10.14	12.789
20:4n-6	1.57	2.512	3.611	4.553
20:5n-3	4.71	7.536	10.83	13.659
22:6n-3	4.83	7.728	11.11	14.007
Σ Saturates	18.85	30.16	43.355	54.665
Σ Mono-unsaturates	5.74	9.184	13.202	16.646
Σ n-6 PUFA	14.66	23.46	33.718	42.514
Σ n-3 HUFA	13.95	22.32	32.085	40.455
n-6/n-3 ratio	1.05	1.05	1.05	1.05

[35]. The total lipid [36] and fatty acid [37] composition of the tissue samples were analyzed through Gas Chromatography. FAME was analyzed using a Hewlett-Packard 5890 gas liquid chromatograph equipped with DEGS (Diethylene Glycol Succinate) column and Flame Ionization Detector (FID) using nitrogen as the carrier gas. The column temperature was 180 °C and injection temperature was 200 °C. The detector response was recorded. The FAME were identified and quantified by comparison of peak area and retention time of standard fatty acids.

Measurement of fecundity and egg quality

The maturation stages of the brooders were classified according to their ovary size and color observed through the carapace [38]. On third day of spawning, the eggs were manually removed from the abdomen of each female; clutch and female weight were determined separately. Three independent clutch weight samples at 50 mg and the number of eggs in each sample were counted. Fecundity was estimated as the number of eggs per clutch (eggs/g female). The changes during three consecutive reproductive cycles of three females from each broodstock diet experiment diets were measured to record the total weight and then sacrificed to find out Midgut Gland Somatic Index (MSI) and Gonado Somatic Index (GSI) at reproductive stage V. The reproductive performance of females fed with four broodstock diets were quantified by studying the changes in fecundity, clutch weight, gonado somatic index, and midgut gland somatic index for three consecutive spawning cycles. The fatty acid profiles of midgut gland, ovary, and eggs were also quantified. The fatty acid analyses revealed that appeared many fatty acids in trace level. These fatty acids in trace amounts, however, are not known to have any significant role neither in terms of nutrition nor embryonic development [39]; hence they have not been considered further.

Statistical analysis

Data on intermoult cycle, fecundity, female body weight, gonadosomatic index, midgut gland somatic index, and fatty acid

Table 4: Total lipid (% dry weight) and principal fatty acid content (mg g^{-1} dry weight) of midgut gland, ovary, and eggs of females *M. rosenbergii* (mean ± S.D; n = 3) fed with four broodstock diet during the first spawning.

	Diet I	Diet II	Diet III	Diet IV
Midgut gland				
Total lipids	26.7±1.31^a	30.6±1.09^b	32.8±1.01^c	34.7±1.09^d
14:00	17.99±0.07^a	29.58±0.63^b	41.34±0.08^c	50.08±0.05^d
16:00	12.47±0.06^a	19.13±0.05^b	29.79±0.06^c	36.13±0.05^d
18:00	16.03±0.06^a	23.49±0.06^b	36.88±0.07^c	44.56±0.06^d
18:1 n-9	13.20±0.06^a	21.13±0.05^b	29.05±0.07^c	36.63±0.06^d
18:2 n-6	30.77±0.06^a	49.44±0.07^b	69.26±0.06^c	87.33±0.09^d
18:3 n-3	10.23±0.06^a	16.52±0.05^b	23.34±0.07^c	29.55±0.07^d
20:4 n-6	3.65±0.05^a	5.90±0.06^b	8.60±0.04^c	10.52±0.06^d
20:5 n-3	6.12±0.05^a	9.80±0.07^b	14.09±0.08^c	16.40±0.07^d
22:6 n-3	5.79±0.08^a	9.74±0.06^b	13.35±0.03^c	16.82±0.05^d
Σ Saturates	46.46±0.06^a	72.53±0.05^b	108.03±0.04^c	130.73±0.04^d
Σ Mono-unsaturated	13.21±0.05^a	21.13±0.05^b	29.05±0.05^c	36.63±0.05^d
Σ n-6 PUFA	34.42±0.05^a	55.34±0.04^b	77.85±0.06^c	97.84±0.04^d
Σ n-3 HUFA	22.14±0.05^a	36.03±0.04^b	50.74±0.05^c	62.74±0.05^d
Ovary				
Total lipids				
14:00	30.7±1.25^a	33.6±0.964^b	35.7±0.984^c	37.5±1.178^d
16:00	19.42±0.05^a	30.50±0.07^b	42.99±0.06^c	54.23±0.06^d
18:00	12.96±0.06^a	20.73±0.05^b	27.50±0.08^c	37.56±0.06^d
18:1 n-9	16.9±0.8^a	27.80±0.07^b	38.42±0.06^c	46.50±0.04^d
18:2 n-6	13.78±0.05^a	22.96±0.03^b	31.70±0.08^c	38.60±0.07^d
18:3 n-3	32.11±0.04^a	51.74±0.120^b	74.98±0.09^c	95.66±0.04^d
20:4 n-6	10.81±0.07^a	17.29±0.05^b	24.36±0.02^c	30.06±0.06^d
20:5 n-3	4.25±0.07^a	7.05±0.06^b	10.49±0.02^c	12.76±0.07^d
22:6 n-3	8.96±0.08^a	14.32±0.04^b	20.01±0.05^c	23.23±0.05^d
Σ Saturates	9.39±0.07^a	13.93±0.05^b	21.10±0.06^c	25.21±0.07^d
Σ Mono-unsaturated	49.07±0.06^a	79.01±0.05^b	108.89±0.06^c	138.25±0.06^d
Σ n-6 PUFA	13.78±0.04^a	22.96±0.05^b	31.68±0.06^c	38.60±0.07^d
Σ n-3 HUFA	36.38±0.09^a	58.81±0.08^b	85.49±0.09^c	108.42±0.05^d
Eggs	29.12±0.05^a	45.51±0.03^b	65.43±0.06^c	78.49±0.06^d
Total lipids				
14:00	31.73±1.150^a	34.5±1.01^b	36.4±0.954^c	39.6±1.044^d
16:00	20.15±0.08^a	31.65±0.06^b	43.83±0.06^c	55.28±0.09^d
18:00	13.45±0.05^a	22.32±0.05^b	30.95±0.08^c	38.27±0.06^d
18:1 n-9	17.37±0.06^a	28.35±0.09^b	39.96±0.06^c	48.45±0.07^d
18:2 n-6	14.36±0.06^a	23.90±0.07^b	33.02±0.05^c	39.97±0.09^d
18:3 n-3	34.05±0.09^a	55.51±0.07^b	80.40±0.06^c	102.51±0.06^d
20:4 n-6	11.92±0.06^a	18.36±0.07^b	25.37±0.08^c	34.56±0.10^d
20:5 n-3	5.83±0.06^a	9.07±0.09^b	12.65±0.06^c	15.50±0.07^d
22:6 n-3	11.79±0.06^a	18.86±0.08^b	28.19±0.06^c	34.16±0.07^d
Σ Saturates	12.09±0.06^a	20.11±0.07^b	30.01±0.08^c	36.44±0.07^d
Σ Mono-unsaturated	50.96±0.05^a	82.28±0.07^b	114.70±0.04^c	141.96±0.03^d
Σ n-6 PUFA	14.35±0.05^a	23.90±0.06^b	33.01±0.05^c	39.96±0.06^d
Σ n-3 HUFA	39.89±0.07^a	64.59±0.06^b	93.04±0.04^c	118.02±0.05^d
	35.78±0.06^a	57.29±0.06^b	83.55±0.08^c	105.11±0.05^d

Within row the asterisks represent significant differences ($P < 0.05$).

profiles were analyzed by one way Analysis Of Variance (ANOVA) and significant difference compared at ($P < 0.05$); the Least Significance Differences (LSD) test was applied.

Results

Proximate composition in diets

The analyzed proximate composition of lipid and protein composition of the broodstock diets were designed to comprise 9.0% and 45% of the dry weight (Table 2). The n-6 and n-3 polyunsaturated fatty acids levels were increased with increasing percentage inclusion of algal mix indicative of the high level of docosahexaenoic acid (DHA 4.8–14.0mg g^{-1} DW) and Eicosapentaenoic acid (EPA 4.7–13.6mg g^{-1} DW) in the diets (Table 3). Relatively high levels of saturated and n-6 polyunsaturated fatty acids were present in the diets. The n-6/n-3 ratio of all the diets was approximately 1. The fatty acid profile of midgut gland, ovary, and eggs of brooders fed with four broodstock diets for three consecutive spawning has been presented in Table 4,5 and 6.

First spawning

Total lipid content of the midgut gland, ovary, and eggs of females fed with broodstock diets were found to be accumulating among diets with increasing concentration of algal mix. Such an increase in the accumulation is statistically significant ($P<0.05$) (Table 4). The increasing accumulations of total lipids elucidate the maximum utilization of the broodstock diet. The fatty acid profiles of the organs indicate that saturated fatty acids are predominately found over than the n-6PUFA and n-3HUFA. There is a significant difference observed in the accumulation of the principal fatty acids on the basis of the diets ($P<0.05$). Over accumulation of saturated fatty acids such as myristic (14:0), palmitic (16:0), and stearic (18:0) acids emphasize their nutritive role in embryonic stage. The percentage accumulation of various types of fatty acids varies among the organs, which differed from one another. For example in midgut gland the range for saturated 2.2 to 2.6, mono-unsaturated 2.2 to 2.3, n-6 polyunsaturated 2.30 to 2.38, and n-3 highly unsaturated fatty acids 1.19 to 2.34, respectively. Similarly ovarian fatty acid profile revealed that more percentage accumulation of fatty acids, such as saturated 2.4 to 2.7, monounsaturated 2.3 to 2.5, n-6 polyunsaturated 2.45 to 2.9, and n-3 highly unsaturated fatty acids 1.7 to 2.45, which is differed significantly ($P<0.05$). It is interesting to note that when compare with midgut gland and ovary, the accumulation of lipids is higher in eggs. The accumulation of various types of fatty acids, such as saturated 2.5 to 2.8, monounsaturated 2.4 to 2.6, n-6 polyunsaturated 2.6 to 3.7, and n-3 highly unsaturated fatty acids 2.5 to 2.7 in the eggs of prawns fed with four diets are also differed statistically ($P < 0.05$). Increased accumulation of total n-6 polyunsaturated and n-3 highly unsaturated fatty acids in eggs is due to satisfy the nutrient requirement of the growing embryos.

Second spawning

The fatty acid profile of prawns fed with four broodstock diets at second spawning revealed that there is a significant difference in accumulation ($P<0.05$). The accumulation range of fatty acids aree saturated (2.25 to 2.65), monounsaturated (2.25 to 2.35), n-6 polyunsaturated (2.36 to 2.45), and n-3 highly unsaturated fatty acids (1.25 to 2.53) in the midgut gland among four diets were differed significantly ($P<0.05$). Regarding the fatty acid profile of ovary, there

Table 5: Total lipid (% dry weight) and principal fatty acid content (mg g^{-1} dry weight) of midgut gland, ovary, and eggs of females *M. rosenbergii* (mean ± S.D; n = 3) fed with four broodstock diet during the second spawning.

	Diet I	Diet II	Diet III	Diet IV
Midgut gland				
Total lipids	28.7±0.802^a	31.56±1.305^b	33.93±1.250^c	35.8±1.054^d
14:00	18.34±0.07^a	30.50±0.06^b	42.19±0.08^c	51.11±0.07^d
16:00	12.71±0.07^a	19.54±0.07^b	30.36±0.08^c	36.86±0.09^d
18:00	16.19±0.08^a	24.06±0.07^b	37.66±0.08^c	45.54±0.07^d
18:1 n-9	13.33±0.06^a	21.58±0.05^b	29.71±0.06^c	37.47±0.08^d
18:2 n-6	31.83±0.07^a	51.13±0.09^b	73.77±0.06^c	91.12±0.07^d
18:3 n-3	11.14±0.10^a	17.87±0.08^b	25.58±0.09^c	31.97±0.04^d
20:4 n-6	3.73±0.06^a	5.99±0.09^b	8.76±0.08^c	10.94±0.05^d
20:5 n-3	6.23±0.07^a	10.19±0.09^b	14.63±0.06^c	17.08±0.07^d
22:6 n-3	6.34±0.05^a	9.68±0.08^b	13.91±0.07^c	18.22±0.05^d
Σ Saturates	47.19±0.04^a	74.07±0.05^b	110.18±0.07^c	133.44±0.05^d
Σ Mono-unsaturated	13.34±0.05^a	21.60±0.07^b	29.71±0.04^c	37.46±0.07^d
Σ n-6 PUFA	35.53±0.04^a	57.08±0.05^b	82.50±0.04^c	102.05±0.05^d
Σ n-3 HUFA	23.72±0.04^a	37.69±0.06^b	54.09±0.07^c	67.26±0.06^d
Ovary				
Total lipids				
14:00	32.1±1.258^a	34.7±1.058^b	36.8±1.10^c	39.7±0.850^d
16:00	19.77±0.05^a	30.51±0.06^b	43.84±0.07^c	55.26±0.05^d
18:00	13.20±0.06^a	20.34±0.07^b	28.06±0.05^c	38.29±0.06^d
18:1 n-9	17.04±0.05^a	28.34±0.06^b	39.19±0.04^c	47.48±0.08^d
18:2 n-6	14.07±0.05^a	23.44±0.08^b	32.35±0.06^c	39.13±0.07^d
18:3 n-3	34.04±0.06^a	54.89±0.07^b	79.79±0.06^c	104.40±0.06^d
20:4 n-6	11.29±0.04^a	18.00±0.07^b	24.86±0.05^c	33.28±0.09^d
20:5 n-3	3.79±0.06^a	5.54±0.05^b	8.77±0.09^c	11.08±0.08^d
22:6 n-3	9.18±0.05^a	14.72±0.07^b	20.05±0.05^c	23.92±0.06^d
Σ Saturates	9.66±0.05^a	15.17±0.06^b	21.69±0.09^c	25.92±0.05^d
Σ Mono-unsaturated	49.99±0.05 ^{a}	79.15±0.06 ^{b}	111.07±0.05^c	140.99±0.04^d
Σ n-6 PUFA	14.08±0.06 ^{a}	23.43±0.05 ^{b}	32.34±0.05^c	39.13±0.04^d
Σ n-3 HUFA	37.80±0.05^a	60.41±0.05^b	88.52±0.04^c	115.46±0.06^d
Eggs	30.14±0.05^a	47.83±0.04^b	66.56±0.06^c	83.06±0.04^d
Total lipids				
14:00	32.83±1.01^a	35.8±1.054^b	38.7±0.721^c	40.6±0.700^d
16:00	20.49±0.06^a	31.66±0.08^b	45.50±0.07^c	53.17±0.05^d
18:00	13.69±0.06^a	22.73±0.07^b	31.50±0.05^c	39.01±0.08^d
18:1 n-9	17.72±0.05^a	28.87±0.04^b	42.74±3.44^c	49.39±0.06^d
18:2 n-6	14.65±0.06^a	24.81±0.07^b	33.66±0.05^c	40.80±0.08^d
18:3 n-3	36.00±0.06^a	58.23±0.07^b	83.99±0.05^c	112.00±0.07^d
20:4 n-6	11.47±0.05^a	18.47±0.06^b	25.86±0.05^c	33.66±0.06^d
20:5 n-3	4.34±0.07^a	6.66±0.05^b	12.83±0.04^c	11.17±0.06^d
22:6 n-3	12.03±0.07^a	19.23±0.06^b	28.74±0.08^c	34.84±0.05^d
Σ Saturates	12.33±0.06^a	20.50±0.08^b	30.56±0.05^c	37.15±0.09^d
Σ Mono-unsaturated	51.88±0.04^a	83.22±0.05^b	117.69±0.05^c	141.56±0.06^d
Σ n-6 PUFA	14.65±0.05^a	24.80±0.04^b	33.67±0.05^c	40.79±0.06^d
Σ n-3 HUFA	40.32±0.05^a	64.89±0.06^b	96.83±0.05^c	123.14±0.04^d
	35.81±0.05^a	58.13±0.04^b	85.14±0.05^c	105.61±0.06^d

Within row the asterisks represent significant differences ($P < 0.05$).

was significant difference (P < 0.05) found in saturated (2.45 to 2.75), monounsaturated (2.35 to 2.55), n-6 polyunsaturated (2.40 to 2.65), and n-3 highly unsaturated (1.75 to 2.60) when compared to first spawning. However, eggs are found to have a maximum accumulation of fatty acids when compared to midgut gland and ovary, which is statistically significant (P<0.05) (Table 5).

Third spawning

The individual fatty acids studied in the third spawning indicates that there was significant difference in the percentage of accumulation in all organs with reference to diets. Increase in the level of fatty acids with regard to spawning cycles is statistically significant at 5% level. In midgut gland similar observation was found in the case of n-6 PUFA and n-3 HUFA. Similar trend was observed in ovary and eggs of three consecutive spawning cycles, the accumulation of fatty acids are in the following order: eggs < ovaries < midgut gland. The four broodstock diet has no influence (P>0.05) on the duration of the intermoult period and breeding frequency but had an influence (P<0.05) on GSI and MSI (Table 7). The diets had significant influence (P<0.05) on the fecundity and clutch weight. The number of eggs produced per spawn varied considerably among diets. But there was direct relationship exist between the number of eggs produced or spawn and the diets. The relationship between the number of eggs per spawning event (NES) and female size (W; in g) in each diet is: Diet I = 955+1147 W (r^2 = 0.84); Diet II = 909+2088 W (r^2 = 0.962); Diet III = 1000+1210 W (r^2 = 0.809), and Diet IV = 811+6931 W (r^2 = 0.731) among three consecutive spawning cycles. The egg production efficiency of brooders fed with four broodstock diets were ranged from 914 eggs g^{-1} body weight in diet I and 982 eggs g^{-1} body weight in diet IV in the first spawning cycle, which is significantly increased in the second spawning (P<0.05) but no such difference observed (P>0.05) in the third spawning. The regression between female size and egg production efficiency (number of eggs per female wt; eggs g^{-1}) resulted in low relationship coefficient among diets; Diet I (r^2 = 0.044); Diet II (r^2 = -.226); Diet III (r^2 = -.164), and Diet IV (r^2 = -.378) among three spawning cycles.

Discussion

Nutrition is an important factor which influences the maturation of wild as well as captive crustacean broodstock [2,40]. The growth and fecundity of an organism depends upon a number of environmental and physical factors, including the impacts of food quantity, composition, and seasonal variability [41]. Recent researches on nutrition were mainly focused on the use of highly unsaturated fatty acids on the reproductive performance. Marine fish oil is the main source of highly unsaturated fatty acids. In view of the inconsistent availability of fish oil to satisfy the n-3 highly unsaturated fatty acids and other sources are being tested. Marine algae are an alternate to replace conventional source and satisfy the current requirement of highly unsaturated fatty acids. In this regard, many research being carried out with algae as a nutrient source for fishes [42] and larvae of *P. vannamei* [43].

In the present study incorporation of heterotrophically grown *Scizochytrium* and *Crypthecodinium* sp. in the diet augmented the reproductive performance of *M. rosenbergii* brooders; this resulted in a significantly increased fecundity and three consecutive berried

Table 6: Total lipid (% dry weight) and principal fatty acid content (mg g^{-1} dry weight) of midgut gland, ovary, and eggs of females *M. rosenbergii* (mean ± S.D; n = 3) fed with four broodstock diet during third spawning.

	Diet I	Diet II	Diet III	Diet IV
Midgut gland				
Total lipids	30.53±0.874*	33.43±0.757*	35.5±0.793c	36.8±0.954d
14:00	18.70±0.06*	31.09±0.08*	43.85±0.09c	53.17±0.05d
16:00	12.96±0.06*	19.96±0.09*	30.94±0.05c	37.57±0.08d
18:00	16.72±0.06*	25.14±0.07*	38.44±0.08c	47.48±0.10d
18:1 n-9	14.08±0.07*	22.50±0.06*	31.04±0.07c	39.13±0.06d
18:2 n-6	33.39±0.06*	53.64±0.08*	78.30±0.07c	96.81±0.05d
18:3 n-3	11.69±0.07*	18.69±0.06*	26.39±0.09c	33.90±0.07d
20:4 n-6	3.79±0.07*	6.09±0.06*	9.95±0.07c	11.63±0.06d
20:5 n-3	6.84±0.05*	10.95±0.07*	15.73±0.06c	18.45±0.05d
22:6 n-3	6.63±0.06*	10.45±0.07*	15.00±0.08c	18.93±0.06d
Σ Saturates	48.35±0.05*	76.11±0.06*	113.18±0.07c	138.20±0.06d
Σ Mono-unsaturated	14.07±0.05*	22.52±0.06*	31.04±0.06c	39.15±0.04d
Σ n-6 PUFA	37.18±0.07*	59.72±0.06*	88.24±0.06c	108.43±0.05d
Σ n-3 HUFA	25.15±0.05*	40.06±0.06*	57.08±0.05c	71.26±0.07d
Ovary				
Total lipids				
14:00	33.7±1.201*	35.6±1.250*	37.6±1.30c	40.46±0.611d
16:00	20.14±0.05*	32.24±0.08*	43.00±0.07c	56.17±0.05d
18:00	13.47±0.08*	20.74±0.06*	29.23±0.05c	39.00±0.07d
18:1 n-9	17.37±0.06*	28.89±0.08*	40.72±0.05c	48.44±0.05d
18:2 n-6	14.65±0.06*	23.90±0.07*	33.68±0.06c	39.97±0.07d
18:3 n-3	36.65±0.05*	58.66±0.07*	84.30±0.06c	106.30±0.07d
20:4 n-6	11.44±0.08*	18.36±0.07*	25.41±0.07c	33.28±0.08d
20:5 n-3	3.94±0.05*	6.29±0.07*	9.24±0.08c	11.86±0.07d
22:6 n-3	9.43±0.06*	14.35±0.08*	21.14±0.07c	25.29±0.06d
Σ Saturates*	11.13±0.06*	15.46±0.05*	22.25±0.07c	27.34±0.08d
Σ Mono-unsaturates**	50.95±0.06*	81.80±0.06*	112.39±0.07c	143.73±0.05d
Σ n-6 PUFA	14.65±0.06*	23.88±0.04*	33.66±0.03c	39.94±0.04d
Σ n-3 HUFA	40.60±0.06*	64.94±0.05*	93.51±0.05c	118.15±0.07d
Eggs	32.01±0.06*	48.14±0.05*	68.72±0.07c	85.83±0.05d
Total lipids				
14:00	34.5±0.945*	36.5±0.800b	38.4±1.054c	42.6±0.700d
16:00	20.85±0.05*	32.80±0.07b	45.49±0.06c	55.29±0.08d
18:00	14.20±0.06*	23.54±0.07b	32.65±0.05c	39.00±0.06d
18:1 n-9	18.38±0.06*	29.40±0.07b	40.72±0.06c	51.36±0.09d
18:2 n-6	15.22±0.06*	24.35±0.07b	35.00±0.07c	42.44±0.05d
18:3 n-3	39.29±0.07*	62.84±0.06b	90.34±0.07c	113.89±0.05d
20:4 n-6	12.36±0.06*	19.42±0.08b	26.90±0.07c	35.19±0.06d
20:5 n-3	4.72±0.05*	7.68±0.06b	10.85±0.07c	13.69±0.09d
22:6 n-3	12.50±0.07*	19.97±0.04b	29.80±0.05c	36.20±0.08d
Σ Saturates	12.80±0.08*	21.26±0.06b	31.68±0.05c	38.51±0.07d
Σ Mono-unsaturated	53.42±0.04*	85.70±0.07b	118.84±0.05c	145.60±0.06d
Σ n-6 PUFA	15.23±0.05*	24.35±0.06b	35.00±0.04c	42.45±0.05d
Σ n-3 HUFA	43.99±0.05*	70.50±0.05b	101.16±0.03c	127.55±0.06d
	37.64±0.06*	60.64±0.0b	88.35±0.06c	109.88±0.05d

Within row the asterisks represent significant differences (P < 0.05).

Table 7: Reproductive performance, GSI and MSI of females *M. rosenbergii* (mean ± S.D; n = 3) fed four experimental diets during consecutive three spawning.

	Diets			
	I	II	III	IV
Intermoult period First spawning	26.9±0.83*	25.9±1.35*	26.0±1.60[a]	26.5±1.07*
(days) Second spawning	27.3±1.04*	26.9±1.34*	28.0±1.07[a]	27.0±1.07*
Third spawning	28.0±1.07*	28.1±0.83*	28.4±0.52[a]	28.0±0.76*
GSI (%) First Spawning	9.83±0.026*	9.99±0.035*	10.26±0.055[bc]	10.45±0.050*
Second Spawning	10.26±0.038*	10.27±0.045*[b]	10.31±0.066[bc]	10.19±0.036*
Third Spawning	10.23±0.05*	10.21±0.051*	10.19±0.04[bc]	10.26±0.05*
MSI (%) First Spawning	4.91±0.040*	5.156±0.297*	5.13±0.05[ab]	5.24±0.045*
Second Spawning	5.03±0.025*	5.133±0.035*	5.16±0.04[ab]	5.13±0.045*
Third Spawning	5.15±0.050*	5.096±0.050*	5.12±0.05[ab]	5.18±0.050*

Within row the asterisks represent significant differences (P < 0.05).

moults as evident from earlier studies. In corporation of fatty acids in midgut gland, ovary, and eggs of females in the present study is in agreement previous study in the same species [1,3]. The egg production efficiency of *M. rosenbergii* studied in the farms before conducting the experiment over a 218 *M. rosenbergii* females ranged from 767.1 eggs g^{-1} to 874.4 eggs g^{-1} body weight. When fed with formulated broodstock experimental diets significantly increased (P<0.05) from 914 eggs g^{1} (diet I) to 982 eggs g^{-1} (diet IV) in the first spawning. This is the first study such comparative analyzed in *M. rosenbergii* to improve the brooder quality. At the same time compare to the wild brooder with the present result revealed that the average efficiency of egg production was 844.41 eggs g^{1} body weight of wild *M. rosenbergii* [34].

In the present study significantly increase the fecundity of experimental brooders when compare to the wild brooders. The efficiency of egg production also increases with female size [44] as reported in *M. rosenbergii* [1,45,46]. Previous studies carried out in *M. rosenbergii* for a period of 180 days on maturation diet fortified with fish oil based different levels of n-6 and n-3 HUFA showed an improved fecundity and larval viability [1,3]. Addition of higher amounts of linoleic acid (18:2n-6) from 3 to 13mg g^{-1} improved the efficiency of egg production from 1282.5±254.3 to 1570±203.1. Moreover, high levels of 18:2n-6 and n-3 HUFA (13 and 15mg g^{-1} DW respectively) improved the fecundity, egg hatchability, and overall quality of the larvae.

The dietary n-6/n-3 ratios of these diets are 0.94, 2.64, and 0.70; of these 0.94 produced a higher fecundity and larval quality. In this present study with algal fatty acids indicates that the increased amount of linoleic acid (13.09 to 37.96 mg g^{-1} DW) and n-3 HUFA (13.95 to 40.45mg g^{-1} DW) in the experimental diets significantly improved the fecundity and enhanced egg production efficiency. The spurt in egg production efficiency in first spawning followed by the stagnation in the subsequent spawning may be due to the maximum level attained that was maintained. The maturation performance, offspring quality, and lipid composition of *M. rosenbergii* females fed with chosen levels of phospholipids do not have any specific role on reproduction, however, linoleic acid significantly enhanced the fecundity, but higher level of n-3 HUFA had no such effect [1].

The egg production is not only determined by the linoleic acid content alone, these variations in fecundity may be due to different conditions of female maintenance in the laboratory, physiological conditions of the experimental animals, and season (personal communication, Sorgeloos). Normally female *M. rosenbergii* mature when they reach a size of 15-20g body weight while, broodstock ponds they mature earlier (minimum size of 6.5-10g) [29]. Use of such precociously mature females in hatcheries results in poor egg and larval quality; subsequently offspring of these females mature even more precociously still worsening the quality of offspring. In the wild the size at first maturity ranges from 20-40g [30]. Quality of eggs spawned by a captive broodstock remains a major constraint for the production of viable post larvae in commercial hatcheries [47], which is to a great extent influenced by the quality of diet [48]. Therefore in the present study suggested that algal fatty acids supplemented broodstock diets have improved the broodstock performance in fecundity of farm reared brooders and its for sustainable aquaculture practice. This study indicates that an algal fatty acids supplement instead of fish oil on the nutritional requirement of broodstock of grow-out crustaceans and even widely cultured organisms. Also further investigation on the broodstock performance at fecundity level before recommended the alage fatty acids supplementation instead of fish meal in aquaculture is warranted.

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