

Effect of Various Dietary Levels of Docosahexaenoic and Arachidonic Acids and Different n-3/n-6 Ratios on Biological Performance of Pacific White Shrimp, *Litopenaeus vannamei*, Raised in Low Salinity

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Abstract

A 3 × 3 factorial study was conducted to evaluate the effect of three fixed levels of docosahexaenoic acid meal (DHAM) and arachidonic acid meal (ARAM), produced by using a meal that had high levels of the desired fatty acid (0.23% DHAM–0.05% ARAM, 0.50% DHAM–0.10% ARAM, and 0.75% DHAM–0.15% ARAM), and three n-3/n-6 dietary ratios (0.3, 0.8, and 1.8), as well as their potential interaction on growth, survival, and fatty acid composition of hepatopancreas and muscle tissue of juvenile Pacific white shrimp, *Litopenaeus vannamei*, cultured in low salinity. Two additional reference diets with menhaden fish oil or soy and flax oils (n-3/n-6 ratios of 1.8 and 1.7, respectively) were tested. No significant differences (at $P < 0.05$) and no significant interactions were observed among treatments for final weight, weight gain, or survival after 6-wk feeding. This study confirmed that supplementation of DHA and ARA from alternative sources to fish oil is effective in promoting growth and survival of juvenile *L. vannamei*. The fatty acid profile and n-3/n-6 ratio of shrimp tissue reflected that of dietary lipids, although more studies are required to elucidate how the n-3 and n-6 fatty acid balance in the diet relates to shrimp growth.

Fish oil and fish meal are ingredients commonly used in balanced feed formulations for shrimp culture. They provide not only essential fatty acids (EFA) and amino acids but also attractability and palatability to the diet (Tacon and Akiyama 1997). According to Bell (1998), aquaculture uses over 17% of the global fish oil and fish meal production. Because their production will not increase in the near future, reliance on these fisheries products has to be reduced as aquaculture production continues to intensify. Replacing these ingredients in balanced feeds using vegetable sources of protein and oil has become a tendency (Davis and Arnold 2000; González-Félix et al. 2002a; Samocha et al. 2004), also justified by the cost, scarcity, and, at times, inconsistent quality of these ingredients.

However, various studies have confirmed that marine fish oils such as menhaden oil have a higher nutritional value for juvenile Pacific white shrimp, *Litopenaeus vannamei*, compared with other vegetable lipid sources because they provide EFA, particularly n-3 highly unsaturated fatty acids (HUFA) such as eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3), which are required for maximum growth (Lim et al. 1997; González-Félix et al. 2002a). González-Félix et al. (2003a) also emphasized the value of arachidonic acid (ARA, 20:4n-6) as an n-6 EFA for this species. They observed no differences in the growth-promoting effect of dietary DHA, EPA, ARA, or an n-3 HUFA mixture and concluded that for *L. vannamei*, no preferential EFA activity for the n-3 over the n-6 family was evident because values for weight gain of shrimp fed the various HUFA were remarkably similar. D'Souza and Loneragan (1999) identified ARA as an EFA

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for penaeid larval development, which is considered essential for growth, survival, reproductive quality of sperm and eggs, larval quality, and the immune response regulation (Bell and Sargent 2003). It also regulates cortisol synthesis in stress tests and improves the physiological response to abrupt salinity changes (Van Anholt et al. 2004).

Observations on the requirements for the polyunsaturated linolenic acid (LNA, 18:3n-3) and linoleic acid (LOA, 18:2n-6) indicate that shrimp possibly require some combination of n-3 and n-6 fatty acids in their diet at a ratio that may vary depending on the species (Xu et al. 1994; Chandge and Paulraj 1998; Glencross and Smith 1999). For this open-thelycum species, González-Félix et al. (2003b) observed a lower LNA and LOA requirement than those of other closed-thelycum species but suggested that balanced dietary ratios of fatty acids are probably critical for maximizing the growth response of shrimp.

Inland aquaculture using low-salinity well waters to raise *L. vannamei* has become a new trend in many countries. The mineral composition of the water is critical for the success of the culture because minerals play an important role in the physiological state of the animals and osmoregulation (McGraw et al. 2002; Gong et al. 2004). However, lipids also are of great importance for shrimp cultured in low salinity because it is presumed that metabolic requirement for energy to be used in osmoregulation, ecdysis, and growth increases. Gong et al. (2004) managed to improve a commercial diet used for *L. vannamei* cultured in low salinity by fortifying it with potassium chloride, magnesium oxide, sodium chloride, and two essential lipids for shrimp, phospholipids and cholesterol. At the end of the culture cycle, larger shrimp and better survival of animals with an enhanced osmoregulatory capacity were achieved. Supplementing these essential lipids not only provided a source of phosphorous, EFA, and energy but also facilitated lipid metabolism, transport, and storage in the hepatopancreas, which partly explained the overall better performance of shrimp. Moreover, Palacios et al. (2004a) observed a beneficial effect of supple-

menting HUFA, mostly EPA and DHA, on survival of *L. vannamei* after a salinity stress test, which was associated to a modification on the fatty acid composition of gills and a larger gill area, resulting in enhanced osmoregulatory mechanisms, specifically Na^+/K^+ -ATPase and carbonic anhydrase activities. Evidently, a need to focus research on re-defining shrimp's nutritional requirements for HUFA when raised in low salinity is vital.

The purpose of this study was to evaluate the effect of various dietary inclusions of DHA and ARA and various n-3/n-6 ratios on the biological performance of *L. vannamei* cultured in low salinity.

Materials and Methods

Experimental Diets

Eleven semipurified experimental diets having the same basal composition were manufactured in the laboratory of Auburn University, Department of Fisheries and Allied Aquacultures, and tested in this trial (Table 1). Two diets served as reference diets, the first diet was formulated exclusively with menhaden fish oil (5.02%) and an n-3/n-6 ratio of 1.8, while the second reference diet lacked fish oil but was formulated instead with soy (0.60%) and flax (4.40%) oil and a similar n-3/n-6 ratio of 1.7. The diets were formulated to contain 35% protein with an analyzed lipid content of 9%. In order to assess the requirement for DHA and ARA by juvenile *L. vannamei* cultured in low salinity (4‰), nine diets were formulated to contain three fixed levels of each fatty acid, expressed as percentage of diet dry weight, using a combination of two commercially available products, AquaGrow-DHA-*Schizochytrium*® and AquaGrow-ARA-*Mortierella*® (Martek Biosciences Corp., Columbia, MD, USA), provided as meals that contained about 40% lipid and 40% of the desired lipid. The three levels of meal inclusion were as follows: (1) 0.23% Docosahexaenoic acid meal (DHAM)–0.05% Arachidonic acid meal (ARAM), (2) 0.50% DHAM–0.10% ARAM, and (3) 0.75% DHAM–0.15% ARAM. To assess the optimal n-3/n-6 dietary ratio, diets were formulated to contain three ratios, 0.3, 0.8, and 1.8. Soy and flaxseed oil content of the diets was

TABLE 1. *Composition of experimental diets.*

Ingredient	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7	Diet 8	Diet 9	Diet 10	Diet 11
Soybean meal solvent extracted ¹	40.50	40.50	40.50	40.50	40.50	40.50	40.50	40.50	40.50	40.50	40.50
Whole wheat ²	26.00	26.00	26.00	26.00	26.00	26.00	26.00	26.00	26.00	26.00	26.00
Poultry by-product meal ³	16.00	16.00	16.00	16.00	16.00	16.00	16.00	16.00	16.00	16.00	16.00
Corn gluten meal ⁴	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Wheat starch ²	1.61	1.63	1.55	1.55	1.55	1.63	1.63	1.63	1.63	1.63	1.63
Oil AquaGrow-DHA ⁵	—	—	0.23	0.23	0.23	0.50	0.50	0.50	0.75	0.75	0.75
Oil AquaGrow-ARA ⁵	—	—	0.05	0.05	0.05	0.10	0.10	0.10	0.15	0.15	0.15
Menhaden fish oil ⁶	5.02	—	—	—	—	—	—	—	—	—	—
Soybean oil ⁷	—	0.60	4.20	2.40	0.60	3.90	2.20	0.50	3.70	2.05	0.40
Flaxseed oil ⁸	—	4.40	0.60	2.40	4.20	0.50	2.20	3.90	0.40	2.05	3.70
Trace mineral premix ⁹	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Vitamin premix ¹⁰	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Vitamin C ¹¹	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07
CaP-dibasic ²	2.60	2.60	2.60	2.60	2.60	2.60	2.60	2.60	2.60	2.60	2.60
Lecithin (soy refined) ²	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Cholesterol ²	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Total	100	100	100	100	100	100	100	100	100	100	100
Estimated n-3/n-6 ratio	1.8	1.7	0.3	0.8	1.7	0.3	0.8	1.8	0.3	0.8	1.9

DHA = docosahexaenoic acid; ARA = arachidonic acid.

¹ Dehulled solvent-extracted soybean meal; Southern States Cooperative, Inc., Richmond, Virginia, USA.

² MP Biochemicals, Inc., Aurora, Ohio, USA.

³ Griffin Industries, Inc., Cold Springs, Kentucky, USA.

⁴ Wheat gluten; Grain Processing Corporation, Muscatine, Iowa, USA.

⁵ AquaGrow-DHA (*Schizochytrium* sp. algae meal) and AquaGrow-ARA (*Mortierella* sp.); Advanced BioNutrition Corp., Columbia, Maryland, USA.

⁶ Omega Protein, Inc., Reedville, Virginia, USA.

⁷ Clarkson Grain Co., Inc., Cero Gordo, Illinois, USA.

⁸ Sigma, St. Louis, Missouri, USA.

⁹ ICN, Aurora, Ohio, USA. g/100 g premix: cobalt chloride 0.004, cupric sulfate pentahydrate 0.250, ferrous sulfate 4.0, magnesium sulfate heptahydrate 28.398, manganous sulfate monohydrate 0.650, potassium iodide 0.067, sodium selenite 0.010, zinc sulfate heptahydrate 13.193, and filler 53.428.

¹⁰ Fisher Scientific, Pittsburgh, Pennsylvania, USA. g/kg premix: thiamin HCl 0.5, riboflavin 3.0, pyridoxine HCl 1.0, DL-Ca-pantothenate 5.0, nicotinic acid 5.0, biotin 0.05, folic acid 0.18, vitamin B12 0.002, choline chloride 100.0, inositol 5.0, menadione 2.0, vitamin A acetate (20,000 IU/g) 5.0, vitamin D3 (400,000 IU/g) 0.002, DL-alpha-tocopheryl acetate (250 IU/g) 8.0, and alpha-cellulose 865.266.

¹¹ Chelated Minerals Corporation, Salt Lake City, Utah, USA. 250 mg/kg active C supplied by Stay C® (L-ascorbyl-2-polyphosphate 35% active C) and Roche Vitamins, Inc., Parsippany, New Jersey, USA.

manipulated to achieve the desired n-3/n-6 ratios and to bring them to the desired total lipid content. Diets were prepared by mixing the dry ingredients in a mixer (Hobart, Troy, OH, USA) for 30 min. Subsequently, lipids were blended into the dry mix, and warm water was then added to the mixture until the appropriate consistency for pelleting was obtained. After that, the diets were passed through a meat grinder and a 2-mm die. The pellets were dried at 45 C in a forced-air oven to a moisture content of less than 10%.

All diets were stored at -30 C until commencement of experimental trials, when they were mechanically crumbled and sieved to desired size. Duplicate samples of each diet were used for lipid analysis.

Experimental Animals and System

A 6-wk growth trial was conducted at the Wet Laboratory of Aquaculture Nutrition and Biotechnology of the Kino Bay Experiment Station, University of Sonora at Kino Bay, Sonora,

Mexico. *L. vannamei* postlarvae (PL) were obtained from the shrimp larviculture laboratory "AcuaPacific, S.A. de C.V.," Mazatlan, Sinaloa, Mexico. They were maintained in a 10-m³ fiberglass circular tank filled with seawater and fed a commercial shrimp feed (Camaronina; Agribrands Purina®, Ciudad Obregon, Sonora, Mexico) with a 40% protein content until they reached an average weight of 1 g. At that time, animals were transferred to an acclimation tank of 1 m³ provided with aeration and heaters (110 V, 300 W Thermajust H15094; NJ, USA) to keep an adequate level of dissolved oxygen (DO; 5.0 ± 0.56 mg/L) and temperature (29.4 ± 0.64 C). Acclimation was carried out by gradually reducing salinity with previously aerated and dechlorinated freshwater in the acclimation tank at a constant rate of 1 g/L/h during 8 h/d until the salinity of 4 g/L was reached. The organisms were subsequently transferred to the experimental system for initiation of the study at a mean body weight of 1.00 ± 0.05 g. Two identical indoor recirculating water systems connected through their sump tanks and sharing the same recirculating water were used, each one of them with fifty 19-L circular polyethylene tanks (bottom area: 0.07 m² and 30 cm diameter). They were filled with 17 L of 4 g/L water, provided with aeration, and an 8% daily water exchange. A randomized design was used to assign each dietary treatment to nine experimental tanks. Four shrimp, very similar in size, were blotted dry, weighed as a group, and stocked into the tanks. Initial and final weights were calculated by dividing the group weight by the number of shrimp weighed. Feeding rate was adjusted to feed shrimp slightly in excess, half of the daily ration was provided in the morning (0900 h) and the rest in the afternoon (1700 h). Uneaten feed, fecal waste, and molt exuviae were removed daily before the first feeding. Temperature, salinity, and DO were monitored daily, while ammonia, nitrite, nitrate, and pH were monitored weekly. After termination of the feeding trial, shrimp were frozen and stored under nitrogen (-30 C). Before lipid analysis, they were thawed and pooled into three composite samples per dietary treatment. Samples of hepatopancreas (midgut gland) and tail muscle were removed.

Each composite sample consisted of tissues from three shrimp.

Lipid Analyses

Experimental diets and samples of hepatopancreas and muscle tissue were analyzed for lipid and fatty acid compositions. Lipids were extracted by the method of Folch et al. (1957) and quantified gravimetrically after drying an aliquot under nitrogen. Total lipid content was expressed as percentage of wet tissue or dry diet. Fatty acids (FA) were transesterified with boron trifluoride, and fatty acid methyl esters (FAME) were analyzed with a Varian 3800 gas chromatograph equipped with a 30-m \times 0.25-mm fused silica capillary column and a flame ionization detector as previously described (Lochmann and Gatlin 1993). FA were identified by comparison of retention times to those of known standards, and they were quantified by using an internal standard (heptadecanoic acid, 17:0); they were expressed as percentage of the total FAME and in mg/g of wet weight.

Statistical Analysis

Final weight, weight gain (expressed as percentage of initial weight), and survival were the indexes used to evaluate shrimp performance. Survival was transformed by arcsine square root before statistical analysis. Data were analyzed by two-way ANOVA to investigate the effect of DHAM-ARAM level and n-3/n-6 ratio and their interaction. In the absence of interactions, one-way ANOVA of single factors was performed. Duncan's multiple range test was used as the mean separation procedure ($P < 0.05$). Statistical analyses were carried out using SAS (version 8.1; SAS Institute, Cary, NC, USA).

Results

Temperature and salinity were controlled at 29.05 ± 0.57 C and 4.10 ± 0.19 g/L, respectively. DO averaged 4.91 ± 0.58 mg/L. Ammonia, nitrite, nitrate, and pH averaged 0.20 ± 0.28 mg NH₄-N/L, 0.65 ± 0.60 mg NO₂-N/L, 2.12 ± 1.78 mg NO₃-N/L, and 7.34 ± 0.25 , respectively. No significant differences among treatments were observed for survival, and no significant

interactions were observed for any of the responses. Likewise, no significant differences in final weight and weight gain were observed at the end of the trial. In spite of the absence of significant differences, animals fed the diets with the fixed level of 0.50% DHAM–0.10% ARAM attained the highest final weight (4.23 g) and weight gain (428.47% of initial weight) among the treatments. The n-3/n-6 dietary ratio of 0.56 also produced the highest final weight (4.25 g) and weight gain (423.15% of initial weight) among the treatments, although they were not significantly different (Table 2).

Total lipid content of experimental diets was relatively constant and averaged $9.07 \pm 0.32\%$; therefore, they were considered isolipidic. The test diets reflected the fatty acid profile of the oils used in their formulation. Reference diet with

menhaden oil had a higher content of DHA than the rest of the diets. The DHA content increased as the dietary level of inclusion augmented, and within each inclusion level, it generally tended to increase as the n-3/n-6 ratio decreased (Table 3). The highest content of ARA was attained in diets with the highest inclusion level, 0.15% of diet. Because the n-3/n-6 desired dietary ratio was attained by manipulation of the dietary inclusion of soy and flaxseed oil, the dietary content of LOA and LNA reflected the formulation; as the dietary ratio increased, LNA increased and LOA decreased; however, the actual dietary ratios were lower than the theoretical ratios estimated in the formulations and averaged 0.22, 0.56, and 1.06 in the experimental diets and 0.88 and 1.04 in the reference diets containing menhaden and soy with flaxseed oil, respectively.

TABLE 2. Initial weight, final weight, weight gain, and survival of *Litopenaeus vannamei* raised in low salinity and fed different dietary levels of DHAM, ARAM, and n-3/n-6 ratio.

Treatment ¹	Initial weight (g)	Final weight (g)	Weight gain (% of initial weight)	Survival (%)
Diet 1	0.99 ± 0.05	4.54 ± 0.72	460.20 ± 81.00	69.4 ± 20.8
Diet 2	1.02 ± 0.06	3.87 ± 0.66	381.95 ± 73.24	44.4 ± 20.8
Diet 3	0.98 ± 0.04	3.72 ± 0.75	379.43 ± 84.64	58.3 ± 21.7
Diet 4	1.02 ± 0.04	4.32 ± 0.78	424.44 ± 82.55	61.1 ± 18.2
Diet 5	0.98 ± 0.06	3.95 ± 0.71	402.92 ± 61.11	72.2 ± 26.4
Diet 6	0.99 ± 0.06	4.21 ± 0.46	424.50 ± 56.06	66.7 ± 12.5
Diet 7	0.97 ± 0.05	4.17 ± 0.65	427.99 ± 66.08	62.5 ± 18.9
Diet 8	1.00 ± 0.07	4.31 ± 0.50	432.86 ± 61.24	66.7 ± 17.7
Diet 9	1.02 ± 0.04	4.25 ± 0.53	413.82 ± 56.96	58.3 ± 21.7
Diet 10	1.01 ± 0.04	4.26 ± 0.52	417.55 ± 44.17	72.2 ± 8.3
Diet 11	1.02 ± 0.04	4.05 ± 0.44	399.42 ± 42.72	66.7 ± 12.5
%DHAM–%ARAM ²				
0.23–0.05%	0.99 ± 0.05	3.99 ± 0.76	402.26 ± 76.17	63.9 ± 22.3
0.50–0.10%	0.99 ± 0.06	4.23 ± 0.52	428.47 ± 58.66	65.4 ± 15.9
0.75–0.15%	1.02 ± 0.04	4.19 ± 0.49	410.26 ± 47.15	65.7 ± 15.7
n-3/n-6 Ratio ²				
0.3	1.00 ± 0.05	4.05 ± 0.62	405.92 ± 67.48	61.1 ± 18.8
0.8	1.00 ± 0.05	4.25 ± 0.64	423.15 ± 63.61	65.4 ± 15.9
1.8	1.00 ± 0.06	4.10 ± 0.56	411.73 ± 55.67	68.5 ± 19.1
ANOVA ($P > F$) ²				
%DHAM–%ARAM	0.1076	0.3247	0.3109	0.9280
n-3/n-6	0.8952	0.4917	0.5979	0.3343
%DHAM–%ARAM × n-3/n-6	0.2572	0.4181	0.7924	0.4700

DHAM = docosahexaenoic acid meal; ARAM = arachidonic acid meal.

¹ Values are means of nine replicates ± SD and a one-way ANOVA; see Table 1 for diet designation.

² Values are means of 27 replicates ± SD from a two-way ANOVA where reference treatments Diets 1 and 2 were not included.

TABLE 3. Total lipid (% of diet) and fatty acid compositions (mg/g wet weight) of experimental diets.¹

Fatty acid	Reference diets		0.23% DHAM-0.05% ARAM			0.50% DHAM-0.10% ARAM			0.75% DHAM-0.15% ARAM		
	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7	Diet 8	Diet 9	Diet 10	Diet 11
Total lipid	9.23	9.17	8.81	9.07	9.27	8.91	9.18	9.16	8.98	9.42	8.56
16:0	13.54	8.68	10.58	11.26	8.45	12.15	10.16	7.69	10.94	9.94	10.69
18:0	2.86	2.86	3.43	1.76	2.36	3.69	3.15	2.35	3.35	2.82	3.20
18:1	11.55	16.23	19.03	21.17	15.14	20.43	17.83	13.72	18.71	16.63	18.52
18:2n-6	10.74	15.82	25.27	24.45	14.81	27.33	20.61	13.27	24.37	18.89	17.71
18:3n-3	1.40	16.63	4.29	12.41	15.33	4.34	10.06	13.12	3.56	8.94	17.63
20:4n-6	0.50	0.12	0.37	0.43	0.33	0.59	0.55	0.44	0.73	0.63	0.66
20:5n-3	4.14	0.00	0.04	0.16	0.08	0.26	0.22	0.09	0.05	0.19	0.18
22:6n-3	3.58	0.00	1.17	0.86	0.60	1.46	1.52	1.18	2.33	2.08	1.94
Saturates ²	21.24	11.75	14.37	13.42	11.15	16.64	13.96	10.56	15.18	13.67	14.80
Monounsaturates ³	17.64	17.58	20.69	23.11	16.60	22.37	19.47	15.07	20.54	18.31	20.42
PUFA and HUFA ⁴	22.32	32.56	31.25	38.66	31.15	34.24	33.20	28.33	31.34	30.99	38.33
Total n-3 ⁵	9.88	16.63	5.50	13.48	16.01	6.11	11.82	14.46	6.00	11.28	19.78
Total n-6 ⁶	11.30	15.93	25.70	24.99	15.14	28.02	21.27	13.79	25.22	19.63	18.49
n-3/n-6 Ratio	0.87	1.04	0.21	0.54	1.06	0.22	0.56	1.05	0.24	0.57	1.07

ARAM = Arachidonic acid meal; DHAM = Docosahexaenoic acid meal; HUFA = highly unsaturated fatty acids; PUFA = polyunsaturated fatty acids.

¹ Values represent averages of duplicate samples; see Table 1 for diet designation.

² Saturates: 12:0, 14:0, 16:0, 18:0, 20:0, and 22:0.

³ Monounsaturates: 16:1, 18:1, 20:1, and 22:1.

⁴ PUFA and HUFA: 16:3, 16:4, 18:2n-6, 18:3n-3, 20:2, 20:3n-6, 20:4n-6, 20:3n-3, 20:5n-3, 22:2, 22:3, 22:4, 22:5n-3, and 22:6n-3.

⁵ Total n-3: 18:3n-3, 20:3n-3, 20:5n-3, 22:5n-3, and 22:6n-3.

⁶ Total n-6: 18:2n-6, 20:3n-6, and 20:4n-6.

Total lipid in shrimp muscle decreased from an initial content of 2.20% to values that ranged from 1.21 to 1.42%, and in hepatopancreas, total lipid increased from an initial 7.29% to values that ranged from 9.36 to 13.29%, showing no significant differences among treatments (Tables 4 and 5). The fatty acid composition of the test diets was reflected to a certain extent in the fatty acid composition of hepatopancreas and muscle tissue of shrimp. For instance, DHA was significantly higher in both tissues of shrimp fed diets with menhaden oil and with the highest dietary level of inclusion of this particular fatty acid (Fig. 1). ARA also was higher in hepatopancreas and muscle tissue of shrimp fed the diets with the highest dietary inclusion of this fatty acid (Fig. 2). The n-3/n-6 ratios of the initial muscle sample were 1.71 and 1.09 for hepatopancreas; then, the ratios observed in shrimp tissues also tended to reflect the dietary ratios of the experimental diets fed, particularly in hepatopancreas. However, higher ratios than those of the diets were observed in muscle

of animals fed the 0.22 and 0.56 dietary ratios and slightly lower ratios in muscle of shrimp fed the 1.06 dietary ratio (Tables 4 and 5). The n-3/n-6 ratio observed in muscle of animals fed the menhaden reference diet was the exception because it was particularly high compared with the dietary ratio fed to these animals or the other treatment ratios for this tissue.

Discussion

After 6 wk under these experimental conditions, the highest final weight (4.54 g) and weight gain (460.2%) were attained in animals fed the menhaden fish oil reference diet (Diet 1). Menhaden fish oil contains approximately 8% DHA, 14% EPA, and 1% ARA (US Food and Drug Administration 2002); hence, it adequately supplies these EFA for optimal shrimp growth. Final weight (3.87 g), weight gain (381.95%), and survival (44.4%) for animals fed the soy and flaxseed oil reference diet (Diet 2) were numerically very poor, indicating a need for a dietary source of HUFA. However, it is clear

TABLE 4. Total lipid (%) and fatty acid compositions (mg/g wet weight) of *Litopenaeus vannamei* muscle tissue.¹

Fattyacid	Reference											
	Initial ²	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7	Diet 8	Diet 9	Diet 10	Diet 11
Total lipid	2.20	1.34 ± 0.08	1.33 ± 0.18	1.41 ± 0.04	1.28 ± 0.08	1.25 ± 0.14	1.21 ± 0.03	1.42 ± 0.12	1.34 ± 0.05	1.36 ± 0.04	1.24 ± 0.05	1.22 ± 0.08
16:0	8.94	10.34 ± 0.20 ^{ab}	5.69 ± 0.46 ^f	7.56 ± 0.47 ^e	8.70 ± 0.88 ^{de}	8.27 ± 1.10 ^{de}	9.20 ± 0.01 ^{bcd}	8.82 ± 0.41 ^{cde}	8.74 ± 0.95 ^{de}	8.86 ± 0.87 ^{cde}	11.17 ± 0.40 ^a	9.63 ± 0.33 ^{bc}
18:0	4.01	5.44 ± 0.08 ^a	3.73 ± 0.50 ^c	4.08 ± 0.16 ^{bc}	5.54 ± 1.06 ^a	5.57 ± 1.20 ^b	5.24 ± 0.12 ^{ab}	5.37 ± 0.22 ^a	5.22 ± 0.75 ^{ab}	5.23 ± 0.03 ^{ab}	6.29 ± 0.33 ^a	5.82 ± 0.26 ^a
18:1	5.67	7.37 ± 0.21 ^b	5.26 ± 0.58 ^c	7.69 ± 0.67 ^b	8.74 ± 1.22 ^b	8.90 ± 1.79 ^b	7.79 ± 0.39 ^b	8.10 ± 0.19 ^b	8.39 ± 1.08 ^b	8.20 ± 0.94 ^b	10.53 ± 0.44 ^a	8.57 ± 0.42 ^b
18:2n-6	4.19	4.92 ± 0.21 ^d	6.50 ± 1.25 ^d	11.58 ± 0.87 ^{ab}	11.60 ± 1.80 ^{ab}	9.30 ± 1.24 ^c	11.19 ± 0.60 ^{ab}	10.29 ± 0.36 ^{bc}	8.83 ± 0.93 ^c	11.25 ± 0.80 ^{ab}	12.47 ± 0.23 ^a	8.81 ± 0.61 ^c
18:3n-3	0.76	0.26 ± 0.01 ^f	3.49 ± 0.83 ^{bcd}	1.27 ± 0.15 ^e	3.19 ± 0.64 ^{cd}	4.71 ± 0.76 ^a	0.93 ± 0.08 ^{ef}	2.83 ± 0.29 ^d	4.30 ± 0.50 ^{ab}	0.93 ± 0.09 ^{ef}	2.96 ± 0.13 ^d	3.92 ± 0.41 ^{abc}
20:4n-6	1.14	1.66 ± 0.08 ^e	1.38 ± 0.18 ^f	1.99 ± 0.08 ^d	2.20 ± 0.26 ^{cd}	1.96 ± 0.07 ^d	2.89 ± 0.07 ^b	2.42 ± 0.10 ^c	2.26 ± 0.24 ^e	2.72 ± 0.01 ^b	3.34 ± 0.13 ^a	2.75 ± 0.04 ^b
20:5n-3	3.93	7.90 ± 0.04 ^a	1.57 ± 0.55 ^f	1.77 ± 0.05 ^{def}	1.89 ± 0.09 ^{def}	1.72 ± 0.23 ^{ef}	2.17 ± 0.50 ^{de}	1.88 ± 0.24 ^{def}	1.76 ± 0.04 ^{def}	2.26 ± 0.25 ^{cd}	2.76 ± 0.15 ^{bc}	2.41 ± 0.16 ^{bc}
22:6n-3	3.99	6.24 ± 0.18 ^a	1.32 ± 0.38 ^e	2.81 ± 0.10 ^d	3.04 ± 0.23 ^d	2.70 ± 0.04 ^d	4.78 ± 0.16 ^b	4.18 ± 0.22 ^c	4.10 ± 0.53 ^c	5.26 ± 0.11 ^b	6.46 ± 0.47 ^a	5.20 ± 0.08 ^b
Saturates ³	12.96	16.10 ± 0.29 ^{ab}	9.42 ± 0.96 ^d	11.68 ± 0.69 ^c	14.30 ± 1.93 ^b	13.88 ± 2.27 ^{bc}	14.46 ± 0.13 ^b	14.23 ± 0.41 ^b	14.02 ± 0.41 ^b	14.15 ± 0.83 ^b	17.52 ± 0.71 ^a	15.54 ± 0.54 ^{ab}
Monounsaturates ⁴	6.05	8.33 ± 0.62 ^b	5.53 ± 0.56 ^c	8.14 ± 0.81 ^b	9.32 ± 1.29 ^b	9.52 ± 1.91 ^b	8.17 ± 0.39 ^b	8.69 ± 0.21 ^b	9.01 ± 1.18 ^b	8.75 ± 0.99 ^b	11.29 ± 0.52 ^a	9.28 ± 0.46 ^b
PUFA and HUFA ⁵	17.10	25.03 ± 1.43 ^b	17.14 ± 1.71 ^c	22.34 ± 1.21 ^b	25.89 ± 3.58 ^b	24.38 ± 2.15 ^b	25.05 ± 0.96 ^b	24.78 ± 0.55 ^b	24.57 ± 2.61 ^b	25.52 ± 0.63 ^b	32.13 ± 0.87 ^a	26.22 ± 1.09 ^b
Total n-3 ⁶	9.22	15.61 ± 0.66 ^a	7.05 ± 0.75 ^f	6.17 ± 0.11 ^f	8.80 ± 1.03 ^{de}	10.05 ± 0.66 ^{cd}	8.42 ± 0.42 ^c	9.45 ± 0.20 ^{de}	10.97 ± 1.19 ^c	8.73 ± 0.23 ^{de}	12.90 ± 0.48 ^b	12.29 ± 0.44 ^b
Total n-6 ⁷	5.39	6.66 ± 0.26 ^c	7.89 ± 1.22 ^c	13.58 ± 0.95 ^{bc}	13.92 ± 2.08 ^b	11.37 ± 1.22 ^d	14.14 ± 0.53 ^{ab}	12.83 ± 0.43 ^{bcd}	11.21 ± 1.19 ^a	14.10 ± 0.80 ^{ab}	15.98 ± 0.24 ^a	11.68 ± 0.59 ^{cd}
n-3/n-6 Ratio	1.71	2.34	0.89	0.45	0.63	0.88	0.60	0.74	0.98	0.62	0.81	1.05

ARAM = Arachidonic acid meal; DHAM = Docosahexaenoic acid meal; HUFA = highly unsaturated fatty acids; PUFA = polyunsaturated fatty acids.

¹ Values represent means of three composite samples ± SD, each consisting of tissue from three shrimp. Means within rows with the same letter are not significantly different (Duncan's alpha = 0.05).

² Initial values represent a single pooled sample of 20 shrimp. See Table 1 for diet designation.

³ Saturates: 12:0, 14:0, 16:0, 18:0, 20:0, and 22:0.

⁴ Monounsaturates: 16:1, 18:1, 20:1, and 22:1.

⁵ PUFA and HUFA: 16:3, 16:4, 18:2n-6, 18:3n-3, 20:2, 20:3n-6, 20:4n-6, 20:3n-3, 20:5n-3, 22:2, 22:3, 22:4, 22:5n-3, and 22:6n-3.

⁶ Total n-3: 18:3n-3, 20:3n-3, 20:5n-3, 22:5n-3, and 22:6n-3.

⁷ Total n-6: 18:2n-6, 20:3n-6, and 20:4n-6.

TABLE 5. Total lipid (%) and fatty acid compositions (mg/g wet weight) of *Litopenaeus vannamei hepatopancreas*.¹

Fattyacid	Initial ²	Reference										
		Diet 1	Diet 2	Diet 3	Diet 4	0.23% DHAM-0.05% ARAM		0.50% DHAM-0.10% ARAM		0.75% DHAM-0.15% ARAM		
Total lipid	7.29	10.34 ± 1.89	9.78 ± 1.59	10.45 ± 1.93	11.32 ± 3.39	9.36 ± 4.68	13.29 ± 0.73	11.77 ± 1.20	12.06 ± 1.95	10.69 ± 1.51	10.70 ± 1.99	11.50 ± 1.33
16:0	14.11	17.10 ± 0.79 ^a	12.13 ± 1.25 ^b	11.87 ± 1.29 ^c	11.84 ± 0.83 ^c	11.49 ± 2.73 ^c	13.50 ± 0.20 ^b	13.94 ± 0.61 ^b	13.21 ± 0.40 ^b	12.27 ± 0.26 ^b	14.40 ± 1.83 ^b	13.18 ± 0.07 ^b
18:0	6.02	2.91 ± 0.15	3.05 ± 0.25	3.45 ± 0.20	2.83 ± 0.31	2.64 ± 1.12	2.92 ± 0.50	2.90 ± 0.12	2.93 ± 0.13	2.83 ± 0.07	3.08 ± 0.47	3.35 ± 0.53
18:1	16.19	17.48 ± 0.61	23.70 ± 1.37	22.80 ± 4.92	22.54 ± 0.94	23.09 ± 5.12	24.43 ± 0.15	25.09 ± 1.24	25.19 ± 0.55	23.21 ± 0.24	26.01 ± 3.55	24.43 ± 1.66
18:2n-6	10.13	10.77 ± 0.63 ^a	16.47 ± 0.77 ^d	22.47 ± 4.51 ^{ab}	19.48 ± 0.35 ^{bcd}	16.25 ± 3.95 ^d	24.36 ± 3.06 ^a	21.74 ± 1.14 ^{ab}	17.25 ± 0.90 ^{cd}	21.01 ± 2.04 ^{abcd}	21.39 ± 2.88 ^{abc}	18.93 ± 2.54 ^{abcd}
18:3n-3	3.91	0.69 ± 0.03 ^a	10.34 ± 0.73 ^a	2.43 ± 0.61 ^c	6.15 ± 0.18 ^b	9.67 ± 2.11 ^a	2.75 ± 0.21 ^c	6.66 ± 0.59 ^b	10.33 ± 0.22 ^a	1.98 ± 0.01 ^{cd}	5.94 ± 0.47 ^b	11.18 ± 1.16 ^a
20:4n-6	1.01	0.70 ± 0.11	0.62 ± 0.10	0.72 ± 0.25	0.55 ± 0.04	0.80 ± 0.37	0.73 ± 0.11	0.76 ± 0.04	0.85 ± 0.16	0.92 ± 0.12	0.97 ± 0.23	1.04 ± 0.10
20:5n-3	3.99	3.50 ± 0.30 ^a	0.10 ± 0.18 ^d	0.18 ± 0.19 ^{cd}	0.16 ± 0.04 ^{cd}	0.27 ± 0.18 ^{bcd}	0.22 ± 0.03 ^{bcd}	0.19 ± 0.16 ^{cd}	0.38 ± 0.05 ^{bcd}	0.46 ± 0.07 ^b	0.50 ± 0.13 ^b	0.49 ± 0.06 ^b
22:6n-3	3.78	4.32 ± 0.10 ^a	0.08 ± 0.14 ^d	1.00 ± 0.26 ^c	0.82 ± 0.04 ^c	1.00 ± 0.40 ^c	1.65 ± 0.25 ^d	1.72 ± 0.11 ^d	2.02 ± 0.18 ^d	2.58 ± 0.50 ^e	2.85 ± 0.48 ^b	3.21 ± 0.44 ^b
Saturates ³	20.14	22.00 ± 0.99 ^a	15.24 ± 1.09 ^b	14.37 ± 3.24 ^b	14.84 ± 1.16 ^b	14.26 ± 3.69 ^b	15.67 ± 2.34 ^b	17.13 ± 0.70 ^b	16.39 ± 0.46 ^b	15.34 ± 0.25 ^b	17.79 ± 2.31 ^b	18.19 ± 2.75 ^b
Monounsaturates ⁴	16.57	21.65 ± 0.50	25.55 ± 1.47	24.54 ± 5.37	24.13 ± 0.87	24.97 ± 5.39	22.64 ± 2.95	27.14 ± 1.37	26.80 ± 0.04	24.98 ± 0.26	28.08 ± 3.80	29.05 ± 4.91
PUFA and HUFA ⁵	25.91	23.12 ± 0.80 ^c	30.72 ± 2.23 ^{abc}	28.70 ± 6.58 ^b	29.40 ± 0.13 ^b	31.27 ± 7.59 ^{abc}	32.82 ± 5.67 ^{ab}	33.55 ± 1.36 ^{ab}	34.52 ± 0.18 ^{ab}	30.29 ± 1.21 ^{abc}	34.52 ± 4.76 ^{ab}	37.85 ± 4.90 ^b
Total n-3 ⁶	12.22	9.30 ± 0.46 ^a	12.20 ± 1.28 ^b	3.91 ± 1.29 ^c	7.96 ± 0.16 ^d	12.52 ± 2.87 ^b	4.65 ± 0.93 ^c	9.32 ± 0.58 ^d	14.09 ± 0.16 ^{ab}	5.12 ± 0.57 ^e	10.19 ± 1.25 ^{cd}	16.17 ± 1.87 ^a
Total n-6 ⁷	11.20	11.71 ± 0.72 ^a	17.09 ± 0.71 ^c	23.26 ± 4.77 ^{ab}	19.93 ± 0.05 ^{abc}	17.13 ± 4.39 ^c	24.46 ± 5.20 ^a	22.59 ± 1.04 ^{ab}	18.79 ± 0.30 ^b	23.42 ± 0.26 ^{ab}	22.51 ± 3.13 ^{ab}	20.14 ± 2.66 ^{abc}
n-3/n-6 Ratio	1.09	0.79	0.71	0.17	0.40	0.73	0.19	0.41	0.75	0.22	0.45	0.80

ARAM = Arachidonic acid meal; DHAM = Docosahexaenoic acid meal; HUFA = highly unsaturated fatty acids; PUFA = polyunsaturated fatty acids.

¹ Values represent means of three composite samples ± SD, each consisting of tissue from three shrimp. Means within rows with the same letter are not significantly different (Duncan's alpha = 0.05).

² Initial values represent a single pooled sample of 20 shrimp. See Table 1 for diet designation.

³ Saturates: 12:0, 14:0, 16:0, 18:0, 20:0, and 22:0.

⁴ Monounsaturates: 16:1, 18:1, 20:1, and 22:1.

⁵ PUFA and HUFA: 16:3, 16:4, 18:2n-6, 18:3n-3, 20:2, 20:3n-6, 20:4n-6, 20:3n-3, 22:2, 22:3, 22:4, 22:5n-3, and 22:6n-3.

⁶ Total n-3: 18:3n-3, 20:3n-3, 20:5n-3, 22:5n-3, and 22:6n-3.

⁷ Total n-6: 18:2n-6, 20:3n-6, and 20:4n-6.

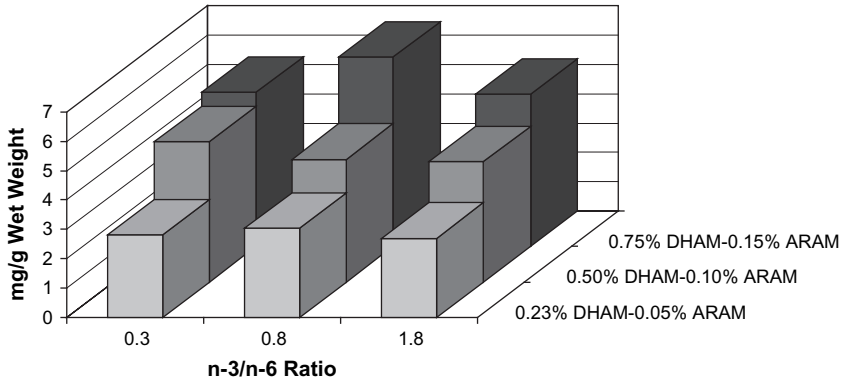


FIGURE 1. Docosahexaenoic acid meal (DHAM) in muscle of *Litopenaeus vannamei* raised in a low-salinity culture system. Values represent means of three replicate observations at three dietary levels of inclusion.

that the rest of the dietary treatments (those with HUFA supplements) performed just as well and attained very similar results in terms of shrimp growth and survival with no significant differences detected, suggesting that the dietary inclusion of DHA and ARA meals was efficient in promoting a similar growth performance in this species compared with animals fed the reference diet with menhaden oil.

In spite of the absence of significant differences, a subtle trend of numerically higher weight gains with the dietary inclusion of 0.50% DHAM–0.10% ARAM was observed, as well as with the n-3/n-6 ratio of 0.56 (Table 2). González-Félix et al. (2002b) evaluated the effect of three dietary

lipid levels on the quantitative requirement for EFA by *L. vannamei* cultured at 25‰ through a factorial experiment with three dietary lipid levels, 3, 6, and 9%, and three inclusion levels of an n-3 HUFA mix of 0.5, 1, and 2%. They reported that the 0.5% inclusion of the n-3 HUFA mix apparently satisfied the nutritional requirements for these EFA and promoted a significantly higher final weight; in fact, significantly depressed growth was observed in animals fed the 2% dietary level of the n-3 HUFA mix, which had already been reported for other species such as *Farfantepenaeus aztecus* when exceeding a 2% inclusion of LNA (Shewbart and Mies 1973) or for *Marsupenaeus japonicus* when including 2% of HUFA in the

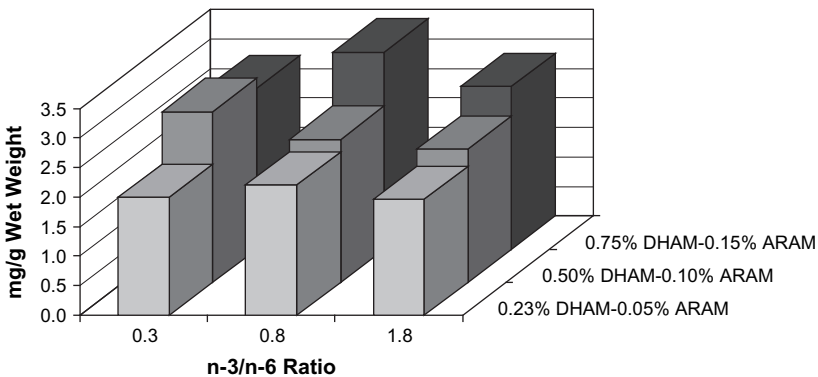


FIGURE 2. Arachidonic acid meal (ARAM) in muscle of *Litopenaeus vannamei* raised in a low-salinity culture system. Values represent means of three replicate observations at three dietary levels of inclusion.

diet (Kanazawa et al. 1985). In another study, González-Félix et al. (2002c) also reported that the instantaneous growth rate of juvenile *L. vannamei* cultured at 25‰ was better when including 0.25% of the n-3 HUFA mix (5.71%/d) and was not significantly different from the 0.5% inclusion (5.93%/d). Results from this study are very similar to the previous reports that suggest the HUFA requirement is close to 0.5% of the diet. Once again, even if no significant differences between the 0.23% DHAM–0.05% ARAM and the 0.50% DHAM–0.10% ARAM dietary inclusions were detected, this last level of inclusion promoted greater growth (3.99 vs. 4.23 g, respectively) of *L. vannamei* when cultured at 4.10 g/L, indicating a very similar requirement for this species when cultured at 25 g/L.

On the other hand, it is known that fatty acids from the n-3 and n-6 families can be synthesized *de novo* by crustaceans, but the limited rate of synthesis compromises their maximum growth. D'Abramo (1997) suggested that the nutritional value of each family resides in that they are provided at an adequate ratio, which is probably that of their natural food. Chandge and Paulraj (1998) reported an optimum growth for *Farfantepenaeus indicus* with an LOA/LNA of 1, each at 0.5% of diet; Xu et al. (1994) also reported the best growth for *Farfantepenaeus chinensis* with the same ratio of LOA/LNA and the same dietary inclusion level. Glencross and Smith (1999) reported an optimum LOA/LNA ratio of 2:3 for *Penaeus monodon*. González-Félix et al. (2003b) reported improved growth for *L. vannamei* fed a diet with an LOA/LNA ratio of close to 1, each at 0.25% of the diet, compared with animals fed each single fatty acid at 0.25% of the diet, but no significant differences were observed between animals fed diets with a 1, 3, or 9 LOA/LNA ratio. In a similar way, this study showed no significant differences between the three dietary ratios evaluated, although the n-3/n-6 ratio of 0.56 promoted greater growth (4.25 g), followed by the 1.06 (4.10 g) and the 0.22 (4.05 g) ratios. In spite of this, it is apparent from a number of studies that an adequate dietary n-3/n-6 fatty acid ratio is important to maximize the growth response of the animals, given that biological mecha-

nisms will affect the utilization of fatty acids depending on the ratio at which they are provided in the diet, particularly the biosynthetic processes of elongation and desaturation of fatty acids by the substrate's competitive inhibition for the Δ^5 and Δ^6 desaturase systems (Henderson and Tocher 1987; Sargent et al. 1993), or the structure of cell membranes, among others (Glencross et al. 2002). An understanding of how the n-3 and n-6 fatty acid balance is interacting with shrimp growth is far from clear; most studies limit the n-3/n-6 ratio to two individual fatty acids from each family, which is not the case of natural or balanced feeds; surely, more research is still needed to elucidate this problem.

Relatively low percentages of survival were attained in this study (44.4–72.2%). However, other studies have reported survival rates for this species in similar salinities, 3.4–5.6 g/L, ranging from 5 to 80% (Saoud et al. 2003) and up to 84% at 5.0 g/L (Gong et al. 2004). Some reports mention that nitrogenous metabolites such as ammonia, nitrite, and nitrate become more toxic in low-salinity than in marine systems. Nitrite concentration in this study (0.65 mg $\text{NO}_2\text{-N/L}$) was below the critical level reported by Lin and Chen (2003) of 6.1 mg/L for water of 15 g/L, which is probably lower at 4 g/L, but it was above the safe level reported by Gross et al. (2004) of 0.45 mg/L in low-salinity brackish water; perhaps, to a certain extent, this may have contributed to mortalities.

Total lipid analysis in the initial muscle sample was 2.20%, and in final samples, it ranged from 1.21 to 1.42% without significant differences among treatments, very similar to those values reported by González-Félix et al. (2003b), which ranged from 0.96 to 1.31% in muscle of *L. vannamei* fed various dietary levels of LNA and LOA and various n-3/n-6 ratios in salinity of 25 g/L or 1.15–1.97% in another study with the same species and salinity, fed various dietary phospholipid levels and n-3 HUFA or DHA (González-Félix et al. 2002c). In hepatopancreas, total lipid ranged from 9.36 to 13.29%, comparable to values reported by González-Félix et al. (2002a) of 5.60–12.26%, or 5.16–10.42% (González-Félix et al. 2002b)

also in salinity of 25 g/L. Palacios et al. (2004a, 2004b) suggested that a low-salinity environment may cause a lipid mobilization in shrimp to meet the requirements for energy imposed by osmoregulation, changing the lipid content as well as the fatty acid profile of cell membranes of gills, muscle, and hepatopancreas. This may explain the lower lipid content in muscle of *L. vannamei* observed at the end of this study at 4.10 g/L of salinity compared with the lipid content observed at the beginning, and because the DHA and ARA dietary requirement appears to be similar when this shrimp species is cultured at low or marine salinities, it is perhaps the total dietary lipid content the one that requires more attention to avoid a drastic lipid mobilization from muscle tissue to meet the energy demand imposed on shrimp by osmoregulation at low salinity. Hepatopancreas, on the other hand, is a dynamic organ in synthesizing and storing lipids; thus, its total lipid content and fatty acid profile vary greatly and can be directly influenced by the energy demand of the organism and its diet.

Fatty acid analysis of experimental diets reflected the composition and dietary inclusion level of the lipids used in each formulation, and in time, they also were reflected in shrimp tissues. The reference diet with menhaden oil (Diet 1) showed the highest level of DHA (3.58 mg/g) and an important ARA content (0.5 mg/g). Muscle of shrimp fed this reference diet also showed the highest content of DHA, followed by muscle of shrimp fed the highest inclusion level for this fatty acid (0.75% DHAM; Fig. 1) and decreased as the dietary inclusion level decreased. For ARA, the highest content in muscle was observed in animals fed the highest dietary inclusion level (0.15% ARAM; Fig. 2), which also decreased as the dietary level decreased (Table 4). These trends in fatty acid profiles confirm what other studies have indicated concerning the great influence of the dietary fatty acid profile on shrimp tissues (Xu et al. 1993, 1994; González-Félix et al. 2002a, 2002b, 2002c, 2003a, 2003b). It also helps explain the significant differences in the content of individual fatty acids in shrimp tissues, derived from the composition and fatty

acid profile of the experimental diets. Although no significant differences in the production parameters were observed in this study, it is apparent that DHA and ARA supplementation is beneficial because the reference diet without menhaden oil (Diet 2) or these EFA showed the poorest survival (44.4%) and low final weight (3.87 g), indicating they should be included in the diet, even when their origin is from an alternative source different to fish oil, which is good news for the balanced feed industry.

Under these experimental conditions, this study confirmed that supplementation of DHA and ARA from alternative sources to fish oil is effective in promoting growth and survival of juvenile *L. vannamei*, comparable to a lipid source commonly used in balanced shrimp feeds, such as menhaden fish oil. In addition, we observed that the fatty acid profile and n-3/n-6 ratio of shrimp tissue reflected that of dietary lipids, although more studies are required to elucidate how the n-3 and n-6 fatty acid balance in the diet relates to shrimp growth.

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