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## Replacement of fish oil in plant based diets for Pacific white shrimp (*Litopenaeus vannamei*)

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## ABSTRACT

Due to economic pressures from high fish oil prices and from buyers and consumers requiring sustainable practices, the use of high levels of fish oils in aquafeeds is no longer desirable. The present study evaluated the replacement of marine fish oil (MFO) with alternative oils in a plant based diet. *Litopenaeus vannamei* juveniles (1.55 g) were stocked into 650 L circular tanks at 26 shrimp tank<sup>-1</sup> and fed 13 experimental diets over a 58-day growth trial. Diets were formulated with soybean oil (SO) as replacement for MFO at inclusion ratios of 100:0, 50:50, 40:60, 30:70, 20:80, and 10:90 as MFO:SO. The next series of diets were formulated to keep n-3/n-6 ratios close to the ratio attained at 50% MFO replacement while removing MFO, this was done by increasing linolenic acid content through the use of linseed oil (LO), resulting in the following MFO:SO:LO ratios, 40:53.4:6.6, 30:56.9:13.1, 20:60:20, and 10:63.7:26.3. Three additional diets were evaluated which included a high LO (10:90 as MFO:LO) and a high soybean oil diet using a low linolenic acid acid soybean oil (LLSO) at a 10:90 ratio of MFO:LLSO. The final diet was a commercial diet which served as a reference. The results showed no statistically significant differences in final mean weight, growth, survival or FCR values of shrimp fed the various diets. Fatty acid (FA) profiles of tail muscle from shrimp fed the various lipid sources in general conformed to the lipids of the feed. Shrimp fed Diet 11, with 19.81 mg of linolenic acid per gram of diet had the highest amount of this FA in shrimp tail muscle (5.61 mg g<sup>-1</sup> wet tissue) and a relatively high n-3/n-6 ratio of 1.15, but at the same time, practically the lowest content of eicosapentaenoic (4.07 mg g<sup>-1</sup> wet tissue) and docosahexaenoic (2.04 mg g<sup>-1</sup> wet tissue) acid among the dietary treatments. This response is typical for animals that cannot elongate and desaturate poly-unsaturated into highly-unsaturated FA (HUFA). As shrimp production was not influenced by lipid source or n-3/n-6 ratio, clearly a range of lipids could be used to support growth. However, as the optimal dietary approach for humans is to consume preformed n-3 HUFA by eating seafood, it would be best for farmed shrimp to retain high levels of n-3 HUFA and high n-3/n-6 ratios as found in wild caught shrimp.

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### 1. Introduction

According to the Food and Agriculture Organization of the United Nations (FAO, 2009), the world's production of captured and farmed shrimp is approximately 6 million tonnes. 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 tonnes) of the world's total shrimp production was from farming. As world population continues to grow, a future increase in demand for seafood is expected.

With the contribution from fisheries at the maximum sustainable level, aquaculture will be the only source for meeting the growing demand for seafood in general and for shrimp in particular.

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

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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). Nevertheless, to replace marine fish oils in commercial feeds, one must have a complete strategy that allows for the replacement of essential FA and enhance the palatability of the diet at the same time.

From an animal production standpoint, as we replace fish oil with alternative oils (e.g., soybean oil), we must ensure that we meet the animals' essential FA requirement. Partial replacement of fish oil in the grow-out period may be possible with little adverse effect on shrimp growth (Patnaik et al., 2006; Samocha et al., 2009). However, in the early growth period, growth and immunity may be compromised. In any case, substitution is likely to reduce the valuable long chain n-3 FA in shrimp tissues. It is also likely to introduce high levels of n-6 FA which are not appropriate from a human nutrition standpoint. Many Western diets are already excessively high in these FA, with n-3/n-6 ratios of 1/15 or lower (Simopoulos, 2002). Published data suggest this excessive amount of n-6 poly-unsaturated fatty acids (PUFA) promotes the pathogenesis of many diseases and, at the same time, describe the health benefits of n-3 highly-unsaturated fatty acids (HUFA) in preventing cardiovascular disease, cancer, inflammatory autoimmune diseases, and supporting brain development and function (Ruxton et al., 2007; Lands, 2008; Chapkin et al., 2009). Seafood is recommended in the diet to increase the n-3/n-6 ratio and promote human health; for that reason, it is critical to ensure that farmed shrimp retain high levels of n-3 HUFA and high n-3/n-6 ratios as found in wild caught shrimp.

Lipid content and the associated C18 PUFA, linoleic (18:2n-6) and linolenic (18:3n-3) acids, as well as n-3 and n-6 HUFA, eicosapentaenoic acid (EPA, 20:5n-3), docosahexaenoic acid (DHA, 22:6n-3), and arachidonic acid (ARA, 20:4n-6) are required in shrimp and other crustacean feeds at levels between 5 and 10 g/kg (Akiyama et al., 1992; González-Félix et al., 2002a). This is an important consideration when replacing fish oil with other substitutes. In addition, a number of laboratory based lipid trials have demonstrated that the fatty acid profile of shrimp tissues parallels the shift in FA profiles of the diet (Deering et al., 1997; González-Félix et al., 2002b, 2003a; Glencross et al., 2002). Given that many plant based feed ingredients contain a low n-3/n-6 ratio (Turchini et al., 2009), the challenge is to promote good shrimp performance while maintaining a high n-3/n-6 ratio ideal for human health. The purpose of this study was to evaluate the replacement of fish oil by soybean oil, as well as the combination of soybean with linseed oil for increasing the n-3/n-6 ratio in practical diets for Pacific white shrimp, *Litopenaeus vannamei*.

## 2. Materials and methods

### 2.1. Source of shrimp and experimental system

Research was conducted at the Texas AgriLife Research Mariculture Laboratory in Corpus Christi, TX, USA. Pacific white shrimp, *L. vannamei*, postlarvae were donated by Shrimp Improvement Systems (Islamorada, FL) and nursed for about 53 days. At the conclusion of the nursery phase, juvenile shrimp ( $1.55 \pm 0.03$  g) were hand sorted for uniform size and stocked into sixty-five partially shaded 650 L circular tanks (bottom area:  $0.85 \text{ m}^2$ ) at 26 shrimp tank<sup>-1</sup> ( $40 \text{ m}^{-3}$  or  $31 \text{ m}^{-2}$ ). Group weight was recorded by tank and a one-way ANOVA was performed to ensure there were no statistically significant differences between treatments in mean shrimp weight at the time of stocking.

All tanks were covered with netting to prevent shrimp losses due to jumping. Each tank was provided with two air-stones having similar air flows of 8–10 L min<sup>-1</sup>. Tanks were filled with natural seawater and chlorinated (5 ppm); chlorine was allowed to dissipate prior to the start of the study. Culture water was circulated between all tanks at a rate of 1.9 L min<sup>-1</sup> to provide one full turnover of water exchange every six hours. Naturally induced primary production was

present and no external biofiltration was provided. Municipal freshwater was added to offset evaporative losses and to maintain salinity. Physicochemical parameters including pH, temperature, salinity and dissolved oxygen (DO) were measured twice daily in eight random tanks. Other water quality indicators including total ammonium-N (TAN, as  $\text{NH}_3 + \text{NH}_4$ ), nitrite-N ( $\text{NO}_2\text{-N}$ ), nitrate-N ( $\text{NO}_3\text{-N}$ ), and reactive phosphorus ( $\text{PO}_4\text{-P}$ ) were measured once a week in eight representative tanks.

### 2.2. Experimental feeds and feeding

Diets were produced in the Nutrition laboratory at the Department of Fisheries and Allied Aquacultures, Auburn University, Auburn, AL, USA. They were formulated to contain 35% protein using solvent extracted dehulled soybean meal and corn gluten meal as the primary protein sources, and different combinations of Menhaden fish oil (MFO) and soy and linseed oils to achieve 6% lipid content. Experimental diets were formulated with six levels of soybean oil (SO) as replacement for MFO at inclusion ratios of 100:0, 50:50, 40:60, 30:70, 20:80, and 10:90 as MFO:SO, and designated as Diets 1 through 6. Another set of diets was formulated to increase the n-3/n-6 ratios and maintain values close to the ratio attained at 50% MFO replacement, while removing MFO at the same time. This was done by increasing the content of linolenic acid with linseed oil (LO), in combination with SO for the replacement of fish oil at inclusion ratios of 40:53.4:6.6, 30:56.9:13.1, 20:60:20, and 10:63.7:26.3 as MFO:SO:LO; they were designated as Diets 7 through 10. Three additional diets were evaluated which included Diet 11 with a high LO content and a MFO to LO ratio of 10:90, Diet 12 with a high soybean oil diet using a low linolenic acid soybean oil (LLSO) at a 10:90 ratio of MFO:LLSO. Finally, Diet 13 was a commercial diet (35% crude protein, 8% crude fat; Rangen, Buhl, ID, USA) which served as a reference. Feed formulations and ingredients are shown in Table 1. Samples of each diet were submitted to New Jersey Feed Laboratory, Inc. (Trenton, NJ, USA) for proximate analysis (Table 1); additional samples were frozen and stored at  $-20^\circ\text{C}$  for duplicate fatty acid analysis.

Dietary treatments were randomly assigned using a double blind experimental design with five replicates per treatment. Initial rations were calculated assuming 100% survival, FCR of 1:1.4 and an estimated growth of  $1.5 \text{ g week}^{-1}$ . One tank from each treatment was equipped with a feed tray to estimate feed consumption. These same tanks were sampled weekly (group weight of five shrimp per tank). Information from the feed trays and the weekly sampling was used to adjust rations during the study.

### 2.3. Lipid and fatty acid analysis

At the end of the 58-day growth trial, the shrimp were harvested, weighed, and counted. Sub-samples of five frozen shrimp from each experimental tank were shipped to Auburn University for FA analysis. Before lipid analysis they were thawed and muscle from the five animals was pooled into a composite sample per tank, ground, and analyzed in duplicate for lipid and fatty acid composition. Experimental diets were analyzed for fatty acid composition as well. Lipids were extracted by the method of Folch et al. (1957) and quantified gravimetrically after drying under nitrogen. Total lipid content was expressed as percent of wet tissue. FA were transesterified with boron trifluoride and fatty acid methyl esters (FAME) were analyzed with a Shimadzu GC-17A gas chromatograph (Shimadzu Scientific Instruments Inc., Portland, OR, USA) equipped with a capillary column (Omegawax 530,  $30 \text{ m} \times 0.53 \text{ mm} \times 0.5 \mu\text{m}$  film thickness, Supelco 2-4019, Sigma-Aldrich, Oslo, Norway) using helium as the carrier gas and a flame-ionization detector as previously described (Quintero et al., 2009). FA were identified by comparison of retention times to those of known standards and they were quantified by using an internal standard, nonadecanoic acid methyl ester (C 19:0, Sigma-Aldrich, St. Louis, MO, USA); they were expressed as  $\text{mg g}^{-1}$  of diet or wet weight, and as percent of the total identified FAME.

**Table 1**  
Ingredient composition (g 100 g<sup>-1</sup> of feed) of experimental diets.

	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7	Diet 8	Diet 9	Diet 10	Diet 11	Diet 12	Diet 13
Soybean meal solvent extracted <sup>a</sup>	54.40	54.40	54.40	54.40	54.40	54.40	54.40	54.40	54.40	54.40	54.40	54.40	–
Whole wheat <sup>b</sup>	28.38	28.38	28.38	28.38	28.38	28.38	28.38	28.38	28.38	28.38	28.38	27.81	–
Corn gluten meal <sup>c</sup>	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00	–
Vitamin premix <sup>d</sup>	1.80	1.80	1.80	1.80	1.80	1.80	1.80	1.80	1.80	1.80	1.80	1.80	–
Trace mineral premix <sup>e</sup>	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	–
Choline chloride <sup>b</sup>	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	–
Stay C 250 mg/kg (25%) <sup>f</sup>	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	–
CaP-dibasic <sup>g</sup>	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	–
Lecithin (deoiled 53% lipid) <sup>h</sup>	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	–
Cholesterol <sup>i</sup>	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	–
Corn Starch <sup>c</sup>												0.47	–
Menhaden fish oil <sup>j</sup>	4.57	2.29	1.83	1.37	0.91	0.46	1.83	1.37	0.91	0.46	0.46	0.46	–
High linolenic acid soy oil <sup>k</sup>		2.29	2.74	3.20	3.66	4.11	2.44	2.60	2.75	2.91			–
Linseed oil <sup>l</sup>							0.30	0.60	0.91	1.20	4.11		–
Low linolenic acid soy oil <sup>k</sup>												4.11	–
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	–
Estimated n-3/n-6 ratio	0.91	0.52	0.40	0.31	0.24	0.18	0.52	0.52	0.52	0.52	2.99	0.09	–
Protein (%)	37.40	36.50	37.20	37.70	37.60	37.80	35.30	36.10	37.10	38.70	37.70	37.00	36.20
Fat (%)	7.06	6.97	7.24	7.40	7.51	7.39	6.95	6.68	7.00	7.01	7.11	7.33	10.78
Fiber (%)	3.22	3.16	3.67	3.27	3.33	3.05	2.97	2.94	3.02	3.12	3.31	3.11	2.92
Ash (%)	7.06	6.94	7.17	7.14	7.13	7.10	6.72	6.70	6.78	7.03	6.95	7.03	7.70
Moisture (%)	7.06	7.85	5.50	5.18	5.03	4.71	9.53	9.85	8.59	5.02	5.42	4.84	8.81

<sup>a</sup> Faithway Feed Co., Guntersville, AL, USA.  
<sup>b</sup> MP Biochemicals Inc., Solon, OH, USA.  
<sup>c</sup> Grain Processing Corporation, Muscatine, IA, USA.  
<sup>d</sup> Vitamin premix (g kg<sup>-1</sup>): thiamin HCl 0.5, riboflavin 3.0, pyridoxine HCl 1.0, DL Ca-pantothenate 5.0, nicotinic acid 5.0.  
<sup>e</sup> Trace mineral premix (g 100 g<sup>-1</sup>): cobalt chloride 0.004, cupric sulphate pentahydrate 0.250, ferrous sulfate 4.0, magnesium sulfate heptahydrate 28.398, manganous sulphate monohydrate 0.650, potassium iodide 0.067, sodium selenite 0.010, zinc sulfate heptahydrate 13.193, filler 53.428.  
<sup>f</sup> Stay C®, (L-ascorbyl-2-polyphosphate), Roche Vitamins Inc., Parsippany, NJ, USA.  
<sup>g</sup> Fisher Scientific, Fair Lawn, NJ, USA.  
<sup>h</sup> Solae Company, St. Louis, MO, USA.  
<sup>i</sup> USB Biochemicals, Cleveland, OH, USA.  
<sup>j</sup> Omega Protein, Inc. Reedville, VA, USA.  
<sup>k</sup> American Soybean Association, St. Louis, MO, USA.  
<sup>l</sup> Sigma-Aldrich Co., St. Louis, MO, USA.

2.4. Data analysis

Differences in final mean weights, growth, survival (arcsine transformed), and FCR, 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 SAS statistical software version 9.1 (SAS Institute Inc., Cary, NC, USA).

3. Results

The results of the daily and weekly water quality monitoring (Tables 2–3.) showed no statistically significant differences between treatments, and the observed values indicate that culture water parameters were within the suitable range for grow out of this species.

Mean final weights ranged from 15.9 to 17.1 g, survival from 90.4 to 99.1%, growth per week 1.76 to 1.91 g week<sup>-1</sup>, FCR 1.16 to 1.30, and yields ranged from 0.592 to 0.658 kg m<sup>-3</sup>. Harvest data are presented in Table 4, along with overall harvest means. One-way ANOVA showed no

**Table 2**  
Summary of daily water quality parameters during the 58-day nutrition study.

		Temperature °C	Salinity ppt	DO mg L <sup>-1</sup>	pH
AM	Mean	27.34	31.00	6.19	7.82
	Max	29.18	31.71	7.24	8.09
	Min	22.9	28.43	4.99	7.32
PM	Mean	28.97	31.02	6.23	7.90
	Max	30.95	31.78	7.3	8.22
	Min	25.09	29.7	5.31	7.59

significant differences ( $P < 0.05$ ) between treatments, including the commercial diet (Table 4).

Total lipid content of experimental diets was relatively constant, consequently they were considered isolipidic (Table 1), except for the commercial reference diet where total lipid content was higher (10.78%). The diets reflected the fatty acid profile of the oils used in their formulation. Diets with more menhaden oil (e.g., diets 1, 13 and 2, respectively) had a higher content of long chained n-3 HUFA (Table 5), including EPA (4.65, 4.49, and 2.63 mg g<sup>-1</sup> diet, respectively) and DHA (2.58, 1.98, 1.42 mg g<sup>-1</sup> diet, respectively). They also contained the highest percentage of ARA (0.46, 0.48, 0.25 mg g<sup>-1</sup> diet, respectively). Linolenic acid was higher in diets with more inclusion of linseed oil (diets 11, 10 and 9 with 19.81, 10.16, and 9: 7.29 mg g<sup>-1</sup> diet, respectively).

After the 58-day feeding trial, total lipid content of shrimp muscle was not significantly affected by dietary treatment. However, fatty acid composition of shrimp muscle tissue reflected the fatty acid profile of the experimental diets fed (Table 6). The n-3 HUFA, DHA and EPA, were always significantly higher in tissues of shrimp fed diets with more menhaden oil (Diet 1: 7.89 mg EPA and 5.62 mg DHA g<sup>-1</sup> wet tissue; Diet 13: 8.21 mg EPA and 5.30 mg DHA g<sup>-1</sup> wet tissue; Diet 2: 7.54 mg EPA and 4.45 mg DHA g<sup>-1</sup> wet tissue) (Fig 1). Linolenic acid was significantly higher in muscle tissue of shrimp fed diets 11, 10, and 9 (5.61, 2.59, and

**Table 3**  
Summary of weekly quality parameters during the 58-day nutrition study.

	TAN mg L <sup>-1</sup>	NO <sub>2</sub> -N mg L <sup>-1</sup>	NO <sub>3</sub> -N mg L <sup>-1</sup>	PO <sub>4</sub> -P mg L <sup>-1</sup>
Mean	0.36	2.5	0.86	0.6
Max	1.54	6.3	4.21	1.8
Min	0.00*	0.0*	0.00*	0.00*

\* Below detectable levels.

**Table 4**  
Harvest data collected after 58-day nutrition study.

Treatment	Mean weight (g)	Survival (%)	Growth (g week <sup>-1</sup> )	FCR	Yield (kg m <sup>-3</sup> )
Diet 1	16.90	92.32	1.88	1.24	0.624
Diet 2	16.65	96.16	1.85	1.19	0.640
Diet 3	16.61	93.84	1.85	1.24	0.620
Diet 4	16.62	99.05	1.85	1.16	0.658
Diet 5	16.18	94.62	1.80	1.26	0.613
Diet 6	16.42	98.08	1.83	1.18	0.644
Diet 7	16.23	98.46	1.81	1.19	0.639
Diet 8	15.90	93.08	1.76	1.30	0.592
Diet 9	17.08	90.40	1.91	1.25	0.613
Diet 10	16.02	96.92	1.78	1.24	0.622
Diet 11	16.33	96.16	1.82	1.22	0.627
Diet 12	16.22	96.92	1.80	1.22	0.628
Diet 13	16.78	96.16	1.87	1.19	0.645
P value (ANOVA)	0.304	0.964	0.314	0.926	0.937

1.94 mg g<sup>-1</sup> wet tissue, respectively), with the highest dietary content of linseed oil (Fig. 1). The highest n-3/n-6 ratio in shrimp muscle was observed in those animals fed Diet 11 (1.31), followed by shrimp fed diets 1 (0.86) and 13 (0.71). For the rest of the dietary treatments, the n-3/n-6 ratios observed in shrimp tissue ranged from 0.15 to 0.49 (Table 6, Fig. 1).

#### 4. Discussion

Due to both economic pressures from high fish oil prices and pressures from buyers and consumers requiring sustainable practices, the use of high levels of fish oils in aquafeeds is no longer

desirable. In the current economic and social climate, feed mill manufacturers and producers are taking a pragmatic approach by looking into practices that will not only reduce feed production costs, but also improve their public image. The most logical approach to reducing the present dependence on fish oil is to increase the use of a combination of ingredients, including SO and soybean lecithin that will balance the formulations. Because any failure of diet formulations on an industry level can result in a negative response from the shrimp producers, it is important that nutrient limitations be identified. This requires that graded levels of nutrient supplementation be tested under semi-controlled production conditions, followed by testing of the resulting diets under pond production conditions. Hence, the present study was designed to evaluate the replacement of the majority of the marine fish oil with alternative oils. This included simple dilution with SO as well as using a mixture of SO and LO to improve the n-3/n-6 ratio.

The results of the present study showed no statistically significant differences in final mean weights, growth, survival or FCR values of shrimp fed diets with SO at different levels of MFO replacement, or diets with higher n-3/n-6 ratios using SO in combination with LO for replacement of MFO. Furthermore, in terms of production, the results were equivalent to those of a 0.71 n-3/n-6 commercial diet which was compared as a reference. The present study confirms that shrimp can be reared in an outdoor system where they have access to natural foods, using a plant based diet and successfully replace up to 90% of the marine oil using a variety of lipid sources. No indication of feed rejection or reduced growth with removal of fish oil and their constituent n-3 HUFA was evident under this experimental conditions, even up to a replacement level of 90% of MFO.

**Table 5**  
Fatty acid composition (mg g<sup>-1</sup> diet and % of FAME) of experimental diets<sup>1</sup>.

Selected FA	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7	Diet 8	Diet 9	Diet 10	Diet 11	Diet 12	Diet 13
<i>mg g<sup>-1</sup> diet</i>													
16:0	10.09	8.98	8.95	8.19	8.07	7.63	9.04	7.82	7.18	7.93	6.17	8.53	12.27
18:0	1.90	2.16	2.28	2.18	2.33	2.32	2.28	2.07	2.00	2.32	1.80	2.44	2.65
18:1n-9	5.49	8.60	9.50	9.38	10.20	10.52	9.51	8.95	9.12	10.99	8.86	11.89	12.05
18:2n-6	11.90	19.78	21.05	21.77	21.97	21.77	20.50	19.90	20.57	23.15	16.00	25.95	12.93
18:3n-3	1.71	2.91	3.25	3.25	3.50	3.65	4.97	5.91	7.29	10.16	19.81	2.73	1.50
20:4n-6	0.46	0.25	0.20	0.14	0.11	0.07	0.21	0.15	0.10	0.05	0.06	0.05	0.48
20:5n-3	4.65	2.63	2.17	1.50	1.10	0.59	2.20	1.50	0.99	0.59	0.62	0.61	4.49
22:6n-3	2.58	1.42	1.19	0.86	0.62	0.33	1.26	0.86	0.56	0.33	0.35	0.33	1.98
Saturates <sup>2</sup>	15.69	13.05	12.85	11.50	11.17	10.36	12.93	11.00	9.92	10.71	8.42	11.44	18.70
Monounsaturates <sup>3</sup>	11.67	12.24	12.70	11.87	12.19	11.97	12.70	11.33	10.92	12.49	10.10	13.50	18.64
PUFA <sup>4</sup>	14.72	23.29	24.79	25.38	25.77	25.61	25.99	26.18	28.11	33.48	36.02	28.87	15.52
HUFA <sup>5</sup>	9.54	5.38	4.47	3.16	2.33	1.27	4.59	3.14	2.10	1.31	1.34	1.32	8.56
Total n-3 <sup>6</sup>	10.73	8.03	7.49	6.24	5.71	4.85	9.34	8.89	9.29	11.41	21.12	3.98	9.53
Total n-6 <sup>7</sup>	12.43	20.08	21.30	21.96	22.15	21.89	20.76	20.09	20.69	23.22	16.09	26.04	13.48
n-3/n-6	0.86	0.40	0.35	0.28	0.26	0.22	0.45	0.44	0.45	0.49	1.31	0.15	0.71
<i>% of FAME</i>													
16:0	19.54	16.63	16.33	15.76	15.68	15.51	16.08	15.12	14.08	13.67	11.04	15.48	19.98
18:0	3.68	4.00	4.16	4.19	4.52	4.71	4.05	4.01	3.93	4.00	3.23	4.43	4.31
18:1n-9	10.63	15.92	17.33	18.07	19.82	21.38	16.91	17.32	17.87	18.96	15.85	21.56	19.60
18:2n-6	23.06	36.69	38.43	41.96	42.70	44.24	36.48	38.55	40.27	39.91	28.65	47.08	21.03
18:3n-3	3.30	5.39	5.92	6.27	6.81	7.43	8.84	11.43	14.27	17.52	35.44	4.96	2.44
20:4n-6	0.90	0.46	0.37	0.28	0.22	0.14	0.37	0.28	0.19	0.09	0.10	0.10	0.79
20:5n-3	9.01	4.87	3.95	2.89	2.13	1.20	3.91	2.91	1.94	1.02	1.11	1.10	7.32
22:6n-3	5.00	2.62	2.17	1.65	1.20	0.67	2.25	1.66	1.10	0.56	0.62	0.60	3.24
Saturates <sup>2</sup>	30.38	24.17	23.43	22.14	21.71	21.05	23.00	21.29	19.45	18.47	15.07	20.76	30.45
Monounsaturates <sup>3</sup>	22.61	22.67	23.17	22.86	23.69	24.32	22.59	21.93	21.39	21.54	18.08	24.48	30.34
PUFA <sup>4</sup>	28.52	43.18	45.25	48.92	50.08	52.04	46.24	50.70	55.05	57.73	64.47	52.37	25.24
HUFA <sup>5</sup>	18.49	9.97	8.15	6.08	4.52	2.58	8.17	6.08	4.12	2.25	2.38	2.39	13.97
Total n-3 <sup>6</sup>	20.80	14.87	13.67	12.02	11.09	9.85	16.60	17.21	18.19	19.69	37.79	7.22	15.54
Total n-6 <sup>7</sup>	24.10	37.24	38.88	42.31	43.03	44.48	36.94	38.91	40.52	40.04	28.82	47.24	21.93

<sup>1</sup> Values represent averages of duplicate samples.<sup>2</sup> Saturates: 14:0, 15:0, 16:0, 18:0, 20:0, 22:0. Internal standard, 19:0, was not considered.<sup>3</sup> Monounsaturates: 15:1, 16:1 18:1, 20:1.<sup>4</sup> PUFA: 16:2, 16:3, 18:2, 18:3, 20:2, 20:3, 22:2.<sup>5</sup> HUFA: 18:4, 20:4, 20:5, 22:4, 22:5, 22:6.<sup>6</sup> Total n-3: 18:3n-3, 20:3n-3, 20:4n-3, 20:5n-3, 22:5n-3, 22:6n-3.<sup>7</sup> Total n-6: 18:2n-6, 20:3n-6, 20:4n-6.

**Table 6**  
Total lipid (%) and fatty acid composition (mg g<sup>-1</sup> wet weight and % of FAME) of shrimp muscle tissue<sup>1</sup>.

Selected FA	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7	Diet 8	Diet 9	Diet 10	Diet 11	Diet 12	Diet 13
Total lipid (%)	1.37 <sup>a</sup>	1.37 <sup>a</sup>	1.34 <sup>a</sup>	1.37 <sup>a</sup>	1.37 <sup>a</sup>	1.40 <sup>a</sup>	1.43 <sup>a</sup>	1.47 <sup>a</sup>	1.42 <sup>a</sup>	1.43 <sup>a</sup>	1.43 <sup>a</sup>	1.40 <sup>a</sup>	1.42 <sup>a</sup>
<i>mg g<sup>-1</sup> wet weight</i>													
16:0	9.78 <sup>a</sup>	9.46 <sup>ab</sup>	8.96 <sup>abc</sup>	7.93 <sup>cd</sup>	8.70 <sup>bcd</sup>	8.46 <sup>bcd</sup>	8.45 <sup>bcd</sup>	8.27 <sup>cd</sup>	8.31 <sup>cd</sup>	8.07 <sup>cd</sup>	7.61 <sup>d</sup>	8.17 <sup>cd</sup>	9.93 <sup>a</sup>
18:0	6.01 <sup>a</sup>	6.58 <sup>a</sup>	6.31 <sup>a</sup>	5.93 <sup>a</sup>	6.38 <sup>a</sup>	6.38 <sup>a</sup>	5.93 <sup>a</sup>	5.90 <sup>a</sup>	6.09 <sup>a</sup>	6.27 <sup>a</sup>	5.89 <sup>a</sup>	6.17 <sup>a</sup>	6.11 <sup>a</sup>
18:1n-9	5.21 <sup>d</sup>	5.89 <sup>bcd</sup>	5.64 <sup>bcd</sup>	5.43 <sup>cd</sup>	5.99 <sup>bcd</sup>	6.41 <sup>b</sup>	5.42 <sup>cd</sup>	5.58 <sup>cd</sup>	5.79 <sup>bcd</sup>	6.06 <sup>bc</sup>	5.52 <sup>cd</sup>	6.12 <sup>bc</sup>	7.64 <sup>a</sup>
18:2n-6	7.59 <sup>g</sup>	10.78 <sup>de</sup>	11.03 <sup>de</sup>	11.17 <sup>de</sup>	13.07 <sup>bc</sup>	15.12 <sup>a</sup>	9.92 <sup>ef</sup>	10.78 <sup>de</sup>	12.16 <sup>cd</sup>	13.25 <sup>bc</sup>	9.26 <sup>f</sup>	14.32 <sup>ab</sup>	6.51 <sup>g</sup>
18:3n-3	0.48 <sup>fg</sup>	0.69 <sup>efg</sup>	0.71 <sup>efg</sup>	0.81 <sup>efg</sup>	0.97 <sup>def</sup>	1.18 <sup>de</sup>	1.00 <sup>de</sup>	1.40 <sup>d</sup>	1.94 <sup>c</sup>	2.59 <sup>b</sup>	5.61 <sup>a</sup>	0.81 <sup>efg</sup>	0.38 <sup>g</sup>
20:4n-6	1.60 <sup>ab</sup>	1.48 <sup>bc</sup>	1.38 <sup>cd</sup>	1.25 <sup>defg</sup>	1.34 <sup>cde</sup>	1.17 <sup>efg</sup>	1.30 <sup>def</sup>	1.28 <sup>defg</sup>	1.22 <sup>defg</sup>	1.15 <sup>fg</sup>	1.10 <sup>g</sup>	1.20 <sup>defg</sup>	1.74 <sup>a</sup>
20:5n-3	7.89 <sup>a</sup>	7.54 <sup>ab</sup>	6.84 <sup>bc</sup>	5.72 <sup>de</sup>	5.56 <sup>e</sup>	4.20 <sup>f</sup>	6.40 <sup>cd</sup>	5.79 <sup>de</sup>	5.21 <sup>e</sup>	4.16 <sup>f</sup>	4.07 <sup>f</sup>	4.19 <sup>f</sup>	8.21 <sup>a</sup>
22:6n-3	5.62 <sup>a</sup>	4.45 <sup>b</sup>	3.76 <sup>c</sup>	3.03 <sup>de</sup>	2.87 <sup>de</sup>	2.02 <sup>f</sup>	3.58 <sup>c</sup>	3.12 <sup>d</sup>	2.67 <sup>e</sup>	2.07 <sup>f</sup>	2.04 <sup>f</sup>	2.10 <sup>f</sup>	5.30 <sup>a</sup>
Saturates <sup>2</sup>	16.23 <sup>a</sup>	16.38 <sup>a</sup>	15.59 <sup>ab</sup>	14.12 <sup>b</sup>	15.37 <sup>ab</sup>	15.08 <sup>ab</sup>	14.71 <sup>ab</sup>	14.45 <sup>ab</sup>	14.66 <sup>ab</sup>	14.56 <sup>ab</sup>	13.86 <sup>b</sup>	14.59 <sup>ab</sup>	16.40 <sup>a</sup>
Monounsaturates <sup>3</sup>	9.46 <sup>b</sup>	9.19 <sup>b</sup>	8.58 <sup>bc</sup>	7.95 <sup>c</sup>	8.61 <sup>bc</sup>	8.84 <sup>bc</sup>	8.25 <sup>bc</sup>	8.26 <sup>bc</sup>	8.31 <sup>bc</sup>	8.41 <sup>bc</sup>	7.93 <sup>c</sup>	8.46 <sup>bc</sup>	11.37 <sup>a</sup>
PUFA <sup>4</sup>	11.38 <sup>d</sup>	15.42 <sup>c</sup>	15.58 <sup>c</sup>	15.69 <sup>c</sup>	18.20 <sup>ab</sup>	20.49 <sup>a</sup>	14.57 <sup>c</sup>	15.95 <sup>bc</sup>	18.02 <sup>ab</sup>	20.21 <sup>a</sup>	19.33 <sup>a</sup>	19.18 <sup>a</sup>	10.30 <sup>d</sup>
HUFA <sup>5</sup>	16.01 <sup>a</sup>	14.32 <sup>b</sup>	12.76 <sup>c</sup>	10.68 <sup>de</sup>	10.46 <sup>e</sup>	7.95 <sup>f</sup>	12.05 <sup>cd</sup>	10.93 <sup>de</sup>	9.75 <sup>e</sup>	7.92 <sup>f</sup>	7.72 <sup>f</sup>	8.04 <sup>f</sup>	16.20 <sup>a</sup>
Total n-3 <sup>6</sup>	14.90 <sup>a</sup>	13.56 <sup>ab</sup>	12.13 <sup>bcd</sup>	10.28 <sup>ef</sup>	10.16 <sup>ef</sup>	8.05 <sup>g</sup>	11.84 <sup>cde</sup>	11.21 <sup>def</sup>	10.72 <sup>def</sup>	9.75 <sup>f</sup>	13.32 <sup>abc</sup>	7.68 <sup>g</sup>	14.81 <sup>a</sup>
Total n-6 <sup>7</sup>	10.02 <sup>gh</sup>	13.61 <sup>de</sup>	13.82 <sup>de</sup>	13.83 <sup>de</sup>	16.05 <sup>bc</sup>	18.02 <sup>a</sup>	12.47 <sup>de</sup>	13.39 <sup>de</sup>	14.79 <sup>cd</sup>	16.03 <sup>bc</sup>	11.57 <sup>fg</sup>	17.26 <sup>ab</sup>	9.02 <sup>h</sup>
n-3/n-6	1.49	1.00	0.88	0.74	0.63	0.45	0.95	0.84	0.72	0.61	1.15	0.44	1.64
<i>% of FAME</i>													
16:0	18.44 <sup>a</sup>	17.12 <sup>b</sup>	17.07 <sup>bc</sup>	16.38 <sup>de</sup>	16.51 <sup>de</sup>	16.17 <sup>e</sup>	17.04 <sup>bc</sup>	16.67 <sup>cd</sup>	16.36 <sup>de</sup>	15.72 <sup>f</sup>	15.60 <sup>f</sup>	16.23 <sup>de</sup>	18.30 <sup>a</sup>
18:0	11.32 <sup>d</sup>	11.89 <sup>c</sup>	12.03 <sup>abc</sup>	12.22 <sup>ab</sup>	12.11 <sup>abc</sup>	12.18 <sup>ab</sup>	11.96 <sup>bc</sup>	11.90 <sup>c</sup>	12.02 <sup>abc</sup>	12.27 <sup>a</sup>	12.08 <sup>abc</sup>	12.28 <sup>a</sup>	11.26 <sup>d</sup>
18:1n-9	9.81 <sup>g</sup>	10.65 <sup>f</sup>	10.76 <sup>f</sup>	11.21 <sup>de</sup>	11.40 <sup>d</sup>	12.24 <sup>b</sup>	10.94 <sup>ef</sup>	11.26 <sup>d</sup>	11.42 <sup>d</sup>	11.90 <sup>c</sup>	11.28 <sup>d</sup>	12.17 <sup>bc</sup>	14.08 <sup>a</sup>
18:2n-6	14.29 <sup>i</sup>	19.53 <sup>gh</sup>	20.99 <sup>f</sup>	23.09 <sup>e</sup>	24.85 <sup>c</sup>	28.87 <sup>a</sup>	20.05 <sup>g</sup>	21.76 <sup>f</sup>	23.98 <sup>d</sup>	26.38 <sup>b</sup>	19.01 <sup>h</sup>	28.45 <sup>a</sup>	12.00 <sup>j</sup>
18:3n-3	0.91 <sup>i</sup>	1.24 <sup>h</sup>	1.35 <sup>h</sup>	1.67 <sup>g</sup>	1.85 <sup>f</sup>	2.24 <sup>e</sup>	2.02 <sup>f</sup>	2.84 <sup>d</sup>	3.84 <sup>c</sup>	5.12 <sup>b</sup>	11.50 <sup>a</sup>	1.60 <sup>g</sup>	0.71 <sup>j</sup>
20:4n-6	3.02 <sup>b</sup>	2.67 <sup>c</sup>	2.64 <sup>c</sup>	2.57 <sup>c</sup>	2.55 <sup>cd</sup>	2.24 <sup>ef</sup>	2.61 <sup>c</sup>	2.58 <sup>de</sup>	2.39 <sup>de</sup>	2.19 <sup>f</sup>	2.25 <sup>ef</sup>	2.39 <sup>de</sup>	3.22 <sup>a</sup>
20:5n-3	14.87 <sup>a</sup>	13.59 <sup>b</sup>	13.00 <sup>c</sup>	11.78 <sup>d</sup>	10.55 <sup>e</sup>	8.02 <sup>f</sup>	12.90 <sup>c</sup>	11.67 <sup>d</sup>	10.27 <sup>e</sup>	7.91 <sup>f</sup>	8.35 <sup>f</sup>	8.34 <sup>f</sup>	15.13 <sup>a</sup>
22:6n-3	10.60 <sup>a</sup>	8.04 <sup>c</sup>	7.17 <sup>d</sup>	6.26 <sup>e</sup>	5.45 <sup>f</sup>	3.86 <sup>g</sup>	7.22 <sup>d</sup>	6.29 <sup>e</sup>	5.24 <sup>f</sup>	3.96 <sup>g</sup>	4.15 <sup>g</sup>	4.17 <sup>g</sup>	9.75 <sup>b</sup>
Saturates <sup>2</sup>	30.60 <sup>a</sup>	29.64 <sup>bcd</sup>	29.71 <sup>bc</sup>	29.14 <sup>cde</sup>	29.17 <sup>cde</sup>	28.83 <sup>efg</sup>	29.66 <sup>bc</sup>	29.14 <sup>cde</sup>	28.88 <sup>efg</sup>	28.44 <sup>fg</sup>	28.38 <sup>g</sup>	29.02 <sup>def</sup>	30.22 <sup>ab</sup>
Monounsaturates <sup>3</sup>	17.81 <sup>b</sup>	16.60 <sup>cde</sup>	16.36 <sup>ef</sup>	16.42 <sup>ef</sup>	16.35 <sup>ef</sup>	16.89 <sup>c</sup>	16.65 <sup>cde</sup>	16.66 <sup>cde</sup>	16.39 <sup>ef</sup>	16.49 <sup>def</sup>	16.20 <sup>f</sup>	16.82 <sup>cd</sup>	20.95 <sup>a</sup>
PUFA <sup>4</sup>	21.43 <sup>h</sup>	27.91 <sup>g</sup>	29.64 <sup>f</sup>	32.41 <sup>e</sup>	34.61 <sup>d</sup>	39.11 <sup>a</sup>	29.42 <sup>f</sup>	32.18 <sup>e</sup>	35.54 <sup>c</sup>	39.96 <sup>a</sup>	39.63 <sup>a</sup>	38.14 <sup>b</sup>	18.99 <sup>i</sup>
HUFA <sup>5</sup>	30.15 <sup>a</sup>	25.85 <sup>b</sup>	24.29 <sup>c</sup>	22.03 <sup>d</sup>	19.87 <sup>e</sup>	15.17 <sup>g</sup>	24.27 <sup>c</sup>	22.02 <sup>d</sup>	19.19 <sup>e</sup>	15.11 <sup>g</sup>	15.78 <sup>fg</sup>	16.02 <sup>f</sup>	29.84 <sup>a</sup>
Total n-3 <sup>6</sup>	28.07 <sup>a</sup>	24.47 <sup>c</sup>	23.07 <sup>d</sup>	21.22 <sup>e</sup>	19.31 <sup>f</sup>	15.37 <sup>g</sup>	23.86 <sup>c</sup>	22.60 <sup>d</sup>	21.12 <sup>e</sup>	18.81 <sup>f</sup>	27.27 <sup>b</sup>	15.29 <sup>g</sup>	27.27 <sup>b</sup>
Total n-6 <sup>7</sup>	18.88 <sup>i</sup>	24.64 <sup>g</sup>	26.29 <sup>f</sup>	28.57 <sup>d</sup>	30.51 <sup>c</sup>	34.41 <sup>a</sup>	25.19 <sup>g</sup>	27.02 <sup>e</sup>	29.16 <sup>d</sup>	31.71 <sup>b</sup>	23.73 <sup>h</sup>	34.33 <sup>a</sup>	16.62 <sup>j</sup>

<sup>2-7</sup>See footnotes in Table 5.

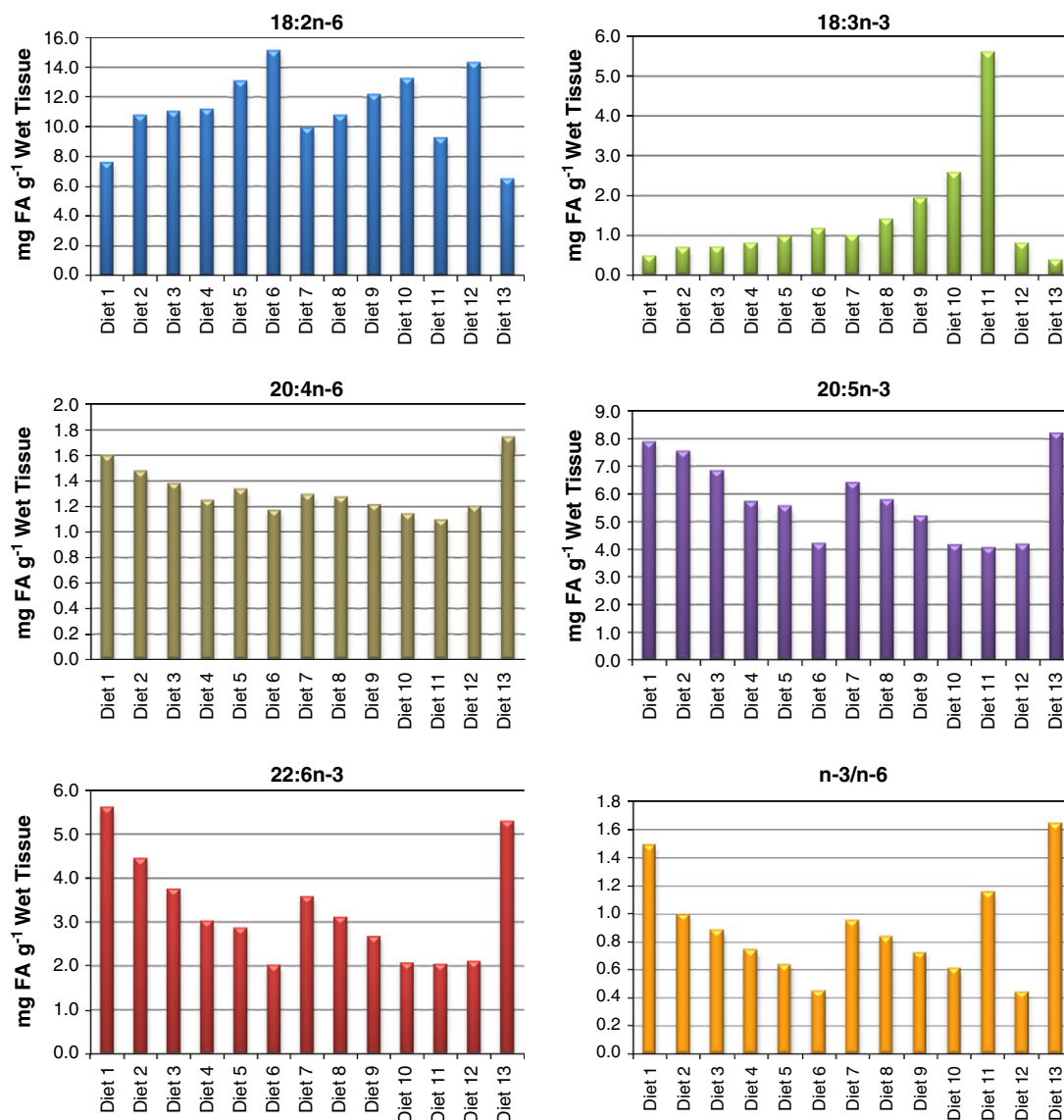
<sup>1</sup> Values represent averages of duplicate samples per tank, and five tanks per dietary treatment. Means within rows with the same letter are not significantly different (Duncan's alpha = 0.05).

Essential FA deficiencies have been induced in outdoor tank systems. Samocha et al. (2009) presumably induced an essential FA deficiency using the same outdoor system when shrimp were offered a plant based diet using soy and linseed oil with or without HUFA supplements from fermentation products. Studies conducted in clear water systems (Lim et al., 1997; González-Félix et al., 2003a; Glencross and Smith, 2001) do report significant reduction in final weight of shrimp fed HUFA-deficient diets. In terms of growth and survival of shrimp in this study, increasing the n-3/n-6 ratio with the use of SO and LO to replace MFO showed no evident improvement, demonstrating that with appropriate diet formulations, the vast majority of the marine ingredients can be removed from shrimp feed. However, a source of HUFA is required even in systems where shrimp may have access to natural foods.

Fatty acid analysis confirmed that linolenic acid, although recognized as the precursor of n-3 HUFA in other organisms, has a very limited role as such in shrimp. Animals fed Diet 11, with 19.81 mg of linolenic acid per gram of diet, showed the highest level of this FA in shrimp tissue, 5.61 mg per gram of wet tissue, but at the same time, practically the lowest content of EPA (4.07 mg g<sup>-1</sup> wet tissue) and DHA (2.04 mg g<sup>-1</sup> wet tissue) among the treatments. Also, a relatively high n-3/n-6 ratio of 1.15 was observed in muscle of shrimp fed Diet 11, comparable to the one observed in those fed Diet 2 (n-3/n-6 = 1.00) where 50% of MFO was replaced by SO, but shrimp fed Diet 2 showed almost double the amount of EPA (7.54 mg g<sup>-1</sup> wet tissue) and DHA (4.45 mg g<sup>-1</sup> wet tissue). This demonstrated the inability of shrimp to elongate and desaturate PUFA into HUFA which has been reported previously in other studies (Kanazawa et al., 1979; Kayama et al., 1980; Lim et al., 1997; González-Félix et al., 2003b). In the case of humans  $\alpha$ -linolenic acid is converted into EPA and DHA,

and linoleic acid is converted into ARA. The enzyme delta-6-desaturase catalyzes the first rate-limiting step of both n-3 and n-6 HUFA synthesis. There is evidence that the synthesis of EPA from  $\alpha$ -linolenic acid is limited, and DHA synthesis is even more limited, because of the common desaturation and elongation enzymes and the competition between the substrates for them (Brenna, 2002). An increase in EPA and DHA synthesis can be achieved by increasing dietary  $\alpha$ -linolenic acid, but the optimal dietary approach for humans is to consume n-3 HUFA preformed by eating seafood (Goyens et al., 2006).

We are then left with few options, and the social pressure and responsibility of finding alternative sources of high quality cost-effective oils, high in n-3 HUFA and n-3/n-6 ratios, to be used in aquafeeds. On the bright side, there is already some progress on this quest. For instance, products obtained by fermentation processes from heterotrophically grown algae have been reported to be a good source of nutrients and essential fatty acids for larval live food enrichment and for formulated broodstock diets of marine teleosts (Harel et al., 2002). In the case of shrimp, Patnaik et al. (2006) demonstrated that fish oil can be successfully replaced in diets for *L. vannamei* using spray-dried cells of *Schizochytrium* sp. and *Mortierella* sp. obtained by a proprietary commercial fermentation process. The same products, used for supplementation of DHA and ARA in practical diets for *L. vannamei*, showed that these alternative sources of HUFA are effective in promoting growth and survival of juvenile shrimp when raised in low salinity (González-Félix et al., 2009). These heterotrophically produced non-marine HUFA-rich products are in a preliminary stage of research as lipid sources in aquafeeds. Additional research on their effect at different life stages of *L. vannamei*, cultured under a variety of environmental conditions, would be helpful to fish oil replacement



**Fig. 1.** Linoleic (18:2-6), linolenic (18:3n-3), arachidonic (20:4n-6), EPA (20:5n-3), DHA (22:6n-3), and n-3/n-6 ratio in muscle of *L. vannamei* during the 58-day nutrition study. Values represent means of duplicate analysis for five replicate observations.

efforts, and would be valuable in determining the economic viability of their large-scale use in shrimp aquafeeds. Another approach to improve n-3 HUFA in farmed animals has been the implementation of a finishing period using a fish oil based diet or finishing diet to restore the original fatty acid profile of farmed fish fillets for instance. However, the disadvantage is that it requires a considerable period of time, up to 16 weeks, to restore an optimal fatty acid profile for human consumption, and this approach still relies on the use of fish oil (Turchini et al., 2007). Finally, the use of genetically modified grain crops rich in n-3 HUFA, or the farming of transgenic fish with greater capability for n-3 HUFA biosynthesis are being explored and have produced exceptional results, but at the same time, it has attracted considerable negative publicity from consumers with respect to the use of this kind of biotechnology in food production (Turchini et al., 2009). All of the aforementioned approaches are valuable contributions in the quest for fish oil replacement, but the goal has not been met, yet.

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