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Comparison of Pond Production Efficiency, Fatty Acid Profiles, and Contaminants in *Litopenaeus vannamei* Fed Organic Plant-based and Fish-meal-based Diets

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Abstract

Reduction or elimination of fish meal and fish oil from aquaculture diets can help to reduce the potential for contamination and dependence of the industry on pelagic fisheries while improving economic competitiveness. However, fish oil provides important omega-3 (n-3) fatty acids (FAs) essential to shrimp health and beneficial to humans. This study evaluated an organic, plant-based diet formulated to replace fish meal and fish oil with plant proteins and docosahexaenoic acid (DHA) produced by algal fermentation. Shrimp cultured in replicate outdoor ponds at 25/m² were fed either a diet composed of organically produced plant ingredients or a conventional commercial fish-meal-based feed. No significant differences were found in production parameters between the conventional fish-meal-based diet and the plant-based diet (production: 4594 and 4592 kg/ha; harvest size: 18.7 and 19.2 g; survival: 93 and 88%; and feed conversion ratio: 1.4 and 1.3, respectively). At harvest, shrimp were analyzed for 147 chemical contaminants and 71 FAs. Contaminant levels were negligible for shrimp raised on both diets. The fish meal and fish oil diet provided significantly higher quantities of eicosapentaenoic acid and DHA than the plant-based diet, and the shrimp fed the conventional diet

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reflected this with higher levels of these beneficial FAs in edible tissues. Differences between feeds and shrimp tissues suggest that essential n-3 FAs may accumulate in shrimp tissues over time or that natural pond productivity may play a role in providing supplemental nutrition. Shrimp raised on the two diets and wild-caught shrimp are clearly distinguishable by their FA profiles. Compared to alternative protein sources like beef, pork, or chicken, differences in lipid profiles of shrimp raised on either diet may be insignificant because both offer increased human health benefits.

The quantity of imported shrimp into the USA continues to grow and has increased 57% during the first 6 mo of 2005 compared to the first 6 mo of 2000 (Harvey 2005). Concurrently, shrimp prices continue to decline, decreasing 31% during this same time period (Harvey 2005). As shrimp consumption has increased, so has consumer awareness and concern over product quality, depletion of natural fisheries, environmental degradation, and potential health risks from chemicals utilized in some shrimp production facilities. Demand for ''environmentally friendly'' and chemical-free ''healthy'' products has increased in all retail markets with consumers willing to pay the additional 20–30% extra for products certified as organic and to actively search for these products. Increased environmental and health consciousness has also caused public concern over animal feed ingredients. Attention has become focused on fish meal in aquaculture feeds, as aquaculture production escalates, operations become more intensive, and the sustainability of marine fish stocks is increasingly more questionable (Naylor et al. 1998; Tidwell and Allan 2001). Furthermore, recent concerns over seafood consumption are the result of fears related to mercury content and possible presence of organochlorine compounds in fish meal fed farmed salmon (Hites et al. 2004). Fish meal is one of the principal ingredients in conventional shrimp feeds, supplying necessary amino acids and fatty acids (FAs). Because of negative public sentiments about bioaccumulation of chemical toxins from contaminated aquaculture feeds and the ecological ramifications of continued depletion of pelagic fisheries, as well as the industry's economic concerns over fluctuating fish meal supplies, developing alternatives to fish meal diet components has become a priority for some feed producers.

In addition to desiring an organic, environmentally friendly product, consumers are increasingly aware of the relationship of diet and human health to the amounts and kinds of dietary fat consumed. Medical recommendations are to lower overall fat intake while increasing the proportion of long-chain polyunsaturated omega-3 (n-3) fatty acids (PUFAs), especially docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), in the diet. The beneficial effects of these FAs in reducing coronary heart disease mortality, nonfatal myocardial infarction, and stroke risk are now well established (Bouzan et al. 2005; König et al. 2005). Low intake of n-3 FAs has been related to depression and suicide (De Vriese et al. 2004). The requirement for DHA in normal development of the brain and retina has been established (Salem and Wark 1993) and the positive relationship between infant consumption of DHA and cognitive development has been quantified (Cohen et al. 2005). Although both omega-6 (n-6) and n-3 FAs play significant roles in human health, the contribution of vegetable oils high in n-6 FAs to total dietary fat intake has increased for Western populations (Cordain et al. 2005). Simopoulos (2002) suggested that this excessive amount of n-6 PUFAs, resulting in a very high n-6 to n-3 ratio ($\sim$15:1), promotes the pathogenesis of many diseases, including cardiovascular disease, cancer, and inflammatory autoimmune diseases.

Shrimp, like most seafood, are a good source of long-chain n-3 FAs. Previous studies have demonstrated that FA composition of shrimp is greatly influenced by their diet (Bragagnolo and Rodriguez-Amaya 2001; Glencross et al. 2002; Gonzalez-Felix et al. 2003). Fish oil is added to conventional commercial shrimp feeds to supply the n-3 FAs essential to the health of the shrimp and beneficial to the humans who consume the shrimp. Until recently, there has been no effective replacement options for fish oil as highly unsaturated FAs are typically not

found at high levels in terrestrial oils. However, several essential long-chain FAs, especially DHA produced by fermentation of heterotrophic marine algae, are now commercially available (Martek BioSciences Corp., Columbia, MD, USA; Advanced BioNutrition Corp., Columbia, MD, USA). Therefore, an opportunity exists to develop contaminant-free, environmentally friendly diets while ensuring shrimp growth and FA content beneficial to human health.

Fish-meal-free and fish-oil-free shrimp feeds are being developed and evaluated against conventional diets already in use. Because plant-based feed ingredients contain a high n-6:n-3 ratio, one challenge in producing shrimp for health-conscious consumers is to ensure that farmed shrimp maintain the very low n-6:n-3 FA ratio typical of wild-caught shrimp. Recent research at the Waddell Mariculture Center (WMC) in Bluffton, South Carolina, USA, conducted in cooperation with Advanced BioNutrition Corp., compared production of shrimp fed a plant-based diet derived primarily from organic, nongenetically modified (non-GM) ingredients with those fed a conventional commercial fish-meal-based feed. This paper summarizes the production results, reports contaminant analyses for 22 metals, 21 pesticides, 25 polyaromatic hydrocarbons (PAHs), and 79 polychlorinated biphenyls (PCBs), and presents the lipid content and FA profiles of diets and shrimp tissues collected at harvest of the 2004 production trial.

Materials and Methods

Shrimp (*Litopenaeus vannamei*) were cultured in six matched 0.1-ha ponds at the WMC. Shrimp in three ponds were fed a commercial pelleted feed containing 35% protein, 7% lipid, and typical levels of fish meal and fish oil. Shrimp in the other three ponds were fed a custom commercially pelleted feed designed to contain 35% protein and 8% lipid formulated almost entirely from plant materials (Table 1). The plant ingredients used in this experimental diet were all non-GM, primarily certified organic products leading to the prospect of having this formulation recognized as a certifiable organic aquaculture feed. Small quantities of squid meal (1%) and liquid fish solubles (1%) were added to the formulation after initial studies indicated that palatability might be a problem. While plant meals and oils can provide all the essential amino acids and most essential FAs, at present certain PUFAs must still be included from alternative sources. The experimental diet included 0.5% AquaGrow-schizochytrium-DHA® and 0.13% AquaGrow-ARA® (Advanced BioNutrition Corp.), derived by fermentation of marine algae and fungus, to provide a source of the essential FAs, DHA and arachidonic acid. A standard mix of vitamins and minerals was also included in the formulation. Both feeds were manufactured by Zeigler Bros., Inc. (Gardners, PA, USA).

TABLE 1. *Formulation of organic certifiable, plant-based grow-out diet for* Litopenaeus vannamei.

Ingredients	Percent by weight
Expelled soybean meal, 42/7, organic	58.1
Whole soft wheat, organic	12
Canadian feed pea meal, organic	10
Corn gluten meal, organic, non-GM	9
Flaxseed oil, organic	2
Dicalcium phosphate	2
Federal vitamin premix #30 w/o choline	1.8
Squid meal	1
Liquid fish solubles	1
AquaGrow-schizochytrium-DHA®	0.5
USFW #3 mineral mix	0.5
Lecithin, organic, non-GM	0.5
BetaFin BT-Danisco	0.5
Kelp meal, Acadian Seaplants Ltd	0.5
Soy oil, no additives, organic	0.2
Choline chloride	0.2
AquaGrow-ARA®	0.13
Stay C	0.07
Total	100
Protein	35%
Total lipid	8%

Non-GM = nongenetically modified.

Two weeks prior to stocking, ponds were filled with water from the Colleton River (salinity ~ 28 ppt) filtered through a 400-μm-size mesh bag and fertilized with liquid inorganic (10-34-0) and pelleted organic fertilizer (alfalfa) to stimulate algal growth. Ponds were managed without water exchange. Aeration was provided by either a 1- or 2-hp paddlewheel mechanical aerator or combination of the two as dissolved oxygen levels dictated. Nursery-reared shrimp

(~0.82 g) were stocked into the ponds at a density of 25 shrimp/m^2, with three ponds per diet treatment. Feed was distributed by commercial feed blower twice daily during the week and once a day on weekends. Feed rates were set and maintained to keep feed conversion ratios (FCRs) below 2:1 based on an estimated growth rate of 1 g/wk and assumed survival. Dissolved oxygen (mg/L), salinity (g/L), temperature (C), and pH were measured daily between 0600 and 0800 h (Table 2). Temperature, salinity, and pH were measured again between 1500 and 1700 h. Shrimp growth in each pond was measured weekly by weighing 50 animals collected by cast net. Shrimp were gravity harvested at 89–90 d by draining the ponds into collection bags attached at the pond outfall drain. Mean shrimp harvest weight was determined to the nearest 0.1 g by weighing 100 randomly selected animals from each pond.

At harvest, approximately two-dozen shrimp were removed from each pond, placed in separate plastic bags, and transferred on ice to the Lipid Chemistry and the Chemical Contaminants laboratories at the National Oceanic and Atmospheric Administration's Center for Coastal Environmental Health and Biomolecular Research and the Hollings Marine Laboratory in Charleston, South Carolina, USA. The samples were stored at −40 C until analyzed during February and March of 2005. A sample of each of the experimental and conventional diets was also collected and analyzed.

Chemical Contaminants Analyses

Shrimp were thawed in a +4 C cooler for approximately 24 h, rinsed with deionized water, deheaded, and peeled so that only edible tissue was analyzed. Five shrimp from each of the three ponds per treatment were pooled (15 shrimp total) into a single sample representative of each diet. A quantitative mass of high-purity water was added to aid in homogenization with a ProScientific Pro 250 titanium homogenizer (PRO Scientific Inc., Oxford, CT, USA). Separate aliquots were sealed in either a precleaned plastic cup for inorganic analysis or a precleaned glass jar for organic analysis and frozen at −40 C until analysis. The percent dry weight determination was made gravimetrically on an aliquot of the wet homogenate.

Total concentrations of 22 elements were measured. Thawed 2.0-g wet-weight subsamples were transferred to Teflon-lined digestion vessels, 10 mL of concentrated nitric acid was added to each, and the samples were allowed to sit at room temperature for 1 h before sealing the vessels. Samples were then heated in a microwave digestion unit with 900 W of power for 1 h. After cooling, 2.0 mL of 30% hydrogen peroxide was added to each, and the samples were again heated in the microwave for an additional 10 min. After cooling, the sample volume was adjusted to 50.0 mL with deionized water. Analyses of 20 elements were performed using a Perkin–Elmer Elan 6100 inductively coupled plasma mass spectrometer (Perkin–Elmer, Inc., Wellesley, MA, USA). A Perkin–Elmer 5100 Zeeman HGA graphite furnace atomic absorption spectrometer (Perkin–Elmer, Inc.) was used to analyze for silver at a wavelength of 328.1 nm with pyrolysis and atomization temperatures of 1100 and 2100 C, respectively.

Separate 0.5-g aliquots of wet homogenate were weighed directly into nickel combustion boats. The sample boats were loaded into an autosampler and analyzed on a Milestone DMA-80 analyzer (Milestone Inc., Shelton, CT, USA). Samples were thermally decomposed in a continuous flow of oxygen with mercury vapors being trapped on a gold amalgamator

TABLE 2. *Water quality (mean ± SD) parameters for ponds subjected to each treatment, averaged over the duration of the study.*

Treatment	Temperature (C)	Salinity (g/L)	Dissolved oxygen (mg/L)	pH
Plant-based diet	29.1 ± 1.8	28.8 ± 2.0	5.0 ± 0.7	7.5 ± 0.4
Fish-meal-based diet	29.1 ± 1.8	27.2 ± 2.1	5.0 ± 0.7	7.4 ± 0.7

and subsequently desorbed for quantification using atomic absorption spectrophotometry at 254 nm.

For each sample, a 4.5-g aliquot of homogenate was weighed in a glass mortar bowl and mixed with approximately 32 g of ashed (450 C) anhydrous sodium sulfate to form a dry powder. The dried homogenate was transferred into a precleaned 33-mL accelerated solvent extraction (ASE) vessel. A measured aliquot (250 μL) of an internal standard mixture containing ^{13}C-labeled PCB 3, 15, 28, 52, 118, 153, 180, 194, 208, and 209; ^{13}C-labeled lindane; heptachlor epoxide; 2,4-dichlorodiphenyldichloroethylene (DDE); mirex; dieldrin, and the following perdeuterated compounds: chlorpyrifos; Endosulfan I; Endosulfan II; 4,4-dichlorodiphenyldichloroethane (DDD); 4,4-dichlorodiphenyltrichloroethane (DDT); naphthalene; acenaphthylene; acenaphthene; fluorene; phenanthrene; anthracene; fluoranthene; pyrene; benzo(a)anthracene; chrysene; benzo(b)fluoranthene; benzo(k)fluoranthene; benzo(e)pyrene; benzo (a)pyrene; perylene; indeno(1,2,3-cd)pyrene; dibenz(a,h)anthracene; and benzo(g,h,i)perylene was added to each sample. The samples were extracted with methylene chloride on the ASE system and the residual water removed by passing the extract through phase separation paper containing a small amount of sodium sulfate. After drying, the extracted sample was concentrated under nitrogen to 1000 μL on an automatic TurboVap concentrator (Caliper Life Sciences, Hopkinton, MA, USA). Lipid and other high-molecular-weight components were removed by size exclusion chromatography. The mobile phase was methylene chloride at 5 mL/min. The volume was reduced to about 1000 μL, and the extract was split into two equal aliquots for cleanup.

Concentrations of 21 pesticides or their degradation products and 79 PCB congeners were measured. Silica solid-phase (500 mg, 3 mL) extraction columns (Phenomenex, Torrance, CA, USA) were placed on a Zymark RapidTrace (Caliper Life Sciences, Hopkinton, MA, USA) and preconditioned with 5 mL of ethyl acetate followed by 5 mL of 60:40 hexane : methylene chloride. The elution solvent for the collected fraction was 1.5 mL of 50:50 ethyl acetate : hexane. Twenty nanograms of δ-hexachlorohexane were added to each sample to allow internal, standard, percent recovery determinations. An Agilent 6890/5973N gas chromatograph/mass spectrometer (Agilent Technologies, Palo Alto, CA, USA) operating in negative chemical ionization mode was used to analyze the samples for pesticides. The PCB analysis was performed on an identical instrument operating in electron impact ionization mode. Both instruments utilize programmable temperature vaporization with large-volume injection ports. An injection volume of 50 μL was used for the PCB and pesticide analyses. A 5% phenyl 30 m × 0.25 mm × 0.25 μm column was used in both GCs. The data were collected in selective ion monitoring mode.

Concentrations of 25 PAHs were measured. Phenomenex silica solid-phase extraction columns (500 mg, 3 mL) were placed on a Zymark RapidTrace and conditioned with 5 mL of a 60:40 hexane : dichloromethane (DCM) mixture followed by 5 mL hexane. The remaining sample aliquot (500 μL) was placed on the column and allowed to flow onto the silica bed. The sample container was rinsed with 0.25 mL hexane and that rinse was also loaded onto the column. The first fraction (saturates) was eluted with a total volume of 0.75 mL of hexane and discarded. The aromatic fraction was eluted from the column with 5 mL of a 60:40 hexane : methylene chloride mixture and collected in a concentrator tube. The fraction was reduced in volume to about 200 μL and transferred to a GC vial for gas chromatograph/ion trap mass spectrometer analysis. Perdeuterated *p*-terphenyl (50.2 ng) dissolved in hexane was added to the fraction to allow internal, standard, percent recovery determinations. A Varian 3400 gas chromatograph (Varian, Inc., Palo Alto, CA, USA) equipped with a high-resolution capillary column (Restek Rtx – 5 ms; 30 m × 0.25 mm × 0.25 μm; Chromatographic Specialties Inc., Brockville, Ontario, Canada) directly interfaced to a Finnigan Magnum ITMS (Thermo Electron Corp., Waltham, MA, USA) was used for quantitative analysis. One-μL injections were made with a Varian 8200 autosampler (Varian, Inc.) in the direct-injection mode, and the

spectrometer was operated in full-scan mode to acquire spectra.

Lipid and FA Analyses

Six shrimp, randomly taken from each pond, were individually analyzed for total lipid content and FA composition. Each shrimp was peeled while partially frozen, deveined, weighed, and transferred to a stainless steel vessel of an OMNI Macro ES homogenizer (Omni International, Marietta, GA, USA). Lipids were extracted using chloroform–methanol as described by Bligh and Dyer (1959). The chloroform extract containing the lipids was dried over anhydrous sodium sulfate, filtered, and made to a known volume. Duplicate 5-mL aliquots of the extract were dried at 60 C and lipid weight determined gravimetrically.

Extracted lipids from each shrimp were analyzed for lipid class composition using an Iatroscan MK-5 TLC-FID Analyzer (Iatron Laboratories, Inc., Tokyo, Japan). Lipids (~5 μg/μL in chloroform) were spotted on SIII chromarods and developed in hexane : diethyl ether : formic acid (85:15:0.25) for 25 min, oven dried at 60 C for 5 min, and the rods scanned. Quantitation was based on five-point calibration curves established for cholesterol oleate, triolein, oleic acid, cholesterol, and phosphatidylcholine (Nu-Chek Prep, Elysian, MN, USA).

An aliquot of extract containing 5–10 mg of lipid was taken for FA analysis. After removing the chloroform under a gentle stream of dry nitrogen, fatty acid methyl esters (FAMEs) were prepared as described by Christopherson and Glass (1969). The esters (~1 μg/μL in isooctane) were analyzed by gas chromatography with splitless injection. Separation was achieved with oven temperature programming as follows: initial temperature of 50 C with a 2-min hold, ramped at 20 C/min to 150 C followed by a 1 C/min ramp to 220 C. With the use of dual injection modules, samples were simultaneously analyzed on dual DB225 ms columns (50%-cyanopropylphenyl-methylpolysiloxane 30 m × 0.25 mm, J&W Scientific Inc., Folsom, CA, USA) with detection by flame ionization (FID) and mass spectrometry (MS). Scans from the MS detector were used, in conjunction with comparison of retention times with those of known standards, to identify FA components. Correction factors were applied to peak areas from the FID and compositions reported as weight percent of FAs.

Data Analysis

Average shrimp weight, weekly growth, survival, production, and FCR for each of the six ponds were calculated and compared by two-way ANOVA.

All dry-weight contaminant concentrations were converted to concentrations per wet weight of edible tissue. All contaminant concentrations in shrimp fed the fish-meal-based diet were each compared directly to the concentration in shrimp fed the plant-based diet. Concentrations of contaminants in the shrimp were also compared to US Environmental Protection Agency (2005) screening values for recreational fishers and to US Food and Drug Administration (2001) action levels. Because inorganic arsenic was not measured directly, it was estimated to be approximately 2% of total arsenic for warm-water (Penaeid) shrimp (UK Food Standards Agency 2005). Toxic equivalent (WHO-TEQ) values were calculated for dioxin-like PCBs and the potency equivalency concentration values were calculated for PAHs (US Environmental Protection Agency 2005).

ANOVAs were used to test for differences in edible tissue weight and total lipid content between diet groups. A series of ANOVAs was used to test for differences in the mean percentages of 18:2n-6 linoleic acid (LA), 18:3n-3 linolenic acid (LnA), 20:4n-6 arachadonic acid (AA), 20:5n-3 EPA, and 22:6n-3 DHA between diet groups. Because of the compositional nature of the data, the FA percentages were log ratio transformed (Aitchison 1986) prior to testing according to the following equation:

$$x_{\text{trans}} = \ln(x_i / c_{\text{r}})$$

where x_i is the weight percent of a given FA, x_{trans} is the transformed weight percent value, and c_{r} is the weight percent of 18:0 stearic acid, the reference FA. Bonferroni correction ($\alpha = 0.05/5 = 0.01$) was used to adjust the

critical value for individual tests, holding the experiment-wise α at 0.05.

To simplify the comparison of FAME profiles of the study shrimp raised on each diet against each other and against several additional reference sources, the dimensionality of the data set was reduced using principal component analysis (PCA). These additional sources provided by Seaborn and Holbrook (Center for Coastal Environmental Health and Biomolecular Research, Charleston, unpublished data) consisted of cultured shrimp (*L. vannamei*) from a previous WMC study, cultured *L. vannamei* imported from Mexico, wild-caught *L. vannamei* imported from Mexico, and wild-caught *Litopenaeus setiferus* taken off the coast of North Carolina, Georgia, and Florida, USA, in 2000 during South Carolina Department of Natural Resources survey trawls (Table 3). The 10 n-6 and n-3 log-ratio-transformed FAs (LA, LnA, 20:2n-6, AA, 20:3n-3, EPA, 22:4n-6, 22:5n-6, 22:5n-3, and DHA) from each of the six sources were subjected to exploratory PCA. Principal component scores for individual animals were plotted to examine similarities and differences among FA profiles (i.e., scores for animals with similar profiles tend to cluster, with distance between clusters directly related to profile differences). Varimax-rotated factor loadings were examined to determine which FAs most influenced these scores. Statistical analyses were performed using JMP 5.0 (SAS Institute Inc., Cary, NC, USA).

Results

Average shrimp weight, growth/wk, survival, production, and FCR for each of the six ponds are shown in Table 4. Mean shrimp weight at

TABLE 3. *Mean percentage of fatty acids (±SE) of wild and cultured shrimp collected at various locations independent of the present study.*

	Cultured		Wild	
Fatty acid	Waddell (n = 3)	Mexico (n = 5)	Southeast Atlantic (n = 5)	Mexico (n = 5)
14:0	0.70 ± 0.06	0.73 ± 0.05	1.33 ± 0.13	1.40 ± 0.04
15:0	0.39 ± 0.01	0.54 ± 0.05	1.79 ± 0.12	0.87 ± 0.03
16:0	22.66 ± 0.40	18.15 ± 0.33	17.27 ± 0.95	14.91 ± 0.15
16:1n-7	1.40 ± 0.06	1.48 ± 0.05	6.66 ± 0.42	5.75 ± 0.15
Iso17:0	0.25 ± 0.02	0.21 ± 0.01	0.91 ± 0.09	0.40 ± 0.02
Aiso17:0	0.10 ± 0.01	0.09 ± 0.00	0.42 ± 0.09	0.20 ± 0.01
17:0	1.10 ± 0.01	1.32 ± 0.05	2.69 ± 0.17	2.54 ± 0.13
17:1n-8	0.15 ± 0.00	0.28 ± 0.02	1.97 ± 0.25	0.97 ± 0.08
18:0	10.37 ± 0.12	12.54 ± 0.22	9.96 ± 0.37	11.19 ± 0.27
18:1n-9	12.72 ± 0.43	12.19 ± 0.14	8.41 ± 0.43	7.88 ± 0.37
18:1n-7	2.60 ± 0.10	2.18 ± 0.06	3.49 ± 0.13	3.63 ± 0.10
18:2n-6	14.98 ± 0.21	11.26 ± 0.10	1.78 ± 0.20	1.11 ± 0.02
18:3n-3	0.46 ± 0.06	0.70 ± 0.02	0.55 ± 0.15	0.24 ± 0.01
20:1n13 + 11	0.01 ± 0.00	0.08 ± 0.00	0.61 ± 0.05	0.43 ± 0.03
20:1n-9	1.21 ± 0.03	0.72 ± 0.01	0.33 ± 0.02	0.43 ± 0.02
20:1n-7	0.10 ± 0.01	0.09 ± 0.00	0.62 ± 0.04	0.39 ± 0.03
20:2n-6	1.32 ± 0.05	1.10 ± 0.08	0.72 ± 0.02	0.84 ± 0.06
20:4n-6	2.57 ± 0.09	4.12 ± 0.05	6.02 ± 0.44	5.05 ± 0.10
20:3n-3	0.09 ± 0.06	0.18 ± 0.01	0.11 ± 0.01	0.15 ± 0.01
20:5n-3	11.65 ± 0.37	16.06 ± 0.31	14.78 ± 0.47	17.15 ± 0.71
22:4n-6	0.04 ± 0.00	0.05 ± 0.00	0.85 ± 0.13	0.69 ± 0.03
22:5n-6	0.46 ± 0.01	0.26 ± 0.01	0.90 ± 0.07	0.76 ± 0.01
22:5n-3	0.67 ± 0.03	0.83 ± 0.02	1.88 ± 0.16	2.44 ± 0.05
22:6n-3	9.76 ± 0.68	10.73 ± 0.23	8.61 ± 0.66	14.23 ± 0.20
Total n-3	22.63	28.50	25.93	34.21
Total n-6	19.37	16.79	10.27	8.45
n-6:n-3 Ratio	0.86	059	0.40	0.49

Values are reported as weight percent of fatty acids.

TABLE 4. *FCR (feed conversion ratio), survival, mean weight at harvest (±SD), and total production for* Litopenaeus vannamei *fed fish-meal-based and plant-based diets.*

Diet	FCR	Survival (%)	Harvest weight (g)	Production (kg/ha)
Fish-meal-based diet (3 ponds)	1.4	91	18.5 ± 3.5[b]	4404
Treatment mean	1.4	100	17.3 ± 2.7[c]	4549
	1.3	87	20.3 ± 2.9[a]	4829
	1.4	93	18.7	4594
Plant-based diet (3 ponds)	1.4	86	18.6 ± 3.0[b]	4223
Treatment mean	1.3	92	18.7 ± 2.8[b]	4782
	1.3	85	20.3 ± 2.8[a]	4772
	1.3	88	19.2	4592

Letters indicate significant difference ($P < 0.05$) among ponds (rows) for average shrimp weight at harvest.

harvest as determined by ANOVA differed among ponds across both treatments; however, there were no statistical differences between the plant-based diet and the fish-meal-based diet for any of the production parameters.

Contaminant levels were negligible in shrimp fed either the fish-meal-based diet or the plant-based diet. No contaminant that exceeded the minimum detection limits of the methods employed came close to exceeding either US Environmental Protection Agency screening values or US Food and Drug Administration action levels. Of contaminants with reportable values, there were no significant differences between shrimp fed the fish-meal-based diet and those fed the plant-based diet. All pesticides, all PAHs, 73 of 79 PCB congeners, and 7 of 22 metals were below detection limits for shrimp from both diet treatments.

Mean lipid content was 1.02 ± 0.02% for shrimp fed the plant-based diet and 1.06 ± 0.02% for shrimp fed the fish-meal-based diet and was not significantly different between diet groups ($F_{1,35} = 1.719$, $P = 0.1985$). Lipid class composition, approximated from analysis of one animal from each pond, was 70–80% phospholipids (membrane lipid), 6–10% sterols, and the remaining 10–24% neutral lipids (storage lipid).

Seventy-one FAs were identified in the feed or shrimp. The 18 FAs that had mean percentages ≥0.5% for either the feeds or the shrimp and together account for >90% of the total FAs in both the feeds and the edible shrimp tissue are shown in Table 5. The plant-based diet contained approximately 16% saturates, 20% monoenes, and 62% PUFAs; the fish-meal-based diet contained 30% saturates, 26% monoenes, and 43% PUFAs. PUFAs in the plant-based feed were 41.1% LA and 19.0% LnA with little 20 and 22 carbon PUFAs. In contrast, the fish-meal-based feed contained 17% LA and 2.5% LnA, with EPA and DHA substantially contributing to the overall PUFA composition at 6.6 and 8.1%, respectively (Fig. 1, Panel A). AA was relatively low in both feeds, at 0.22% of FAs in the plant-based feed and at 0.64% in the fish-meal-based feed.

The major FAs in shrimp from both diet groups were 16:0, 18:0, 18:1, 18:2n-6, 18:3n-3, 20:4n-6, 20:5n-3, and 22:6n-3 (Table 5). Major differences in the FA profiles between the two groups were in the percentages of the PUFAs. LA ($F_{1,35} = 1055$, $P \leq 0.0001$) and LnA ($F_{1,35} = 1069$, $P \leq 0.0001$) were significantly higher in shrimp fed the plant-based diet, while AA ($F_{1,35} = 64$, $P \leq 0.0001$), EPA ($F_{1,35} = 519$, $P \leq 0.0001$), and DHA ($F_{1,35} = 182$, $P \leq 0.0001$) were significantly lower (Table 5; Fig. 1, Panel B). The ratio of total n-6:n-3 FAs was 1.13 in shrimp fed the plant-based diet compared to 0.58 in shrimp fed the fish-meal-based diet. The primary factor contributing to the high n-6:n-3 ratio in the shrimp fed the plant-based diet was the incorporation of a substantial amount of dietary LA resulting in a level in the shrimp of 23%, surpassing the percentages of all other FAs. This was not surprising because LA comprised over 40% of the FAs in the plant-based feed. The level of incorporation of LnA appeared to be lower for these shrimp with 18% in the feed and 4.6% in the shrimp tissue.

TABLE 5. *Mean percentages (±SE) of selected fatty acids (mean values >0.5%) found in shrimp* (Litopenaeus vannamei) *raised on a plant-based diet and shrimp raised on a fish-meal-based diet.*

	Plant-based feed		Fish-meal-based feed	
	Feed	Shrimp (n = 18) Mean ± SE	Feed	Shrimp (n = 18) Mean ± SE
Total lipid (%)[a]	6.7	1.02 ± 0.02	5.7	1.06 ± 0.02
Fatty acid:	Weight % of fatty acids			
14:0	0.55	0.18 ± 0.00	4.90	0.69 ± 0.02
15:0	0.08	0.26 ± 0.00	0.45	0.43 ± 0.01
16:0	10.67	16.08 ± 0.11	17.74	17.17 ± 0.10
16:1n-7	0.43	0.50 ± 0.01	6.04	1.80 ± 0.04
16:2n-4	0.06	<0.01 ± 0.00	0.87	0.03 ± 0.00
17:0	0.13	1.20 ± 0.02	0.48	1.40 ± 0.02
C18:0	4.30	11.58 ± 0.09	4.40	10.69 ± 0.11
18:1n-9	18.08	10.63 ± 0.05	13.13	11.80 ± 0.09
18:1n-7	1.21	2.05 ± 0.02	2.78	3.12 ± 0.04
18:2n-6	41.05	23.27 ± 0.14	16.97	12.52 ± 0.10
18:3n-3	18.97	4.63 ± 0.05	2.48	0.98 ± 0.04
18:4n-3	0.13	0.04 ± 0.00	1.65	0.14 ± 0.01
20:1n-9	0.32	0.46 ± 0.01	1.51	0.89 ± 0.01
20:4n-6	0.22	3.00 ± 0.05	0.64	3.46 ± 0.06
20:4n-3	0.06	0.12 ± 0.00	0.89	0.27 ± 0.00
20:5n-3	0.52	10.81 ± 0.13	6.60	15.77 ± 0.13
22:5n-3	0.10	0.48 ± 0.01	1.33	0.87 ± 0.02
22:6n-3	1.08	8.75 ± 0.12	8.06	11.79 ± 0.16
Total saturated	16.67	31.00 ± 0.00	29.72	32.46
Total monoenes	20.62	14.42	25.90	18.88
Total PUFA	62.53	54.18	42.23	48.11
n-3	20.86	25.38	21.67	30.27
n-6	41.51	28.71	18.45	17.55
n-6:n-3	1.99	1.13	0.85	0.58

PUFA = polyunsaturated omega-3 fatty acid.
[a] for feed "as is"; for shrimp – wet weight.

The strong effect of dietary LA in all cultured shrimp was evident in the plot of principal component (PC) scores. PC1 (68.5% of the variance) separated wild shrimp (positive PC1 scores) from all cultured shrimp (negative PC1 scores). Positive PC1 scores were influenced by high levels of LA in the cultured shrimp and negative PC1 scores by higher levels of the longer-chained n-6 FAs (AA, 22:4n-6, and 22:5n-6) in wild shrimp. PC2 (14.6% of the variance) separated study shrimp fed the plant-based diet from study shrimp fed the fish-meal-based diet. The fish-meal-based diet shrimp clustered with other cultured *L. vannamei* from Mexico and previous Waddell studies along the PC2 axis. PC2 also separated all but one of the wild *L. setiferus* from the wild *L. vannamei* from Mexico. Negative PC2 scores were driven by higher levels of EPA and DHA (Fig. 2).

Discussion

For the consuming public, the appeal of aquaculture diets that contain little or no fish meal and fish oil relates to both environmental and human health concerns. Organic, certifiable, plant-based aquaculture diets have the potential to reduce industry fish meal use, which in turn may lower the current rate of depletion of pelagic fisheries, assuming that use by other sectors remains static. More importantly, such diets also address the concern over chemical contaminants that may accumulate in the food chain, become concentrated in fish meal, and be passed onto the consumer through aquacul-

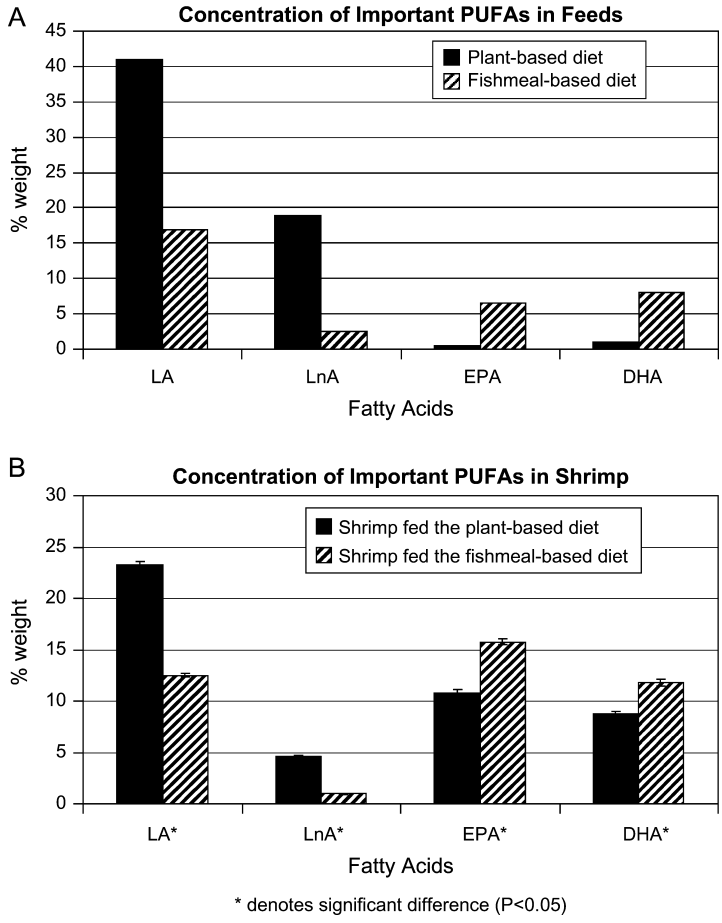

FIGURE 1. *Mean percentages of important PUFAs measured in the organic certifiable, plant-based and fish-meal-based diets (Panel A) and in the edible tissue of shrimp fed these diets (Panel B). "*" indicates a significant difference (*$P < 0.0001$*).*

tured, seafood-fed, fish-meal-based diets. On these two counts, the experimental diet employed in this study was successful.

There were no significant differences among the average weight of shrimp at harvest, the total production per hectare, the growth rate, survival, and FCR of the ponds fed the plant-based diet versus those receiving the fish-meal-based diet (Table 4). This demonstrates that a fish-meal-free, plant-based diet supplemented with sources of DHA and AA and very small amounts of squid meal and liquid fish solubles for palatability can be a fully equivalent or superior shrimp production grow-out feed compared to conventional fish-meal-based diets.

An important reason for eliminating fish meal from aquaculture feeds is to further reduce the potential for contaminant accumulation in the seafood (Hites et al. 2004). In this study, the chemical contaminant loading of shrimp from both diets was essentially nonexistent. Shrimp in general, because of their short life spans, low trophic-level feeding, and low body fat accumulate very few contaminants and are generally one of the healthiest seafoods from this perspective (Mahaffey 2004; Tittlemier et al. 2004; Burger et al. 2005; Rawn et al. 2005). The results of analysis of shrimp from both the conventional fish-meal-based and the plant-based diets in the present study reconfirm

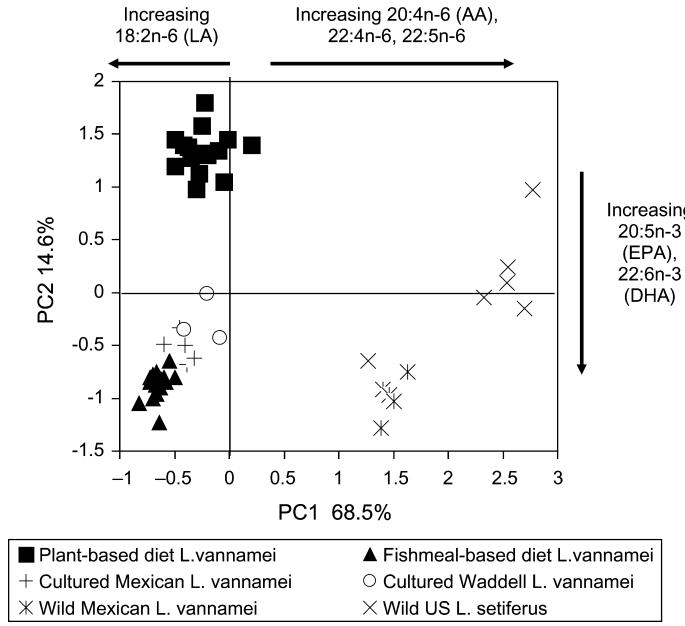

FIGURE 2. *Plot of principal component scores for individual study shrimp (Litopenaeus vannamei) fed a plant-based diet and a fish-meal-based diet. Also shown are scores of shrimp from a previous Waddell Mariculture Center study (L. vannamei), a culture facility in Mexico (L. vannamei), wild caught in Mexican waters (L. vannamei), and wild caught off the southeastern USA (Litopenaeus setiferus). Fatty acids with loadings (>0.80) for each axis are listed with directional arrows.*

the low-contaminant loads of shrimp and further demonstrate the equivalency of the two dietary approaches.

Results indicate that FA profiles of shrimp fed fish-meal-based and plant-based diets were significantly different. This is an important point because the public is aware of and concerned about the health benefits provided by n-3 PUFAs, especially DHA and EPA, as well as the n-6:n-3 ratio of PUFAs in the seafood they consume. Shrimp fed the plant-based diet were equivalent to those fed the fish-meal-based diet in the amount of lipid per weight of tissue. The mean lipid content of 1.0% for the study shrimp fell within the range of values (0.9–1.1 g/100 g) reported by Bragagnolo and Rodriguez-Amaya (2001) for several species of wild marine shrimp and farmed freshwater prawns. These values, however, were lower than the value of 1.73 g/100 g listed in the US Department of Agriculture (2005) nutritional database for mixed species. In all cases, however, shrimp would clearly fall within the category of a low-fat species.

The FA profiles of the study shrimp were qualitatively similar to those reported by other investigators (Bragagnolo and Rodriguez-Amaya 2001; Glencross et al. 2002; Gonzalez-Felix et al. 2003). These authors attribute quantitative differences in shrimp FA profiles primarily to dietary influence, especially for cultured shrimp. Because wild shrimp have a substantially lower percentage of LA than farm-raised shrimp, it is doubtful that shrimp have a requirement for the high levels of this FA typically found in commercial feeds. Bottino et al. (1980) concluded that seasonal changes in the FA profiles of wild *L. setiferus*, *Farfantepenaeus aztecus*, and *Farfantepenaeus duorarum* were likely because of changes in the phytoplankton composition transmitted directly or indirectly to the shrimp through diet rather than a response to temperature change. They further reported that dietary differences had a greater effect on FA composition than species differences, noting similar seasonal changes in composition among species. Because the FA profile of any feed system can be modified, it should

be possible to alter the FA composition of aquacultured shrimp depending upon social and economic considerations.

The predominant difference in the FA composition of the two feed groups in this study was the substantially higher percentages of LA and LnA in shrimp fed the plant-based diet. It appears that dietary LA was readily incorporated into shrimp tissue somewhat proportionally to that contained in their diet. Bottino et al. (1980) reported an increase in LA from 2.3 to 18.1% when laboratory-reared shrimp were placed on a diet containing 32% LA for 1 mo. No further increase was noted at the end of 3 mo, and the authors suggested that there may be limited upper levels for the deposition of unsaturated FAs in shrimp tissues. The present study seems to support this concept as the level of LA in the tissues of shrimp on the plant-based diet was 23% or about 50% of that found in the diet, while tissues of shrimp on the fish-meal-based diet contained 13% LA, 76% of that found in the diet. On the other hand, it is also likely that the very high percentage of LnA in the plant-based diet may have affected the uptake and deposition of LA.

While the mean percentages of LA and LnA were lower in the tissue of shrimp than in their respective diets, those of AA, EPA, and DHA were considerably higher. AA, EPA, and DHA were significantly lower in tissue of the shrimp fed the plant-based diet than those fed the fish-meal-based diet. However, this difference was substantially less than that might be expected based on the differences in levels of these FAs in their respective diets. Although the amounts of AA, EPA, and DHA were $\leq$1.08% each in the plant-based diet, they accounted for 3.0, 10.8, and 8.8%, respectively, of FAs found in shrimp fed this diet; 14, 20, and 8 times that found in their diet. The AA, EPA, and DHA percentages of 3.5, 15.8, and 11.8%, respectively, found in shrimp fed the fish-meal-based diet were only 5.4, 2.4, and 1.4 times that found in their diet (Table 5). Previous studies have shown that shrimp have very limited ability to convert LA to AA (Lilly and Bottino 1981) and LnA to EPA and DHA (Kanazawa et al. 1979). Therefore, AA, EPA, and DHA must be acquired through diet. It is becoming generally recognized that natural pond productivity may contribute significantly to *L. vannamei* nutrition (Moss et al. 1992; Decamp et al. 2002; Moss 2002; Tacon et al. 2002). Bottino et al. (1980) reported 0.2, 2.4, and 0.4% of AA, EPA, and DHA, respectively, for algae recovered from a shrimp-rearing pond and 1.3, 5.7, and 26% for benthos. It is quite possible that natural pond production provided these FAs equally to both feed groups in this study. Additional research is needed to better understand the contribution of natural productivity to the EPA and DHA levels in farmed shrimp.

Most of the published shrimp diet studies focus on establishing an economically feasible shrimp diet formulation that will provide optimum production. The ability to achieve this with a plant-based, no-fish-meal diet has been demonstrated in this study. While this is critical to the industry, the health-conscious consumer's interest extends to the development of a product with higher EPA and DHA and a lower n-6:n-3 ratio. Because of effectiveness and cost, vegetable-based oils with high levels of LA are a common lipid component of aquaculture diets, often exceeding the levels of fish meal in even conventional diets. Shrimp fed the plant-based diet exhibited not only the high levels of LA and LnA reflective of the higher levels in the feed but also displayed significantly lower levels of EPA and DHA than did the shrimp fed the fish-meal-based diet (Fig. 1). This difference in FA profile clearly distinguishes between the shrimp raised on the two diets (Fig. 2). A further result of these distinctions is that the n-6:n-3 ratio is nearly twice as large (1.13 vs 0.58) for shrimp raised on the plant-based diet. Therefore, while the plant-based diet was fully equivalent to the fish-meal-based diet in terms of production efficiencies and equally safe in regard to chemical contaminant risks, the edible product that resulted was inferior in terms of those considerations most important to human health, concentrations of EPA and DHA, and the n-6:n-3 ratio.

Although EPA and DHA levels were significantly lower for the fish-meal-free diet

TABLE 6. *Comparison of levels of total lipids (fats), EPA, and DHA found in the study shrimp to those of other popular meats listed in the online US Department of Agriculture (2005) nutritional database*

	Total lipids (fats), g/100 g	20:5n-3 (EPA), g/100 g	22:6n-3 (DHA), g/100 g
Beef, fresh, variety meats and by-products, raw	23.52	0.00	0.00
Pork, fresh, variety meats and by-products, raw	26.54	0.00	0.00
Chicken, broilers or fryers, meat only, raw	3.08	0.01	0.03
Salmon, farmed Atlantic, raw	10.85	0.62	1.29
Tuna, canned white, in water	2.97	0.23	0.63
Crustaceans, shrimp, mixed species, raw	1.73	0.26	0.22
Conventional fish-meal-based diet study shrimp	1.06	0.12	0.09
Plant-based, fish meal-free diet study shrimp	1.02	0.08	0.06

formulation tested in this study, the human health significance of this difference may be relatively small compared to other popular protein sources. We compared the amounts of EPA and DHA available from the shrimp raised on the two diets with the values from the online US Department of Agriculture (2005) nutritional database for beef, pork, chicken, salmon, tuna, and mixed-species shrimp (Table 6). In order to make these comparisons, the relative amounts of FAs determined for the study shrimp were converted to g FA/100 g tissue using equations published by Weihrauch et al. (1977). Because shrimp have a low-fat content, the absolute amounts of EPA and DHA that they provide are correspondingly low compared to many finfish but clearly exceed those of beef, chicken, and pork. Nevertheless, efforts to develop a no-fish-meal-reared shrimp must be cautious about maintaining an FA profile that is at least equivalent to that of shrimp raised on a fish-meal-based diet and ideally mimics the profile of wild shrimp. Aquaculture should be concerned about following the model of modern industrial-style chicken production. In 1980, a typical chicken contained 170 mg of DHA per 100 g of tissue; today because of changes in diet, the average chicken contains 25 mg DHA/100 g tissue (Ungoed-Thomas 2005). It seems likely that a reduction of LA in shrimp aquaculture diets in combination with enhanced levels of EPA and DHA could result in an exceptionally healthy product with higher percentages of EPA and DHA and a lower n-6:n-3 ratio that would more closely mimic wild shrimp. While the EPA and DHA levels in the shrimp from this study were modest, that is a reflection of the diet formulation. Supplements can be added to feeds to customize diets in order to produce a desired profile; however, such modifications may not be cost effective. Future research into the use of finishing diets at the end of grow out to restore human-health-optimal FA ratios to shrimp raised on fish-meal-free formulations might also prove productive in addressing this issue.

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