Practical Diets for *Litopenaeus Vannamei* (Boone, 1931): Working Towards Organic and/or All Plant Production Diets.

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Abstract

As shrimp prices become more competitive and the profit margins declining, producers are looking to high value markets for their products. Shrimp produced using organic or environmentally friendly production conditions have the potential to bring in higher prices through market differentiation. However, if one is to develop production schemes for such markets, one must also produce an organic feed or one that has minimal levels of marine proteins and oils. In previous research we developed a practical diet formulation without fish meal but containing marine fish oil. Since supplies of fish oil are also limited, this work concentrated on replacement of the marine oil source as well as the testing of an experimental organic diet. In the first experiment, fish oil in two of the diets was substituted by oil originating from commercially produced algae containing approximately 50% oil. These diets as well as a commercial feed were offered to juvenile *Litopenaeus vannamei* (Boone, 1931) over a 15 week growth trial. At the conclusion of the growth trial, survival, final weight, and feed conversion ratio (FCR) were not significantly different among treatments. In the second experiment, the diet previously tested using plant and algae oils was tested against a diet using only plant oils. To examine the potential of an organic diet, a practical diet using primarily organic ingredients was also tested. The three diets were tested in conjunction with a commercial control diet over a 12-week growth period. At the conclusion of this trial, shrimp reared on the organic diet and the diet without algae oil supplements were significantly smaller than those offered the commercial control. This result is presumably due to a lack of HUFA in the diets without algae oils high in DHA and AA. To further test the potential of a feed without fish meal, a commercial version of one of the test diets was produced at a feed mill and tested under pond production conditions. As this was only a demonstration, statistical differences cannot be determined but the feed did appear to produce acceptable results under commercial pond production conditions. Based on the results of these studies, it would appear that both fish meal and marine oil sources can be removed from shrimp feeds if suitable alternative sources of protein and lipids are provided to meet essential amino acid and fatty acid requirements of the shrimp. Although this study confirms the biological feasibility of fish meal and fish oil replacement at the densities tested, commercial application will require further analysis of relative costs and marketing benefits of these technologies.

1. Introduction

As part of the global growth of aquaculture, the world production of crustaceans has experienced continuous expansion that is expected to continue as world population increases and demand for quality sea food continues to rise (FAO, 2003b; Tacon, Dominy & Pruder 2000). Although crustaceans contributed only 5.25 % of the world aquaculture production in 2002, it represented 20.8 % of the total production by value (FAO, 2003a). In 2001, marine shrimp were the second most important world aquaculture species with a value of US$ 8.4 billion (FAO, 2003a). Additionally, marine shrimp production represented about 50 % of the total crustacean production. Other crustaceans groups produced include: crab and sea spiders, 15.0 %; freshwater crustaceans (mainly *Macrobrachium rosenbergii*), 13.7 %; lobster and spiny-rock lobster, 2.7 %; and other marine crustaceans, 18.5 % (FAO, 2003a).

Paralleling the growth of the industry has been an expansion in feed production (Tacon et al., 2000). The majority of finfish and crustaceans species fed with these commercial feeds are omnivorous or carnivorous species (Lee, Blake & Rodrick 1980; Lee and Lawrence, 1997; Tacon and Akiyama, 1997) which demand high quality protein and special organoleptic properties of the feed. Fish meal, as well as other marine meals such as, krill, shrimp, squid and scallop waste, are often included in aquatic feeds as they are considered an excellent source of high quality proteins, highly unsaturated fatty acids, vitamins, minerals and attractants (Tacon and Akiyama, 1997). Due to these properties, fish meal has become one of the primary components of commercial feed formulations. The demand for fish meal in aquatic feeds has been estimated to account for 31 % to 42.5 % of total world fish meal production (Tacon and Berg, 1998). Of this, marine shrimp consumed about 17.6 % of the total amount.

Even though fish meal and other marine meals are excellent sources of protein and other essential nutrients for aquaculture feeds, their demand is subject to competition from other sectors of the agriculture industry. However, the supply, quality, and price often fluctuate from year to year due to both market and environmental constraints. There are also environmental concerns, in terms of pollution, and over-fishing as well as ethical considerations for the use of fish products that could be used directly to feed humans (Chamberlain, 1993; Tacon and

Akiyama, 1997). Due to these constraints replacement of fish meal and other marine proteins with alternative sources of proteins from terrestrial animal or plants has been encouraged (Tacon and Akiyama, 1997).

Animal by-product meals include meat and bone meal, blood meal, poultry by-product meal, feather meal (e.g., hydrolyzed or enzyme treated), and specialized protein. These feed ingredietns are characterized by: higher production than fish meals, cheaper but variable composition, lower nutritional quality than fish meals (e.g., some imbalances in essential amino acids, high ash content, and reduced nutrient digestibility, among others) possible palatability problems, and possible microbial contamination (Bureau and Cho, 1999). With suitable considerations of nutrient profiles and palatability, these meals are often utilized as a substitute for marine protein sources with good success (Davis and Arnold, 2000; Forster, Dominy, Obaldo & Tacon 2003). The primary draw back is that they are often quite variable in quality.

Another source of proteins for aquatic feeds is plant proteins (Li, Robinson & Hardy 2000). From a nutritional stand point, problems are similar to those of using animal by-products. Fish meal and most of the marine meals can be replaced either singularly or in combination with plant protein sources without affecting the physical and nutritional quality of the feeds (Viola, Mokady, Rappaport & Arieli 1982; Viola, Arieli & Zohar 1988; Wu, Rosati, Sessa & Brown 1995; Webster et al., 1995; Tidwell, Webster, Yancey, & D'Abramo 1993; Sudaryono, Hoxey, Kailis & Evans 1995; Davis and Arnold, 2000; Samocha, Davis, Saoud & DeBault. 2004). Plant proteins are produced in larger quantities than fish meals, their production year to year is more stable, they are often less costly, and their expanded use does not threaten overexploitation of a limited resource as can occur with fisheries products. Among different sources of vegetable proteins, soybean meal is most commonly used as a replacement or complement to marine proteins (Hertrampf and Piedad-Pascual, 2000; Olvera-Novoa and Olivera-Castillo, 2000). Other sources of plant proteins, such as cottonseed meal, peanut meal, canola meal, distillers grain with solubles, and some legume meals are commonly utilized (Li et al., 2000). However, the use of plant proteins can be limited due to a variety of factors including, deficiency or imbalance of

essential amino acids, reduced levels of minerals, limited levels of HUFA, presence of anti-nutritional factors or toxins, and decreased palatability.

In addition to being good sources of EAA, marine meals are a good source of marine oils which are rich in highly unsaturated fatty acids (HUFA). Lipid content and the associated C18 poly-unsaturated fatty acids (PUFA), linoleic (18:2 n-6) and linolenic (18:3 n-3) as well as n–3 HUFA (eicosapentanoic acid, EPA: decosahexanoic acid, DHA: and arachidonic acid, AA), are required in shrimp and other crustaceans feeds (Kanazawa, Tokiwa, Kayama & Hirata 1977; Read, 1981; Fenucci, Lawrance & Zein-Eldin 1981; Martin, 1980; Shiau, 1998). It has been demonstrated that these essential fatty acids are required in the diets of crustaceans at levels between 0.5 % and 1 % for penaeid shrimp (for reviews see Lim and Akiyama, 1995). Therefore, their content in the diet should be considered when replacing fish meal and oil. Although one could add marine oils rich in HUFA to counter problems with fatty acid levels and ratios associated with the use of vegetable oils, marine oil sources are also limited and in short supply. Consequently, alternative oil sources should also be pursued.

Many of the before mentioned limitations can be overcome through the use of proper combinations of different types of ingredients to balance essential nutrient profiles (e.g., amino acids and fatty acids), by developing specific processing procedures to inactivate, reduce or eliminate anti-nutritional factors (e.g., heat treatment for heat labile components), and/or by limiting their inclusion in the diet to a level that does not influence animal performance (Li et al., 2000).

Although, nutritional information on shrimp is far from complete, we have a good understanding of primary nutrient requirements to allow the replacement of marine protein and oil sources with non-marine ingredients. However, to do this one has to have a replacement strategy that considers nutritional requirements for essential amino acids, fatty acids, mineral and vitamins. Since most commercial feeds contain complete vitamin and trace-mineral premixes, we probably do not need to worry about these nutrients. However, quite often shifts in essential amino acids,

fatty acids and macro-minerals are not considered. Consequently, if we consider the potential nutritional shifts, we should be able to completely replace fish meal and oil if a suitable replacement strategy is adapted.

2. Objective

The primary objective of the present stud y was to evaluate the effect of fish meal and fish oil replacement strategies using non-marine protein and oil sources and the subsequent influence on growth and survival of the Pacific white shrimp *Litopenaeus vannamei* (Boone, 1931) in a small scale outdoor tank system. A secondary objective was to demonstrate the potential of commercial shrimp feeds using alternative ingredients under commercial pond production conditions.

3. Material and Methods

Two outdoor growth trials in tanks as well as a pond production trial were conducted to evaluate various replacement strategies for the reduction of marine ingredients in practical shrimp feeds. Test diets (Table 1) were produced in the laboratory at the Auburn University, Auburn AL. The oil and dry ingredients were weighed and then mixed in a food mixer (Hobart Corporation, Troy, OH, USA) for 15 min. After mixing, hot water was then blended into the mixture to attain a consistency appropriate for pelleting. Each diet was pressure pelleted using a meat grinder and a 2-mm die. After pelleting, the diets were dried to a moisture content of 8-10 % using a forced air drying oven (< 40 ☜C) and stored at 4 ☜C. Dietary treatments were randomly assigned and each experiment was run using a double blind experimental design.

In experiment 1, a 15-week feeding trial was conducted with juveniles (0.66 ± 0.06 g) of the Pacific white shrimp, *Litopenaeus vannamei* to determine the suitability of three diets in which marine fish meal was replaced with co-extruded soybean poultry by-product meal with egg supplement (CoESPB; Profound^{TM}, American Dehydrated Foods, Inc., Verona, MO, USA). A practical basal diet (Diet 3) which was previously developed as a fish-meal-free diet was formulated to contain 35% protein and 8% lipid (Table 1). To further refine this diet, fish oil in two of the diets (Diet 1 and 2) was replaced with oil originating form two commercially produced heterotrophic marine “algae” (Aqua Grow-Hi DHA, AquaGrow ARA, Advanced BioNutrition Corp., Columbia, MD, USA). A commercial diet (35% crude protein, 8% crude fat; Rangen Inc., Buhl, ID, USA) was offered as a commercial reference or control. For each treatment 5 replicate tanks were stocked with 26 shrimp resulting in a density of 30/m^2 (which corresponds to 46/m^3).

In experiment 2, a 12-week feeding trial was conducted with juvenile shrimp (6.07☽≺0.3 g) to determine the suitability of three diets in which marine fish meal and/or marine fish oil was replaced with CoESPB, algae meals or organic plant protein sources. A practical basal diet (Diet 4) which was previously derived from a diet developed as a fish-meal-free diet was formulated to

contain 35% protein and 8% lipid (Table 1). To confirm the need of HUFA oil supplements, the HUFA oil source was removed from Diet 5. Diet 6 was formulated to replace the Profound™ with organic plant protein sources. A commercial diet (35% crude protein, 8% crude fat; Rangen Inc., Buhl, ID, USA) was offered as a control reference. For each treatment, 5 replicate tanks were stocked with 19 shrimp resulting in a density of 22/m^2 (which corresponds to 34/m^3).

Table 1. Diet formulations expressed as g/100g (as is) for practical diets designed to contain 35 % protein and 8 % lipid using various strategies for the replacement of marine fish meal and oil.

	Experiment I			Experiment II		
	Diet 1 AG2-0.5	Diet 2 AG0.5-0.13	Diet 3 Profound	Diet 4 AG0.5-0.13	Diet 5 w/o MFO	Diet 6 Organic
Profound™[1]	39.00	39.00	39.00	39.00	39.00	
Soybean meal	29.50	30.20	30.50	30.20	30.20	
Soybean meal, organic [3]						58.10
Field Pea Meal [4]						10.00
Corn gluten, organic [5]						9.00
Aqua Grow-Hi DHA [6]	2.00	0.50		0.50		0.50
AquaGrow ARA [6]	0.50	0.13		0.13		0.13
Kelp meal[7]						0.50
Menhaden Fish Oil [8]			3.04			
Soy oil [9]	1.47	1.53		1.53	1.30	
Soy oil, organic [10]						0.20
Flax oil (linseed oil) [11]	0.48	1.23		1.23	1.80	
Flax oil, organic [12]						2.00
Wheat starch [9]	1.98	2.34	2.39	2.34	1.63	
Whole wheat [9]	20.00	20.00	20.00	20.00	21.00	
Whole wheat-organic [10]						14.00
Trace Mineral premix	0.50	0.50	0.50	0.50	0.50	0.50
Vitamin premix	1.80	1.8	1.8	1.80	1.80	1.80
Choline chloride [9]	0.20	0.20	0.20	0.20	0.20	0.20
Stay C 250 mg/kg 14	0.07	0.07	0.07	0.07	0.07	0.07
CaP-diebasic [9]	2.00	2.00	2.00	2.00	2.00	2.00
Lecithin (soy refined) [9]	0.50	0.50	0.50			
Lecithin, organic crude [15]				0.50	0.50	0.50
Betaine-3DP [16]						0.50

1 Profound™ , Co-extruded soybean and poultry by-product meal. American Dehydrated Foods, Inc., Verona, MO, USA.
2 Dehulled Solvent extracted soybean meal, Southern States, Cooperative Inc. Richmand VA, USA.
3 Expeller Pressed soybean meal, Organic Professional Proteins LTD, Washington, IA, USA.
4 Whole Green Peas, feed grade, Popular Valley Organics, Canada.
5 Corn gluten meal 60% protein, Grain Processing Corporation, Muscatine, Iowa. Via Cereal By-product, West Memphis
6 Aquagrow Hi DHA(schizochytrium sp algae meal) Advanced BioNutrition, Columbia, MD USA.
7 *Ascophyllum nodosum* flour, Acadian Seaplants Limited, Nova Scotia, Canada.
8 Omega Protein, Inc., Reedville, VA, USA.
9 United States Biochemical Company, Cleveland, OH, USA.
10 Clarkson Grain Co Inc. Cerro Gordo, IL, USA.
11 Sigma, St. Louis, MO, USA.
12 Sila Nutrition Toronto, Ontario, Canada.
14 Stay C® , (L-ascorbyl-2-polyphosphate 35% Active C), Roche Vitamins Inc., Parsippany, New Jersey, USA.
15 Clarkson Soy Products Inc. Cerro Gordo, IL, USA.

[16] Danisco Animal Nutrition, Carol Stream, IL, USA.

Growth trials for experiments 1 and 2 were conducted at the Shrimp Mariculture Research Facility, Texas Agricultural Experiment Station, Corpus Christi, TX. Each study was conducted in replicated HDPE circular tanks positioned under a shade with roofing made of clear and opaque panels. Each tank had a working volume of 650 L and bottom area of 0.85 m^2. Each tank was equipped with two air stones which delivered air at a rate of 8-10 Lpm and covered with 0.3 mm mesh netting to prevent shrimp from jumping out of the tanks. Natural seawater was used after initial chlorination with salinity adjusted to 30 ppt. One tank in each treatment was provided with a feed tray that covered about 45% of the tank's bottom (about 0.40-m^2) to estimate feed consumption. Five shrimp from each of these tanks were collected weekly to estimate growth (group weights) and to adjust daily rations. Weekly rations were calculated assuming 100 % survival, FCR of 1:1.5 and an estimated growth between 1 g and 1.2 g per week. Daily rations were then calculated based on expected growth and offered four times per day. Each tank was run as a static system with municipal freshwater added to offset evaporation losses. Dissolved oxygen, temperature, salinity and pH were monitored twice a day (morning and afternoon) in each tank. Total ammonia-nitrogen and nitrite-nitrogen were monitored once a week in all tanks. On the day of termination, reactive phosphorus, five-day carbonaceous biochemical oxygen demand ($cBOD_5$) and nitrate-nitrogen were monitored in all tanks. At the conclusion of the fifteen-week growth trial, all shrimp were harvested, counted and weighed. Average final weight and survival for each dietary treatment were determined. Feed conversion ratio values were estimated based on feed inputs. Differences in final average weights, survival (arcsine transformed), and FCR were analyzed using an analysis of variance to determine if significant (p <0.05) differences existed among treatment means. The Student-Neuman-Keuls multiple comparison test was used to determine where significant differences existed between treatment means. Repeated Measures ANOVA tests were used to compare differences in daily water quality indicators and total ammonia nitrogen and nitrite nitrogen between treatments. All statistical analyses were conducted using SPSS statistical software (V. 11 for Windows, SPSS Inc., Chicago, IL, USA).

To demonstrate the potential of diets without fish meal, preliminary pond trials were conducted at the Waddell Mariculture Center, Bluffton, SC using a commercial version of Diet 2 formulated to contain 30 % protein and 8.18 % lipid. This diet was compared to a high quality commercial feed containing 35 % protein with 2.5 % squid. The shrimp were reared in four ponds, two medium (0.25 ha) and two large (0.50 ha). Ponds were filled at least two weeks prior to stocking with a mix of water from the Colleton River (~28 ppt) filtered through a 400 µm size mesh bag and fertilized with organic fertilizer to stimulate algal growth. Ponds were run 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. Shrimp (PL_{10}) were stocked into the ponds on 11 June 2003, at a density of 25.2 shrimp/m^2, with one medium and one large pond per diet treatment. Feed was distributed by commercial feed blower three times a day during the week and two times a day on the weekend. Late in the growing season**,** this schedule was revised to provide feed two times per day. Feed rates were set and adjusted based on growth and estimated survival to maintain food conversion ratios (FCR) below 2:1. Dissolved oxygen (g/L), temperature (C), and pH were measured daily between 6 and 8 AM. Shrimp growth was measured weekly by obtaining weights of at least 25 animals collected by cast net. The shrimp were sampled weekly to determine growth and to adjust feed rates. Shrimp were reared for 62 days in the large ponds while shrimp in the medium sized ponds were reared for the full 111 day growing season. Mean shrimp harvest weight was determined by weighing 100 randomly selected animals to the nearest 0.1 g. Shrimp were harvested by gravity draining the ponds into collection bags attached at the pond outfall drain.

4. Results and Discussion

To capitalize on potential markets for shrimp grown under organic or environmentally sustainable conditions there is considerable interest in the use of organic ingredients as well as diets using ingredients that are from more sustainable sources. Hence a series of growth

trials were conducted to evaluate various replacement strategies for the removal of marine protein and oil sources from shrimp feeds.

Study 1, was terminated after 15 weeks. At the termination of the study there were no significant differences between treatments in daily water quality indicators. Table 2 summarizes the average, standard deviation, maximum and minimum values for these indicators. These values represent acceptable ranges reported for optimal growth and survival of penaeid shrimp.

Table 2. Summary of the daily water quality parameters observed in the first experiment, in which *Litopenaeus vannamei* juveniles were reared over a 15 week period.

	Dissolved Oxygen (mg/L)		Temperature (C)		pH		Salinity
	am	pm	am	pm	am	pm	(ppt)
Average	6.49	6.47	27.2	28.7	7.7	7.9	32
STD[1]	0.36	0.49	0.9	1.25	0.3	0.2	1
Max	7.62	7.81	28.6	30.5	8.4	8.2	36
Min	5.59	5.09	24.3	24.7	6.4	7.4	27

[1] Standard Deviation

Table 3 summarizes the levels of ammonia-nitrogen, nitrite-nitrogen, nitrate-nitrogen, $cBOD_5$, and reactive phosphorus on the last day of the study. No significant differences in total-ammonia-nitrogen, nitrite-nitrogen, $cBOD_5$ and reactive phosphorus concentrations were found between the four treatments. Some statistically significant differences were noticed in nitrate-nitrogen concentrations between treatments; however, since nitrate concentrations did not exceed 7 mg/L, these differences were unlikely to effect animals performance. Table 4 presents the mean values of the ammonia-nitrogen and nitrite-nitrogen over the fifteen-week period. No statistically significant differences were found in these indicators between treatments. In general, the observed water quality indicators were acceptable for good growth and there were no indications the various diets produced different impacts on water quality parameters.

Table 3. Summary (mean ± STD[1]) of daily water quality parameters observed on the day of study termination (Experiment I) [2].

	mg/L				
Treatment	TAN [3]	NO_2-N [4]	NO_3-N [5]	$cBOD_5$ [6]	RP [7]

Diet 1	bdl[7]	0.01 ± 0.01 [a]	4.45 ± 0.76 [a]	8.54 ± 6.13 [a]	3.81 ± 0.86 [a]
Diet 2	bdl[7]	0.01 ± 0.01 [a]	1.77 ± 0.76 [b]	4.59 ± 4.64 [a]	2.73 ± 0.32 [a]
Diet 3	bdl[7]	0.01 ± 0.01 [a]	6.64 ± 1.78 [c]	4.95 ± 2.77 [a]	3.72 ± 0.96 [a]
Control	bdl[7]	0.03 ± 0.05 [a]	2.02 ± 0.53 [b]	4.32 ± 5.34 [a]	3.02 ± 0.63 [a]

[1] Standard deviation
[2] The same superscript letters within a column represent no statistically significant difference (SNK test at 0.05)
[3] Total ammonia-nitrogen
Nitrite-nitrogen
Nitrate-nitrogen
Carbonaceous biological oxidation demand
[7] Reactive phosphorus
[8] Below detection limit

Table 4. Summary (mean ± STD[1]) of the weekly water quality parameters for *Litopenaeus vannamei* juveniles reared over a 15 week period and offered one of four test diets (Experiment I)[2].

Treatment	TAN [3] (mg/L)	NO_2-N [4] (mg/L)
Diet 1	0.13 ± 0.24	0.86 ± 1.46
Diet 2	0.16 ± 0.33	0.21 ± 0.33
Diet 3	0.10 ± 0.19	0.71 ± 1.24
Control	0.13 ± 0.27	0.26 ± 0.58

[1] Standard deviation
[2] Based on ANOVA, no significant differences among treatment means were detected.
[3] Total ammonia-nitrogen
[4] Nitrite-nitrogen

Table 5 summarizes the shrimp average final weights, survival, FCR and yields at the end of the study. No statistically significant differences were found between treatments for these indicators. These results confirm that this practical diet, using no marine protein sources, is comparable to a commercial feed. Additionally, the use of commercially produced algae containing high levels of DHA and AA in combination with plant oil sources promoted acceptable feed utilization, growth and survival when menhaden fish oil was removed from the diet.

Table 5. Summary (mean ± STD[1]) of shrimp final average weights, survival, FCR and yield at the conclusion of a 15 week growth trial in which *Litopenaeus vannamei* juveniles were offered one of four test diets (Experiment I)[2].

Treatment	Average Final Weight (g)	Survival (%)	FCR	Yield/tank (g)
Diet 1 (AG 2-0.5)	17.10 ± 1.13	95.38 ± 4.21	1.55 ± 0.14	424.2 ± 39.4
Diet 2 (AG 0.5-0.13)	17.89 ± 0.41	93.85 ± 11.73	1.52 ± 0.22	436.2 ± 51.8
Diet 3 (MFO)	17.02 ± 0.88	96.92 ± 5.01	1.50 ±0.12	438.7 ± 35.4
Control	18.50 ± 1.04	97.69 ± 3.44	1.40 ±0.12	470.4 ± 39.0
MSE [3]	3.33	3.100	0.069	18.73
P value	0.0621	0.8231	0.4549	0.6774

[1] Standard Deviation
[2] Based on ANOVA, no significant differences among treatment means were detected.

[3] Pooled mean square error.

Study 2, was terminated after twelve weeks. No significant differences were found among treatments in daily water quality indicators. Table 6 summarizes the average, standard deviation, maximum and minimum values for these indicators. These values represent acceptable ranges reported for optimal growth and survival of penaeid shrimp.

Table 6. Summary of the daily water quality parameters observed in the second experiment, in which *Litopenaeus vannamei* juveniles were reared over a 12 week period.

	Dissolved Oxygen (mg/L)		Temperature (C)		pH		Salinity
	am	pm	am	pm	am	pm	(ppt)
Average	6.59	6.69	26.0	27.1	7.7	7.8	29.0
STD	0.69	0.81	2.13	1.88	0.4	0.4	2.5
Max	8.29	9.80	30.0	30.9	8.2	8.3	33.5
Min	5.08	4.81	22.2	23.8	6.8	6.1	20.4

Table 7 summarizes the levels of total ammonia-nitrogen, nitrite-nitrogen, nitrate-nitrogen, $cBOD_5$, and reactive phosphorus on the last day of the study. No significant differences in total ammonia-nitrogen, nitrite-nitrogen, nitrate-nitrogen, $cBOD_5$, and reactive phosphorus concentrations were found between the four treatments.

Table 6. Summary of the daily water quality parameters observed in the second experiment, in which *Litopenaeus vannamei* juveniles were reared over a 12 week period.

	Dissolved Oxygen (mg/L)		Temperature (C)		pH		Salinity
	am	pm	am	pm	am	pm	(ppt)
Average	6.59	6.69	26.0	27.1	7.7	7.8	29.0
STD	0.69	0.81	2.13	1.88	0.4	0.4	2.5
Max	8.29	9.80	30.0	30.9	8.2	8.3	33.5
Min	5.08	4.81	22.2	23.8	6.8	6.1	20.4

Table 7. Summary (mean ± STD[1]) of daily water quality parameters observed on the day of study termination (Experiment 2)[2].

	mg/L				
Treatment	TAN [3]	NO_2-N [4]	NO_3-N [5]	$cBOD_5$ [6]	RP [7]
Diet 4	bdl [7]	0.062 ✂ 0.062 [a]	6.57 ✂ 1.32 [a]	6.67 ✂ 2.69 [a]	2.95 ✂ 0.45 [a]
Diet 5	0.004 ✂ 0.009 [a]	0.119 ✂ 0.092 [a]	7.28 ✂ 0.82 [a]	7.14 ✂ 2.07 [a]	3.29 ✂ 0.74 [a]
Diet 6	0.034 ✂ 0.047 [a]	0.066 ✂ 0.028 [a]	7.64 ✂ 0.41 [a]	5.52 ✂ 1.91 [a]	2.60 ✂ 0.38 [a]
Control	0.028 ✂ 0.041 [a]	0.046 ✂ 0.037 [a]	6.66 ✂ 0.42 [a]	8.22 ✂ 4.09 [a]	3.22 ✂ 1.00 [a]

[1] Standard deviation.

[2] The same superscript letters within a column represent no statistically significant difference (SNK test at 0.05).

[3] Total ammonia-nitrogen.

[4] Nitrite-nitrogen.

[5] Nitrate-nitrogen

[6] Five-day carbonaceous biological oxidation demand

[7] Reactive phosphorus.

[8] Below detection limit.

Table 8 presents the mean values of the total ammonia-nitrogen and nitrite-nitrogen over the twelve-week period. No significant differences were found in these indicators among treatments. As in the first study, the observed water quality indicators were acceptable for good growth and there were no indications the various diets produced different impacts on the measured water quality indicators. Table 9 summarizes the shrimp average final weights, survival rates, FCR and yields at the end of the second study. No significant differences were found among treatment means for survival, FCR or yield due to dietary treatments. However, the average weight of the shrimp maintained on the commercial diet was significantly higher than that of shrimp maintained on Diets 5 and 6. Differences between the commercial control diet and Diet 4 were not statistically significant. The shrimp maintained on the basal diet without a HUFA source (Diet 5) were smaller than those maintained on the same diet with HUFA originating from the algae meal (Diet 4) although the differences were not statistically significant. This trend needs to be verified, since the reduced growth is presumed to be due to EFA. Performance of shrimp maintained on Diet 6 (all organic diet) was the poorest but had reasonable growth and good survival; confirming the potential of an organic diet utilizing plant proteins and oils in combination

with HUFA supplements. All three test-diets appear to support good survival but the basal diet with the algae meals performed the best and was not significantly different from the commercial control.

Table 8. Summary (mean ± STD[1]) of the weekly water quality parameters for *Litopenaeus vannamei* juveniles reared over a 12 week period and offered one of four test diets (Experiment 2)[2].

Treatments	TAN (mg/L) [3]	NO_2-N (mg/L) [4]
Diet 4	0.106 ✂ 0.195	1.194 ✂ 1.570
Diet 5	0.157 ✂ 0.199	1.190 ✂ 0.540
Diet 6	0.172 ✂ 0.274	1.389 ✂ 1.938
Control	0.132 ✂ 0.214	0.487 ✂ 0.698

1 Standard deviation

2 Based on ANOVA, no significant differences among treatment means were detected.

3 Ammonia-nitrogen

4 Nitrite-nitrogen

Table 9. Summary (mean ± STD[1]) of shrimp final average weights, survival, FCR and yield at the conclusion of a 12 week growth trial in which *Litopenaeus vannamei* juveniles were offered one of four test diets (Experiment 2)[2].

Treatment	Average Final Weight (g)	Survival (%)	FCR	Yield/tank (g)
Diet 4 AM 0.5-0.13	17.36 ± 0.37 [a b]	95.8 ± 4.40	1.20 ± 0.07	316.1 ± 17.8
Diet 5 w/o MFO	16.43 ± 0.61 [b]	96.8 ± 7.06	1.23 ± 0.11	308.5 ± 24.7
Diet 6 Organic	15.23 ± 0.71 [c]	94.7 ± 7.44	1.38 ± 0.18	277.9 ± 34.1
Control	17.94 ± 1.00 [a]	90.5 ± 6.86	1.21 ± 0.13	308.6 ± 34.7
MSE [3]	0.318	3.930	0.0574	12.29
P value	0.0001	0.4663	0.1384	0.1698

1 Standard Deviation

2 The same superscript letters within a column represent no statistically significant difference (SNK test at 0.05)

Some authors have found that shrimp fed diets that contain vegetable oils high in linolenic acid promote better growth and survival (Guary, Kayama & Ceccaldi 1976). This response was probably due to n-3/n-6 PUFA ratio. It has been suggested that a n-3/n-6 dietary ratio that resembles that of the crustaceans body, especially of the reproductive tissues, will promote better growth and reproductive performance of crustaceans (Millamena and Quinitio, 2000; Floreto, Bayer& Brown 2000). The vegetable oils present in the main plant protein sources are higher in linoleic acid than linolenic acid (except for the linseed meal)

and do not contain n-3 HUFA, in contrast the oil in fish meal has a higher level of n-3 HUFA and very low levels of n-3 and n-6 PUFA. Since dietary lipids influence the lipid composition of the crustacean body (D'Abramo and Sheen, 1993, Lim et al 1997), replacement of fish meal (and other marine protein sources) for plant protein sources may result in an increase in the levels of PUFA, especially of linoleic acid (n-6) and reduction in the n-3/n-6 ratio and in low levels of HUFA. Floreto et al. (2000) found that the n-3 HUFA and the n-3/n-6 ratio of juvenile lobster (*Homarus americanus*) were lower at higher levels of replacement of soybean meal for fish meal, which was associated with longer molting cycles.

To evaluate the commercial potential of a diet using terrestrial plant and animal proteins and no fish oils, a commercial version of Diet 2 was formulated and produced at Zeigler Brothers Inc., Gardners, PA, USA feed mill using typical pelleting equipment. This diet was then tested at the Waddell Mariculture Center under typical pond production conditions. Results are summarized in Table 10. Under short-term (62 days) as well as long-term (110 & 111 days) growth trials final production was very similar. There was a notable difference in final weights during the extended growth period (15.8 vs 19.2) indicating that further refinement of the test diet may be warranted. However, the final production of 4,140 vs. 4,218 kg/ha were quite similar and within the typical range observed in replicated ponds reared under the same conditions. Based on these results the use of alternative protein and oil sources in commercial rations seems to have considerable promise.

Table 10. Response of *Litopenaeus vannamei* to a practical test diet without fish meal or fish oil as compared to a commercial diet under pond production conditions. Ponds were stocked at 25 postlarvae per meter and cultured under typical commercial production conditions for 111 or 62 days.

	Test diet	Commercial	Test diet	Commercial
Pond size	0.50 ha	0.50 ha	0.25 ha	0.25 ha
Days	62	62	111	111

Temperature (C)	29.0 ± 1.0	29.0 ± 1.0	27.9 ± 2.1	28.5 ± 1.9
Salinity (mg/L)	24.9 ± 1.4	23.6 ± 0.8	19.5 ± 1.6	20.0 ± 0.9
D.O. (g/L)	4.4 ± 0.5	4.3 ± 0.5	4.3 ± 0.8	3.9 ± 0.6
pH	7.7 ± 0.2	7.9 ± 0.2	7.6 ± 0.5	7.6 ± 0.4
FCR	1.06	1.05	1.22	1.12
Survival (%)	105.0	106.0	92.7	85.9
Final mean weight (g)	8.9	8.7	15.8	19.2
Production (kg/ha)	2,277	2,304	4,140	4,218

Conclusions

Under test conditions, the test diets were suitable to support growth and survival of the Pacific white shrimp *Litopenaeus vannamei* in a tank-system and ponds with limited water discharge and in the presence of natural productivity. There were minimal differences in shrimp growth, yield and survival among the test diets and the control commercial diet. Of the diets tested, the organic feed performed the poorest and thus requires further refinement. Based on the results of the present study, both marine protein and oil sources can be replaced in practical shrimp feeds if a suitable replacement strategy is adapted.

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