

The use of HUFA-rich algal meals in diets for *Litopenaeus vannamei*

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Abstract

A 15-week growth trial was conducted with juvenile, Pacific white shrimp *Litopenaeus vannamei* to study the efficacy of using algal meals as a source of highly unsaturated fatty acids in practical diets that are designed to contain no marine protein or oil sources. Based on previous study, a practical diet was designed containing co-extruded soybean poultry by-product meal with egg supplement and soybean meal as the primary protein sources for formulations containing 350 g kg⁻¹ crude protein and 100 g kg⁻¹ lipid. To further refine the diets, the fish oil in two of the diets was completely substituted with plant oils and oil originating from microbial fermentation products rich in docosahexaenoic acid (DHA) and arachidonic acid (ArA). A commercial shrimp feed was also included in the trial for comparison. The mean values for shrimp final weight (17.8 g), yield (537.7 g m⁻² or 703.2 g m⁻³), survival (98.5%) and feed conversion ratio (1.4 : 1) showed no statistically significant differences between diets. The results suggest that co-extruded soybean poultry by-product meal and oil from heterotrophic microalgal fermentation sources can be potential candidates for fish meal and marine oil replacement in shrimp diets.

KEY WORDS: algal meal and algal oil, arachidonic acid, co-extruded soybean poultry by-product meal, docosahexaenoic acid, fish meal, heterotrophic algae, highly unsaturated fatty acids

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Introduction

The world production of farmed shrimp has expanded rapidly over the past two decades and these trends are expected to continue as global population increases and demand for quality sea food continues to rise (Tacon *et al.* 2000; FAO 2003). In 2001, marine shrimp were the second most important world aquaculture species with an estimated value of US\$ 8.4 billion (FAO 2003). Manufactured shrimp diets typically contain approximately 250 g kg⁻¹ fish meal (Tacon & Barg 1998) and typically contain 20–40 g kg⁻¹ of added fish oil. In the year 2000, approximately 372 000 mt of fish meal was used in the production of shrimp feeds accounting for 176 g kg⁻¹ of fish meal used for global aquaculture feeds (Barlow 2000). Due to increasing demand and limited supplies of marine ingredients, if shrimp culture is to remain a viable industry, feed manufacturers must reduce dependence on marine protein and oil sources.

To replace marine protein sources and oils in commercial feeds, one must have a complete strategy that allows for the replacement of required nutrients. With suitable considerations of nutrient profiles and palatability, a variety of animal and plant proteins have been used as substitutes for marine protein sources with good success (Lim & Dominy 1990; Piedad-Pascual *et al.* 1990; Sudaryono *et al.* 1995; Davis & Arnold 2000; Olvera-Novoa & Olivera-Castillo 2000; Forster *et al.* 2003; Samocha *et al.* 2004). However, the use of alternative protein sources is often accomplished in combination with the use of marine oils to supply essential fatty acids and enhance the palatability of the diet.

It is clear that fish meal and most of the marine meals can be replaced either singularly with animal by-product meal or

in combination with plant protein sources without affecting the physical and nutritional quality of the feeds (Viola *et al.* 1982, 1988; Tidwell *et al.* 1993; Sudaryono *et al.* 1995; Webster *et al.* 1995; Wu *et al.* 1995; Davis & Arnold 2000; Samocha *et al.* 2004). These meals are primarily viewed as good sources of essential amino acids; however, fish meals (as well as other marine protein sources) are a good source of marine oils that are rich in highly unsaturated fatty acids (HUFA). Lipid content and the associated C18 polyunsaturated fatty acids (PUFA), linoleic (18:2n-6) and linolenic (18:3n-3) as well as n-3 and n-6 HUFA (EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; ArA, arachidonic acid), are required in shrimp and other crustacean feeds (Kanazawa *et al.* 1977; Martin 1980; Fenucci *et al.* 1981; Read 1981; Shiau 1998). It has been demonstrated that these essential fatty acids are required in the diets of crustaceans at levels between 5 and 10 g kg⁻¹ (Lim & Akiyama 1995). Therefore, their content in the diet should be considered when replacing fish meal and oil with other substitutes.

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 and can contain undesirable contaminants. Consequently, alternative oil sources for shrimp diet formulations should also be pursued. A number of studies have reported the successful inclusion of photosynthetic microalgae in aquaculture feeds (Day *et al.* 1990; Zhou *et al.* 1991; Day & Tsavalos 1996; Langden & Onal 1999). This approach has not found commercial application to replace fish-based ingredients mostly because of their high production cost and culture inefficiency (Chaumont 1993; Wilkinson 1998). Heterotrophically grown microalgae and its extracted oil was successfully used as a source of nutrients and essential fatty acids for larval live food enrichment and in formulated broodstock diets of marine teleosts (Harel *et al.* 2002). Heterotrophic algal groups, such as chrysophytes, cryptophytes and dinoflagellates have been known to produce lipids with high levels of EPA, DHA and ArA (Cohen *et al.* 1995; Behrens & Kyle 1996; Apt & Behrens 1999). The algal species *Schizochytrium* can produce as high as 500 g DHA kg⁻¹ lipid (Behrens & Kyle 1996) and has high potential as fish oil substitute in aquaculture feed.

The primary objective of this study was to evaluate the effectiveness of fish meal and fish oil replacement strategies using terrestrial protein sources and oil from a product rich in DHA and ArA originating from microbial fermentation in the practical diets of *Litopenaeus vannamei*.

Materials and methods

Three test diets (Table 1) were prepared with co-extruded soybean poultry by-product meal (CoESPB; ProfoundTM, American Dehydrated Foods, Verona, MO, USA) as a substitute for fish meal in practical diets for the Pacific white shrimp *L. vannamei*. A practical basal diet, previously developed as a fish meal-free diet (Samocha *et al.* 2004), was formulated to contain 350 g kg⁻¹ of protein and 100 g kg⁻¹ of lipid. To further refine the diets, menhaden fish oil in two of the diets (Diet 1 and Diet 2) was completely replaced with spray-dried heterotrophic marine algal meals high in DHA and ArA (*Schizochytrium* meal and AquaGrow[®]ARA; Advanced BioNutrition Corp., Columbia, MD, USA). The *Schizochytrium* meal (high DHA, 460 g lipid kg⁻¹ meal, 438 g DHA kg⁻¹ lipid) was used as dried intact cells of heterotrophic algae *Schizochytrium* sp. and *Mortierella alpina*, products of commercial microbial fermentation. The AquaGrow[®]ARA (350 g lipid kg⁻¹ meal, 376 g ArA kg⁻¹ lipid) was used after the extraction of the oil and then spray drying of the heterotrophic algae. A commercial diet (Diet 4, with

Table 1 Diet formulations expressed as g kg⁻¹ (as is) for practical diets designed to contain 350 g kg⁻¹ protein and 100 g kg⁻¹ lipid using various strategies for the replacement of marine fish meal and oil

	Diet 1	Diet 2	Diet 3
Profound TM 1	390	390	390
Soybean meal ²	295	302	305
Schizochytrium meal ³	20	5	Nil
AquaGrow [®] ARA ³	5	1.3	Nil
Menhaden fish oil ⁴	Nil	Nil	30.4
Soy oil ⁵	14.7	15.3	Nil
Flax oil (linseed oil) ⁶	4.8	12.3	Nil
Wheat starch ⁵	19.8	23.4	23.9
Whole wheat ⁵	200	200	200
Trace mineral premix	5	5	5
Vitamin premix	18	18	18
Choline chloride ⁵	2	2	2
Stay C 250 mg kg ⁻¹ 7	0.7	0.7	0.7
CaP-dibasic ⁵	20	20	20
Lecithin (soy refined) ⁵	5	5	5

¹ 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., Richmond, VA, USA).

³ DHA GOLD[®]; (*Schizochytrium* sp. algae meal) and AquaGrow[®]-ARA (*Mortierella* sp.) Advanced BioNutrition Corp., Columbia, MD, USA).

⁴ Omega Protein, Inc., Reedville, VA, USA.

⁵ United States Biochemical Company, Cleveland, OH, USA.

⁶ Sigma, St Louis, MO, USA.

⁷ Stay C[®] (L-ascorbyl-2-polyphosphate 35% Active C; Roche Vitamins Inc., Parsippany, NJ, USA).

350 g kg⁻¹ crude protein, 80 g kg⁻¹ crude fat; Rangen Inc., Buhl, ID, USA) was included in the test as a commercial reference. All other known nutritional requirements of *L. vannamei* were met by the experimental diets. Test diets were produced in the laboratory at the Department of Fisheries and Allied Aquacultures (Auburn University, Auburn, AL, USA). 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 80–100 g kg⁻¹ 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. Lipid content (either extraction) and fatty acid profiles (Association of Official Analytical Chemists 1990, no. 963.22) of the three test diets were determined by New Jersey Feed Laboratory, Inc. (Trenton, NJ, USA). Lipids were reported to be 112, 105 and 107 g kg⁻¹. The fatty acid profiles of the three test diets are presented in Table 2.

The 15-week feeding trial was conducted at the Texas Agricultural Experiment Station (TAES), Shrimp Mariculture Research Facility (SMRF), Corpus Christi, TX. Specific pathogen-free nauplii of *L. vannamei* nauplii were obtained from Harlingen Shrimp Farms (Los Fresnos, TX). Postlarvae (PL) were raised at the TAES, SMRF. Five-day-old postlarvae (PL₅) were reared in a nursery facility before attaining the juvenile stage. The juveniles (0.66 ± 0.06 g) used in this study were hand sorted for uniform size upon initiation of the growth trial. The 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 a bottom area of 0.85 m². Each tank was covered with a net to avoid shrimp escape. The tanks were equipped with two air stones each. A constant airflow of 8–10 L m⁻¹ was maintained in all tanks during the entire study period. Natural seawater was used after initial chlorination with salinity adjusted to 30 g L⁻¹. The tanks were stocked at a density of 30 shrimp m⁻² (equivalent to 46 m⁻³). One tank in each treatment was provided with a feed tray that covered about 45% of the tank's bottom area (about 0.40 m²) to estimate feed consumption. Five shrimp from each of these tanks were collected weekly to estimate growth (group weights) and to adjust daily feeding rations. Weekly rations were calculated assuming 100% survival, Feed Conversion Ratio (FCR) of 1:1.5 and an estimated growth between 1 and 1.2 g week⁻¹. Daily rations were then calcu-

Table 2 Fatty acid composition of the test diet reported as percentage of the identified fatty acids

	Diet 1	Diet 2	Diet 3
14:0	1.36	0.56	2.54
14:1	0.08		0.21
15:0	0.10	0.07	0.31
15:1			0.09
16:0	21.14	19.33	22.52
16:1n-7	2.50	2.44	5.38
16:2n-4			0.50
16:3n-4	0.09	0.08	0.53
16:4			0.20
17:0	0.18	0.15	0.43
18:0	6.16	6.39	6.26
18:1n-9	29.10	30.98	26.02
18:1n-7	1.99	2.05	2.73
18:2n-6	23.54	25.25	15.38
18:2n-4			0.12
18:3n-6	0.13	0.07	0.14
18:3n-3	3.94	7.88	1.70
18:4n-3			0.86
20:0	0.21	0.20	0.18
20:1n-9	0.26	0.28	0.63
20:2n-6	0.09	0.09	0.16
20:3n-6	0.18	0.11	0.14
20:3n-3			0.09
20:4n-6	1.61	0.97	0.98
20:4n-3	0.09		0.48
20:5n-3	0.27	0.08	3.57
22:1n-9			0.09
22:0	0.21	0.19	0.14
21:5n-3			0.16
22:5n-6	1.74	0.62	0.39
22:5n-3	0.07		0.87
22:6n-3	3.96	1.14	4.78
24:0	0.12	0.07	
24:1n-9	0.06		0.11
∑n-3	8.34	9.10	12.51
∑n-6	27.28	27.11	17.18

lated based on expected growth and offered four times per day. The entire study was conducted without any water exchange. Occasionally, municipal freshwater was added to the tanks to offset for evaporation losses and to maintain salinity. Physicochemical parameters like pH, temperature, salinity and dissolved oxygen were measured twice daily. Other water quality indicators-like total ammonium-N (NH₃ + NH₄) and nitrite-N (NO₂-N) were measured once a week in each tank. On the day of termination, in addition to total ammonium-nitrogen and nitrite-nitrogen (NO₂-N), culture medium was monitored for nitrate-nitrogen, reactive phosphorus and 5-day carbonaceous biochemical oxygen demand (cBOD₅). Total ammonium-nitrogen and reactive phosphorus were determined using the methods of Artiola (1989); nitrite-nitrogen, nitrate-nitrogen and cBoD5 were analyzed by the methods of Strickland & Parsons (1972);

Hach Water Analysis Handbook (1997) and Standard Methods (1995), respectively. Temperature, pH, dissolved oxygen and salinity were measured using a YSI meter (YSI Inc., Yellow Spring, OH, USA).

At the end of the 15-week growth trial, the shrimp were harvested, tank-wise separately, weighed and counted. Average final weight and survival for each dietary treatment were determined. Feed conversion ratio values were estimated based on feed inputs and final biomass. Differences in final average weights, survival (arcsine-transformed) and FCR were analysed using a one-way ANOVA to determine if significant ($P < 0.05$) differences existed among treatment mean values. The Least Significance Difference (LSD) multiple comparison test was used to determine significant differences between treatment mean values. Repeated measures ANOVA test was used to compare the differences in daily and weekly water quality indicators between treatments. All statistical analyses were conducted using SPSS statistical software (V. 11 for Windows, SPSS Inc., Chicago, IL, USA). Although, the experimental design had five replications, the conclusions are based on four replications to avoid potential bias because of shrimp handling in the control tanks which were used to monitor growth and feed.

Results and discussion

This research was conducted in static water systems designed to mimic pond production conditions. As this is a static system, it is critical to evaluate water quality conditions and their potential effects on production. At the conclusion of the study there were no significant differences in measured water quality parameters between treatments (average values are presented in Table 3). These values represent acceptable ranges reported for optimal growth and survival of penaeid shrimp. As the study was carried out with no water exchange, it was important to monitor ammonium-nitrogen ($\text{NH}_4 + \text{NH}_3$) and nitrite-nitrogen levels on a weekly basis. Again, no statistically significant differences were found

Table 3 Summary of the daily water quality indicators in the diet study with *Litopenaeus vannamei*

	Dissolved oxygen (mg L ⁻¹)		Temperature (°C)		pH		Salinity (g L ⁻¹)
	AM	PM	AM	PM	AM	PM	
	Average	6.49	6.47	27.2	28.7	7.7	
SD	0.36	0.49	0.9	1.25	0.3	0.2	1
Maximum	7.62	7.81	28.6	30.5	8.4	8.2	36
Minimum	5.59	5.09	24.3	24.7	6.4	7.4	27

Table 4 Summary of the treatment mean values (mean \pm SD) and maximum values of the weekly water quality indicators over a 15-week period with *Litopenaeus vannamei* juveniles offered four test diets under no water exchange

Treatment	Ammonium-N ¹ (mg L ⁻¹)		NO ₂ -N ² (mg L ⁻¹)	
	Average	Maximum	Average	Maximum
Diet 1	0.16 \pm 0.25 ^a	1.17 (3)	0.87 \pm 1.46 ^a	6.20 (10)
Diet 2	0.14 \pm 0.25 ^a	1.16 (10)	0.19 \pm 0.33 ^a	1.50 (8)
Diet 3	0.10 \pm 0.16 ^a	0.60 (2)	0.72 \pm 1.24 ^a	4.88 (8)
Control	0.13 \pm 0.28 ^a	1.76 (6)	0.24 \pm 0.58 ^a	2.51 (9)

The numbers in the bracket indicate the corresponding week when the reading occurred. Minimal levels are not given as they were below detectable limits.

Values with the same superscript letter in a column indicate no statistically significant differences (ANOVA at 0.05 level).

SD = Standard Deviation.

¹ Total ammonium-nitrogen ($\text{NH}_3 + \text{NH}_4$).

² Nitrite-nitrogen.

between treatments (Table 4). In general, the observed water quality indicators were acceptable for good growth and there were no indications of dietary effects on water quality. The levels of ammonium-nitrogen, nitrite-nitrogen, nitrate-nitrogen, cBOD₅ and reactive phosphorus on the day of the study termination were also determined. No significant differences in total-ammonia-nitrogen (below detectable limits), nitrite-nitrogen (0.01–0.03 mg L⁻¹), cBOD₅ (4.32–8.54 mg L⁻¹) and reactive phosphorus (2.73–3.81 mg L⁻¹) concentrations were found between the four treatments. Statistically significant differences were observed for nitrate-nitrogen concentrations between treatments. The observed levels (mean \pm SD) for nitrate-nitrogen (mg L⁻¹) at the conclusion of the trial were 4.45 \pm 0.76^a (Diet 1), 1.77 \pm 0.76^b (Diet 2), 6.64 \pm 1.78^c (Diet 3) and 2.02 \pm 0.53^b (control; significant differences indicated by different superscripts). As nitrate levels did not exceed 7 mg L⁻¹, these differences were unlikely to affect animals' performance. Although, the highest level of average ammonium encountered in the tanks fed the Diet 1 and Diet 2 were lower than the ammonium level in tanks fed the control diet, no detectable ammonium was found in any of the treatments by the end of the study. Nitrite levels in one of the tanks fed the Diet 1 reached a concentration of 6.2 mg L⁻¹; however, this level did not appear to result in negative impact on shrimp growth or survival in the present study.

The growth performance of the *L. vannamei* fed three types of formulated diets is summarized in Table 5. No statistically significant differences were found in the average final weight, total shrimp yield, survival and FCR between treatments. The survival of the shrimp was >95% for all of the treatments

Table 5 Summary of the shrimp final average weight (mean \pm SD, $n = 4$), survival, FCR and yield in a diet study with *Litopenaeus vannamei* at the TAES Shrimp Mariculture Research Facility (Corpus Christi, Texas)

Treatment	Mean final weight ¹ (g)	Survival (%)	FCR	Yield (g m ⁻³)	Yield (g m ⁻²)
Diet 1	17.5 \pm 0.9 ^a	95.2 \pm 4.2 ^a	1.5 \pm 0.1 ^a	666.3 \pm 60.9 ^a	509.6 \pm 46.6 ^a
Diet 2	17.8 \pm 0.5 ^a	99.0 \pm 11.7 ^a	1.4 \pm 0.0 ^a	706.7 \pm 5.9 ^a	540.4 \pm 4.5 ^a
Diet 3	17.0 \pm 1.0 ^a	100.8 \pm 5.0 ^a	1.4 \pm 0.1 ^a	694.5 \pm 37.4 ^a	531.1 \pm 28.6 ^a
Control	18.8 \pm 0.9 ^a	99.0 \pm 3.4 ^a	1.36 \pm 0.1 ^a	745.1 \pm 42.1 ^a	569.8 \pm 32.2 ^a
P-value	0.062	0.481	0.455	0.112	0.112

¹ Total number of shrimp – 125 \pm 3.

Values with the same superscript letter in a column indicate no statistically significant differences (ANOVA at $\alpha = 0.05$ level).

confirming that both water quality and nutrient quality of the diets were adequate to support long-term survival. Based on intermittent observations of consumption using a feeding tray, there were no indications of feed rejection or reduced palatability by shrimp fed the various test diets.

Fish meal has been completely substituted by terrestrial protein sources in production diets of various fishes, such as catfish and tilapia (Webster & Lim 2002) and crustaceans, such as *Macrobrachium rosenbergii* (Tidwell *et al.* 1993). However, replacement of marine protein and oil sources in the practical diets for *L. vannamei* has been variable. For example, in previous studies it was demonstrated that solvent-extracted cottonseed meal or soybean meal could replace 40% of a marine protein mix (Lim & Dominy 1990; Lim 1996, respectively). More recently, it has been demonstrated that co-extruded soybean poultry by-product meal with egg supplement could be used successfully as a replacement for fish meal in indoor research systems without primary production (Davis & Arnold 2000) as well as outdoor green-water systems (Samocha *et al.* 2004). Although this replacement strategy removed fish meal, the diets still utilized marine oils to deliver essential fatty acids.

The present study not only used similar diets without fish meal, but also replaced the marine oil with plant oils and oils originating from algae meals rich in DHA and ArA (spray-dried cells of *Schizochytrium* sp. and *Mortierella* sp.), produced from commercial microbial fermentation. As there were no differences in the performance parameters of the shrimp, one can conclude that the lowest level of algae meal tested was adequate to support good growth and survival of the shrimp. The importance of fish oil in the aquaculture diet has been well documented. Fish oil is the major source of essential fatty acids, such as EPA, DHA and ArA. In the past, researchers have tried to substitute fish oil with various types of vegetable oils with limited success. For example, in a study carried out by Morris (2001), 80% of fish oil was substituted with either soy or rapeseed oil in Atlantic salmon. Unfortunately, vegetable oils lack the long chain omega fatty

acids that are contained in the marine oils that are essential for marine fish and shrimp growth.

Researchers in the past have tried to overcome this problem by using live algae rich in HUFA as larval feed supplement (Harel *et al.* 1998; Harel & Place 1999). However, because of high production cost and culture inefficiency, the approach of growing photosynthetic algae for replacing fish meal or fish oil has not found commercial application so far. The alternative approach is to use the heterotrophic algae grown on microbial fermentors. Earlier studies indicated that DHA and ArA enrichment from heterotrophically grown algae of certain species can be used successfully as enrichment for live larval food or the maturation diets of many aquatic species (Barclay & Zeller 1996). The oil from the *Schizochytrium* sp. contains as high as 50% DHA (Barclay & Zeller 1996).

In the present study, growth and survival values were not significantly affected by the replacement of fish meal with co-extruded soybean poultry by-product meal and fish oil with heterotrophic algal sources. The use of heterotrophic algae as an oil source in the feed is a recent concept in the practical diets of shrimp and is still in a preliminary stage of research. Levels of HUFAs are particularly important from the standpoint of the health benefits of the seafood to the consumer. Further studies are needed to determine the fatty acid composition of cultured shrimp in order to study the potential effect of fish oil replacement on the final lipid profile in the shrimp tissue. These findings will assist feed companies in selecting alternatives sources to expensive fish oil in formulating feed for shrimp.

In conclusion, a complete replacement of fish meal and fish oil using plant protein and non-marine oil sources did not show apparent reduction in growth of Pacific white shrimp *L. vannamei* under the present experimental conditions. Based on the findings, co-extruded soybean poultry by-product meal can serve as the primary source of crude protein and essential amino acids in diets of the Pacific white shrimp. In addition, the algal oil can be used as a

suitable fish oil substitute (HUFA source) in the practical diets for *L. vannamei*.

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