



Use of commercial fermentation products as a highly unsaturated fatty acid source in practical diets for the Pacific white shrimp *Litopenaeus vannamei*

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Abstract

Removal or reduction of marine ingredients (MI) from feed formulations is critical to the sustainability of the aquaculture industry. By removing MI, diets may become limiting in several nutrients including highly unsaturated fatty acids (HUFA) such as docosahexaenoic acid (DHA) and arachidonic acid (ArA). To reduce reliance on MI in shrimp diets, two trials were conducted with *Litopenaeus vannamei* juveniles to determine the feasibility of using fermentation meals rich in DHA and ArA as the primary source for HUFA. A practical diet with no MI was formulated with/without DHA and ArA supplements and fed in the first trial. A diet with menhaden fish oil or a combination of plant oil with/without DHA and ArA supplements was used in the second trial. To determine whether HUFA is only needed in the early growth stages, we also fed one group a HUFA-supplemented diet to 5 g and then switched them to a HUFA-supplement-free diet. In both trials, the weights were reduced when HUFA supplements were not provided either throughout the trial or from 5 g to harvest (< 16 g). These results suggest that supplementation of plant oils with DHA- and ArA-rich oils from fermented products is a viable option to replace marine fish oil for *L. vannamei*.

Keywords: DHA, ArA, practical diets, Pacific white shrimp, *Litopenaeus vannamei*

Introduction

Marine fish meals and fish oils are excellent sources of high-quality essential amino acids, lipids, vitamins, minerals and attractants in aquaculture diets (Tacon & Akiyama 1997). However, the unstable prices associated with fluctuations in the supply of these marine ingredients and the sustainability of these practices are of prime concern (Chamberlain 1993; Tacon & Akiyama 1997; Naylor, Goldberg, Primavera, Kautsky, Beveridge, Clay, Folk, Lubchenco, Mooney & Troell 2000). Hence, replacement of these marine ingredients with cost-effective alternative sources of proteins and lipids in aquaculture feeds is a high-priority task for feed mills and aquaculturists (Tacon & Akiyama 1997). Previous studies (Lim 1996; Davis & Arnold 2000; Samocha, Davis, Saoud & DeBault 2004; Menoyo, Lopez-Bote, Obach & Bautista 2005) showed that either animal or plant sources can be used as suitable substitutes for fish meal and fish oil in a small-scale tank system. In their efforts to replace fish meal, researchers used plant protein sources such as soybean meal (Sudaryono, Hoxey, Kailis & Evans 1995; Hertrampf & Piedad-Pascual 2000; Olvera-Novoa & Olivera-Castillo 2000), solvent-extracted cotton seed meal (Lim 1996), lupin meals (Sudaryono *et al.* 1995), legumes, leaf meals (Eusebio & Coloso 1998; Li, Robinson & Hardy 2000) and papaya or camote leaf meal (Penaflores 1995) in feed formulations for aquatic animals, with varying

degrees of success. Several studies have demonstrated that with suitable adjustments, animal by-product meals could be used successfully as fish meal replacements to meet the nutrient and attractability requirements for the target species (Davis & Arnold 2000; Forster, Dominy, Obaldo & Tacon 2003; Samocha *et al.* 2004). Other researchers showed that a combination of animal by-product meal and/or plant protein sources provided promising results as fish meal substitutes without affecting the physical and nutritional quality of the feeds (Wu, Rosati, Sessa & Brown 1995; Viola, Mokady, Rappaport & Arieli 1982; Viola, Arieli & Zohar 1988; Tidwell, Webster, Yancey & D'Abramo 1993; Sudaryono *et al.* 1995; Webster, Yancey & Tidwell 1995; Davis & Arnold 2000; Samocha *et al.* 2004).

Considerable research has also been carried out on fish oil replacement strategies in aquaculture diets. Plant protein and vegetable oil use in aquafeeds without marine fish meal or fish oil is often limited by the potential problems associated with insufficient levels of essential amino and fatty acids, anti-nutritional factors and poor palatability (Francis, Makkar & Becker 2001). Heterotrophically grown algae and products obtained by fermentation processes have been reported to be a good source of nutrients and essential fatty acids for larval live food enrichment and for formulated broodstock diets of marine teleosts (Harel, Koven, Lein, Bar, Behrens, Stubblefield, Zohar & Place 2002). In a recent study, Patnaik, Samocha, Davis, Bullis and Browdy (2006) showed that fish meal and fish oil can be successfully replaced in the diets for *Litopenaeus vannamei* using co-extruded soybean and a poultry by-product meal (Profound™) and spray-dried cells of *Schizochytrium* sp. and *Mortierella* sp. obtained by a proprietary commercial fermentation process. Although we have demonstrated the ability to make these substitutions, the need to include a highly unsaturated fatty acids (HUFA) supplement has not been established especially under conditions when some natural productivity is present. Consequently, the objective of the current study was to evaluate potential replacement of marine oil sources in diets for *L. vannamei* using a mixture of plant oils enriched with HUFA produced through the fermentation process.

Materials and methods

Two growth trials were conducted with juvenile Pacific white shrimp, *L. vannamei*, in an outdoor tank sys-

tem operated with no water exchange. The studies evaluated practical diet formulations in which both marine fish meal and fish oil have been completely replaced with alternative sources of nutrients (Table 1). The basal diets (Diet 1 and Diet 4, for Trial 1 and Trial 2, respectively) were based on diet formulations which showed good growth and survival of this species in previous studies (Davis & Arnold 2000; Samocha *et al.* 2004). Test-diets were formulated to meet all known nutritional requirements for this species and had 35% crude protein (CP) and 8% lipid levels. The HUFA were provided either from fish oil or from a meal made from spray-dried cells of *Schizochytrium* sp. and *Mortierella* sp. (DHA GOLD® and AquaGrow®-ARA, Advanced Bio-Nutrition, Columbia, MD, USA), collectively referred to as a HUFA-rich source, which are the products of proprietary fermentation processes. A commercial diet (35% CP, 8% lipid; Rangen, Buhl, ID, USA) was included in each trial as a reference diet.

The first trial was conducted over a 12-week period using hand-sorted juvenile (6.07 ± 0.3 g) shrimp. Diet 1 ('HUFA-rich' diet) was formulated with the HUFA from the spray-dried cells. Diet 2 ('HUFA-deficient' diet) was formulated with no HUFA supplementation to determine the effect of this deficiency on shrimp performance. A product of co-extruded soybean and poultry by-product meal served as the protein source in Diet 1 and Diet 2 (Profound™, American Dehydrated Foods, Verona, MO, USA.).

The second trial was conducted over a 14-week period using hand-sorted juveniles (0.95 ± 0.04 g). For this series of diets, the poultry by-product meal, rather than the Profound™, served as the primary protein source. Diet 4 ('HUFA-rich' diet) was formulated with the same source of HUFA as that of Diet 1. Diet 3 was prepared with the same ingredients as those used for Diet 4 but the HUFA was provided from menhaden fish oil ('menhaden-rich' diet). Diet 5 ('HUFA-deficient' diet) was prepared with the same ingredients as those used for the formulation of Diet 3 and Diet 4 but without HUFA supplementation. To determine whether dietary HUFA supplementation is only required during the juvenile phase, a sixth treatment was included and will be referred to as 'Diet 6', in which the shrimp were fed a 'HUFA-rich' diet (Diet 4) up to a size of 5 g and the 'HUFA-deficient' diet (Diet 5) from this size until the end of the study. It is important to note that the performances of the diets were compared within the same trial and not between the trials.

The test diets were prepared in the feed laboratory of Auburn University, Auburn, AL, USA, using stan-

Table 1 Diet formulation (% as is basis) for practical diets designed to contain 35% protein and 8% lipid using various fish meal and fish oil replacement strategies

	Trial 1		Trial 2		
	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
	HUFA	w/o HUFA	MFO	HUFA	w/o HUFA
Profound™*	39.00	39.00			
Poultry by-product meal†			16.00	16.00	16.00
Soybean meal‡	30.20	30.20	40.50	40.50	40.50
Corn gluten, organic§			5.00	5.00	5.00
<i>Schizochytrium</i> meal (DHA)¶	0.50			0.50	
AquaGrow®-ARA¶	0.13			0.13	
Soy oil	1.53	1.30		3.15	3.20
Flax oil (linseed oil)**	1.23	1.80		1.60	1.79
Menhaden fish oil††			5.02		
Wheat starch	2.34	1.63	1.61	1.25	1.64
Whole wheat	20.00	21.00	26.00	26.00	26.00
Trace Mineral premix‡‡	0.50	0.50	0.50	0.50	0.50
Vitamin premix§§	2.00	2.00	2.00	2.00	2.00
Stay C 250 mg/kg¶¶	0.07	0.07	0.07	0.07	0.07
CaP-diebasic	2.00	2.00	2.60	2.60	2.60
Lecethin (soy refined)			0.50	0.50	0.50
Lecethin, organic crude	0.50	0.50			
Cholesterol			0.20	0.20	0.20

*Co-extruded soybean and poultry by-product meal (American Dehydrated Foods, Verona, MO, USA).

†Griffin Industries (Cold Springs, KY, USA).

‡Dehulled solvent-extracted soybean meal, Southern States, Cooperative, Richmond, VA, USA.

§Grain Processing, (Muscatine, IA, USA).

¶DHA GOLD® (*Schizochytrium* sp. algae meal) and AquaGrow®-ARA (*Mortierella* sp.) (Advanced BioNutrition, Columbia, MD, USA).

|| United States Biochemical (Cleveland, OH, USA).

**Sigma (St. Louis, MO, USA).

††Omega Protein (Reedville, VA, USA).

‡‡As g 100 g⁻¹ premix: cobalt chloride 0.004, cupric sulphate pentahydrate 0.250, ferrous sulphate 4.0, magnesium sulphate heptahydrate 28.398, manganous sulphate monohydrate 0.650, potassium iodide 0.067, sodium selenite 0.010, zinc sulphate heptahydrate 13.193, filler 53.428.

§§g kg⁻¹ premix: thiamine HCl 0.5, riboflavin 3.0, pyridoxine HCl 1.0, DL Ca-Pantothenate 5.0, nicotinic acid 5.0.

¶¶Stay C® (L-ascorbyl-2-polyphosphate 35% Active C) (Roche Vitamins, Parsippany, NJ, USA).

||| Organic lecithin, Clarkson Grain (Cerro Gordo, IL, USA).

HUFA, highly unsaturated fatty acid; DHA, docosahexaenoic acid; ArA, arachidonic acid.

standard practices. Dry ingredients and oil were mixed in a food mixer (Hobart, Troy, OH, USA) for 15 min. 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, diets were dried to a moisture content of 8–10% and stored at 4 °C. Dietary treatments were randomly assigned and the study was run as a double-blind experiment.

Both trials were conducted in an outdoor tank system at the AgriLife Research Mariculture Laboratory the Texas AgriLife Research and Extension Center, Corpus Christi, TX, USA. Each treatment was randomly assigned to five-replicate high-density polyethylene 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. Tanks were covered with a net to prevent shrimp from escaping. Aeration was provided by two air stones per tank (7–10 L min⁻¹ stone⁻¹) that were fed by a common regenerative air blower. Natural seawater was used after initial chlorination, de-chlorination by aeration and salinity was adjusted to 30 ppt. Tanks were stocked with 26 and 31 shrimp to provide an initial stocking density of 31 shrimp m⁻² (40 shrimp m⁻³) and 36 shrimp m⁻² (48 shrimp m⁻³) for Trial 1 and Trial 2 respectively. Upon reaching the 5 g size in Trial 2, five shrimp from each tank were removed for sub-sampling. The study was resumed, after the removal of these shrimp, assuming

that each tank had 26 shrimp. However, the actual number of shrimp in each tank after the culling was not determined to minimize shrimp stress. One tank in each treatment was provided with a feed tray, which covered about 45% of the tank's bottom area, to estimate feed consumption, and was considered to be the treatment indicator tank. Five shrimp from each of the indicator tanks were collected weekly to estimate growth (group weights) and to adjust rations. Weekly rations were calculated assuming 100% survival, feed conversion ratio (FCR) of 1:1.5 and predicted weekly growth that varied between 1.0 and 1.2 g. Daily rations were divided into four equal portions, which were fed at 08:30, 11:30, 14:30 and 16:30, hours 7 days a week. Both studies were conducted with no water exchange. To offset evaporative losses and to prevent an increase in salinity, chlorinated municipal freshwater was added to each tank when needed. Physicochemical parameters including pH, temperature, salinity and dissolved oxygen were measured twice daily in all the tanks. Total ammonium-nitrogen ($\text{NH}_3\text{-NH}_4$) and nitrite-nitrogen ($\text{NO}_2\text{-N}$) were measured in every tank once a week. On the day of termination, water samples from each tank were analysed for total ammonium nitrogen (TAN), nitrite nitrogen ($\text{NO}_2\text{-N}$), reactive phosphorus (RP) and 5-day biochemical oxygen demand (cBOD_5).

At the conclusion of each trial, shrimp were group-weighted and counted to provide the mean final weight and survival for each tank. Feed conversion ratio values were calculated based on feed inputs and the biomass gain for each tank. Differences in the weekly and daily water quality indicators were analysed using repeated measures ANOVA. Differences among treatments in TAN, $\text{NO}_2\text{-N}$, RP and cBOD_5 on the day of termination were analysed using one-way ANOVA. The same statistical test was used to determine differences between treatment means ($P < 0.05$) in the final mean weight, survival and FCR values. Statistical analyses were performed only on

the test diets; the data for the reference diet were provided for informational purposes. The Student–Newman–Keuls (SNK) test was used as a tool to identify the difference between treatment means. Square root transformation of per cent survival data was also evaluated but did not affect the interpretation of the results; hence, it is not presented. Statistical analyses were conducted using SPSS (V. 13 for Windows, SPSS, Chicago, IL, USA).

Results and discussion

No statistically significant differences were found between treatments for both trials in the daily or the weekly water quality indicators (Tables 2 and 3). These daily values represent acceptable ranges reported for good growth and survival of penaeid shrimp and are typical for this system. It is interesting to note that although there were no significant differences in the weekly water quality indicators between treatments, on Day 56 of the study, one of the tanks experienced a short exposure (for about a week) to a TAN level of 1.94 mg L^{-1} and an $\text{NO}_2\text{-N}$ level as high as 7.3 mg L^{-1} . The fact that there was no significant difference in the mean shrimp final weight within treatment tanks, along with the high survival in this tank (89%), suggests that these levels had no adverse effect on the shrimp in this study.

Shrimp survival rates in both trials were high and typical for this research system. Statistical analyses of the data from Trial 1 indicated significant differences in shrimp final mean weights, with no significant differences in shrimp survival and FCR values (Table 4). The lowest survival value (93.5%) was found for shrimp maintained on the commercial reference diet, which was lower than the 97.5–100% observed for the test diets. A significant reduction was observed in the final weights between shrimp reared on the 'HUFA-rich' diet (Diet 1) and the

Table 2 Summary of the daily water quality indicators (Mean \pm SD¹ and range) from the growth trials conducted in outdoor tanks with *Litopenaeus vannamei*

	Dissolved oxygen (mg L^{-1})		Temperature ($^{\circ}\text{C}$)		pH		Salinity (g L^{-1})
	a.m.	p.m.	a.m.	p.m.	a.m.	p.m.	
Trial 1	6.5 \pm 0.4 (5.6–7.6)	6.5 \pm 0.5 (5.1–7.8)	27.2 \pm 0.9 (24.3–28.6)	28.7 \pm 1.2 (24.7–30.52)	7.7 \pm 0.3 (6.4–8.4)	7.9 \pm 0.2 (7.4–8.2)	32 \pm 1 (27–36)
Trial 2	7.0 \pm 0.5 (6.0–9.3)	6.8 \pm 0.4 (5.8–7.5)	27.4 \pm 0.8 (25.7–29.1)	28.9 \pm 0.9 (26.6–31.3)	7.8 \pm 0.3 (7.3–8.3)	8.1 \pm 0.2 (7.5–8.6)	22 \pm 1 (18–26)

'HUFA-deficient' diet, indicating a possible deficiency of HUFAs.

The second growth trial was initiated with smaller shrimp (0.6 vs. 6 g shrimp), allowing for more tissue replacement and the evaluation of phased feeding of the HUFA diets. As in the first trial, there were no significant differences in survival between shrimp fed

the different diets in Trial 2. Significant differences were observed between treatments in terms of the final shrimp weights and FCR. There were no differences in the final weights between the 'HUFA-rich' diet (Diet 4), the 'menhaden-rich' diet (Diet 3), as well as the commercial reference diet. The final weights of shrimp fed the fish oil diet were significantly higher than those fed the two diets without the HUFA supplements (Diet 5 and Diet 6). Shrimp reared on the 'HUFA-rich' (Diet 4) were not significantly different but numerically larger than those reared on these HUFA-deficient diets (Diet 5 and Diet 6). Shrimp maintained on the 'menhaden-rich' diet (Diet 3) showed a better FCR than those maintained on the HUFA-deficient diet (Diet 5).

In both growth trials, the final weight, survival and FCR values of shrimp receiving diets with HUFA supplements were similar to those observed for the commercial diets. Furthermore, there were no indications of feed rejection, with all diets readily consumed. These results demonstrate that marine ingredients can be removed from practical diets for shrimp reared in outdoor tanks with no water exchange in the presence of natural productivity. The reduced growth of shrimp reared on diets without HUFA supplements would indicate that the supplementation of HUFAs is a critical component of the replacement strategy. While complete replacement of fish meal has been successful in the production diets

Table 3 Summary (mean ± SD*) of the mean weekly water quality parameters (mg L⁻¹) recorded during the growth trials conducted in outdoor tanks with *Litopenaeus vannamei*

	TAN (mg L ⁻¹)	NO ₂ -N† (mg L ⁻¹)
Trial 1		
Diet 1	0.11 ± 0.19	1.19 ± 1.57
Diet 2	0.16 ± 0.20	1.19 ± 0.54
Reference	0.13 ± 0.21	0.49 ± 0.7
P value	0.18	0.07
Trial 2		
Diet 3	0.13 ± 0.43	0.67 ± 1.08
Diet 4	0.10 ± 0.34	0.34 ± 0.75
Diet 5	0.12 ± 0.37	0.87 ± 1.41
Diet 6‡	0.11 ± 0.38	0.50 ± 0.79
Reference	0.13 ± 0.38	0.47 ± 0.87
P value	0.93	0.42

*Standard deviation.

†Nitrite-nitrogen.

‡Shrimp were fed Diet 4 up to the 5 g size. Beyond this size shrimp were switched to Diet 5.

TAN, total ammonium nitrogen.

Table 4 Final weights, survival rates, feed conversion ratio (FCR) for *Litopenaeus vannamei* juveniles reared in outdoor tanks and offered the test diets

	Mean final weight (g)	Survival (%)	FCR
Trial 1*			
Diet 1 (HUFA)	17.4 ^a	97.5	1.20
Diet 2 (w/o HUFA)	16.4 ^b	100	1.23
PSE†	0.385	2.28	0.041
P value	0.0206	0.5161	0.5844
Reference diet	18.0	93.5	1.16
Trial 2‡			
Diet 3 (MFO)	16.4 ^a	98.1	1.28 ^b
Diet 4 (HUFA)	15.7 ^{ab}	97.7	1.34 ^{ab}
Diet 5 (w/o HUFA)	14.6 ^b	92.3	1.53 ^a
'Diet 6' (Diet 4+Diet 5)§	14.0 ^b	98.1	1.44 ^{ab}
PSE	0.478	2.119	0.061
P value	0.0143	0.1637	0.0465
Reference diet	16.7	91.0	1.36

Based on Student–Newman–Keuls mean separation, treatment means with the same superscript letters are not significantly different.

*Data represent the mean of five replicates. Shrimp had a mean initial weight of 6.07 ± 0.3 g.

†Pooled standard error.

‡Data represent the mean of five replicates. Two tanks being offered Diet 3 and Reference diet, have been excluded from the data set due to discrepancy from human error. The shrimp had a mean initial weight of 0.95 ± 0.04 g.

§Shrimp were fed Diet 4 to 5 g size and Diet 5 until the harvest.

of various fish such as catfish and tilapia (Webster & Lim 2002) and crustaceans such as *Macrobrachium rosenbergii* (Tidwell *et al.* 1993), the replacement of marine protein and oil ingredients in practical diets for *L. vannamei* is still under development. Earlier studies have reported partial substitution of fish meal using a solvent-extracted soybean meal (Lim & Dominy 1990) and a solvent-extracted cotton seed meal in a diet formulation for *L. vannamei* (Lim 1996). Similarly, studies with *L. vannamei* using Profound™ as a partial substitute for fish meal have shown encouraging results: Davis and Arnold (2000) demonstrated that 80% of the fish meal in a diet could be substituted by this product without an apparent negative effect on shrimp survival or growth. Samocha *et al.* (2004) also reported a complete replacement of the fish meal by Profound™, with no apparent palatability problems or negative impact on shrimp performance. In a more recent study, Patnaik *et al.* (2006) showed that both fish meal and fish oil can be completely replaced by a commercial fermentation product (DHA GOLD® and AquaGrow®-ARA as HUFA source) without impairing shrimp performance. Furthermore, Browdy, Seaborn, Atwood, Davis, Bullis, Samocha, Wirth and Leffler (2006), in an outdoor pond study at the Waddell Mariculture Center, Bluffton, SC, USA, showed no significant differences in the production parameters between shrimp that were fed an all-plant-based diet with a HUFA supplement and those fed a conventional fish meal-based diet.

The importance of fish oil in the aquaculture diets has already been well documented. Fish oil is the major source of essential fatty acids such as eicosapentaenoic acid, The DHA and arachidonic acid (ArA). Researchers have attempted to substitute fish oil with various types of vegetable oils. The use of vegetable oil in feeds without marine oil sources is often limited by the potential problems associated with insufficient levels of essential fatty acids (Gonzalez-Felix, Gatlin III, Lawrence & Perez-Velazquez 2002). Docosahexaenoic acid- and ArA-rich products created by fermentation process have been used successfully in the past to enrich live larval food or in maturation diets of many aquatic species (Barclay & Zeller 1996). The oil from the *Schizochytrium* sp. has as high as 50% DHA (Barclay & Zeller 1996) and can serve as a potential candidate for replacement of conventional sources for marine HUFA. In the present study, we have demonstrated a successful 100% replacement of fish meal by poultry meal in a practical shrimp diet. Furthermore, similar to Patnaik *et al.*'s (2006)

results, we were able to completely replace the marine oil ingredient using a combination of plant oils supplemented with fermentation products rich in HUFA as a source for the essential fatty acids.

As demonstrated in a previous research and confirmed by these trials, both Profound™ and the poultry by-product meal can serve as primary sources for protein and essential amino acids in practical diets of the Pacific white shrimp. The complete replacement of fish meal and fish oil using non-marine ingredients can be accomplished using plant oils supplemented with fermentation products as the HUFA source. The use of a heterotrophically produced non-marine HUFA-rich product as the lipid source in feed is a recent concept in practical diet formulations for shrimp and is still in a preliminary stage of research. Additional research on the effect of these ingredients on different life stages of *L. vannamei* under various environmental conditions would be beneficial to fish oil and fish meal replacement efforts. And, finally, more studies are needed to determine the economic viability of the large-scale use of these components in shrimp feed formulations.

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