ALTERNATIVE APPROACHES FOR REMOVING FISH MEAL AND OILS FROM FARMED SHRIMP DIETS USING PLANT AND POULTRY MEALS AND MARINE ALGAL PRODUCTS

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One of the most contentious issues relative to the development of national organic standards for aquaculture relates to the inclusion of fish oils and fish meals in fish and shrimp diets. Over the past four years, a multidisciplinary and multi-institutional team of scientists have conducted a series of tank and pond production trials to evaluate the potential for culture of Pacific White Shrimp *Litopenaeus vannamei* on alternative diets using plant and poultry byproduct meals as protein sources and commercially available heterotrophically grown algal meals to provide long chain polyunsaturated fatty acids (PUFAs), docosahexaenoic acid (DHA) and arachadonic acid (ARA). The results of this research provide information on alternative nutritional technologies to replace fish meal and fish oil which currently represent a significant part of total feed for marine shrimp.

The present contribution specifically addresses several important questions regarding the proposed organic standard relative to the production of *L. vannamei*. First an alternate nutritional technology which is particularly suited to the culture of this, the most popular species of farmed shrimp in the world today is described. Second, details on the results of recent research we have carried out demonstrating the feasibility of these technologies is provided. Information on production rates, growth, survivability and feed conversion is provided. The studies have been carried out utilizing diet formulations which would in principle meet the fish meal and fish oil standards for organic production in terms of allowance as a food source. Third, the results of post harvest studies on the quality of the shrimp resulting from these studies is presented comparing human nutritional values for omega three and omega six fatty acids. Finally, conclusions on the significance of the research as a whole relative to the proposed aquaculture standards is provided.

Alternative Nutritional Technology

The marine shrimp *L. vannamei* has become the most popular farmed species almost everywhere in the world (Rosenberry 2006). In 2004, production of this species in Asia and the Pacific region grew to 1.1 million tons added to the 266,000 tons in Latin America and the Caribbean (FAO 2006). Assuming 90% of these shrimp are in fed systems, an average of 2:1 feed conversion ratio and an inclusion rate of 20% for fish meals and 2% for fish oils, a simple calculation would suggest that in 2004 about 500,000 tons of fish meals and 50,000 tons of fish oil went into feeds for this species. Based on estimates by Tacon et al. (2006), marine shrimp feed uses 22.8% of the fish meal used in aquaculture feeds. The dominance of the species in global shrimp aquaculture production may be related to several factors including: 1. Availability of healthy specific pathogen free (SPF) fast growing selectively bred seed stocks, 2. Amenability to intensive culture, and 3. Ability to use lower protein feeds and to take advantage of natural productivity in the culture environment. According to the FAO Cultured Aquatic Species Information Programme website: "*P. vannamei* are very efficient at utilizing the natural productivity of shrimp ponds, even under intensive culture conditions. Additionally, feed costs are generally less for *P. vannamei* than the more carnivorous *P. monodon*, due to their lower requirement for protein (18–35 percent compared to 36–42 percent)" (www.fao.org). The increasing economic importance of *L. vannamei*, the huge amounts of marine meals and oils that are currently being used in feeds for culture of these shrimp and the biological potential for reducing or eliminating inclusion of marine products in diets for the species provided strong justification for our research efforts.

The nutritional technologies tested were based upon use of poultry byproducts or ingredients that were or could be certified as organic plant meals for protein replacements. Animal byproducts can provide a good source of protein which is often available in good supply at a reasonable cost. Although some problems with variable composition, relative nutritional quality and palatability need to be considered in developing marine protein replacement in shrimp diet formulation strategies, good success can be achieved (Davis and Arnold 2000, Forster et al. 2003, Amaya et al. 2007). Similarly, much success has been achieved using plant meals as a protein source in aquatic animal diets. These meals are typically readily available in large quantities at a reasonable cost and most can be sourced to meet organic certification requirements. Nutritional issues similar to those found with animal meals must still be overcome in developing replacement formulations. A large body of literature is developing relative to use of plant meals to replace marine meals and limitations usually stem from amino acid imbalances, mineral levels, anti-nutritional factors, palatability and limited levels of essential highly unsaturated fatty acids (HUFA)(see Gatlin et al. 2007 for a recent review).

Highly unsaturated fatty acids (HUFAs: eicosapentaenoic acid (EPA), ARA and DHA) are required in shrimp feeds (Kanazawa et al. 1977, Read 1981, Fenucci et al. 1981, Martin 1980, Shiau 1998). Eliminating fish meals in diet formulations can result in reductions in levels of these essential fatty acids. Fish oil replacement would also reduce or eliminate sources of essential HUFA requiring addition of alternative sources to meet these requirements. In the present research, commercially available heterotrophically grown microbial meals were applied as an alternative nutritional HUFA source to provide DHA and ARA. Aquagrow® Gold (Advanced BioNutrition Corp., Columbia, MD, USA) is a heterotrophically grown *Schizochitrium sp*. algal meal that contains 18 to 22% DHA by weight. It contains intact algal cells that have been drum dried. The Aquagrow® ARA (Advanced BioNutrition Corp., Columbia, MD, USA) is also a spray dried meal product remaining from an industrial oil extraction process of the heterotrophically grown microfungus *Mortierella alpine* containing about 12% ARA by weight. The inclusion of these non-genetically modified (GMO) nutritional products allows for the sourcing of the vital fatty acids from microbial producers at the lowest levels of natural food chains. The industrial fermentation processes for producing these meals are well developed and amenable to organic certification. These types of nutritional ingredients offer new opportunities to reduce dependence on harvest of marine pelagic fish for these important components of aquatic and terrestrial animal nutrition. The results of our research summarized below suggest that under the conditions of our trials, fish meal and fish oil levels in diets for the marine shrimp *L. vannamei* can clearly be eliminated or reduced well below the proportions in the conventional formulations in use today.

Shrimp production performance

A series of small scale tank trials were conducted to evaluate a variety of feed formulations incorporating the alternative nutritional strategies discussed above in replicated small scale growout microcosms. These studies were carried out in 650 L (0.85 m^2) HDPE circular tanks positioned under a shade with roofing made of clear and opaque panels. Tanks were stocked with juveniles produced from SPF *L. vannamei* broodstock. Tanks were aerated and studies were carried out without water exchange encouraging development of a rich microbial community. In all studies, water quality parameters were carefully monitored and all remained within acceptable ranges reported for optimal growth and survival of penaeid shrimp.

Results of an initial small scale trial was published by Patnaik et al. (2006). In the fifteen-week growth trial, a practical diet was designed containing co-extruded soybean poultry by-product meal with egg supplement (Profound™, American Dehydrated Foods, Inc., Verona, MO, USA) and soybean meal as the primary protein sources for formulations containing 350 g/kg crude protein and 80 g/kg lipid (Table 1). To further refine the diets, the fish oil in two of the diets was completely substituted with plant oils and oil originating from the microbial fermentation products described above. A commercial shrimp feed was also included in the trial for comparison. All tanks were stocked at a density of 30 shrimp/m^2. Detailed tables with water quality parameters can be found in Patnaik et al. (2006). 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 (Table 2). 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. In this study, growth and survival values were not significantly affected by the replacement of fish meal with Profound™ and fish oil with heterotrophic algal sources.

Results of a second small scale trial were published in Davis et al. (2004). This 12-week feeding trial, conducted with juvenile shrimp was designed to determine the suitability of three diets in which marine fish meal and/or marine fish oil was replaced with the Profound™, microbial meals or primarily 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 at a density of $22/m^2$.

Study 2, was terminated after twelve weeks. No significant differences were found between treatments in water quality indicators. 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 parameters. Table 3 summarizes the shrimp average final weights, survival rates, FCR and yields at the end of the 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, the reduced growth is presumed to be due to an essential fatty acid deficiency. Performance of shrimp maintained on Diet 6 (all organic diet) was the poorest but had reasonable growth and good survival; thus, 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.

Based on the results of the small scale trials, commercial scale trials were conducted in 1,000 m^2 ponds at the South Carolina Department of Natural Resources, Waddell Mariculture Center. All ponds were filled with water from the Colleton River (salinity ~28 ppt) filtered through a 400 µm size mesh bag, 2 weeks prior to stocking, and fertilized with liquid inorganic (10-34-0) and pelleted organic fertilizer (alfalfa) to stimulate algal growth. As in previous tank trials ponds were managed without water exchange encouraging a rich microbial community in the systems. Aeration was provided by either a 1 or 2 hp paddlewheel mechanical aerator or combination of the two as dissolved oxygen levels dictated. 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 food conversion ratios (FCR) below 2:1 based on an estimated growth rate of 1 g/wk and assumed survival.

In the first experiment (Browdy et al. 2006) 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. A commercial pelleted feed containing 35% protein, 7% lipid, and typical levels of fish meal and fish oil was compared with a custom commercially pelleted feed designed to contain 35% protein and 8% lipid formulated almost entirely from plant materials (Table 4). The plant ingredients used in this experimental diet were all non-GMO, 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 (Both diets were manufactured by Zeigler Bros., Inc., Gardners, PA USA).

Data on water quality parameters can be found in Browdy et al. (2006). Observed water quality indicators were acceptable for good growth and there were no indications that the various diets produced different impacts on the measured parameters. Average shrimp weight, growth/week, survival, production, and feed conversion ratio (FCR) for each of the six ponds are shown in Table 5. Mean shrimp weight at harvest as determined by ANOVA differed among ponds across both treatments; However, no significant differences were found in production parameters between the conventional fish meal based diet and the plant based diet (production: 4,594 and 4,592 kg/ha, harvest size: 18.7 and 19.2 g, survival: 93% and 88%, and feed conversion rate: 1.4 and 1.3 for fish meal and plant based diets, respectively).

A second pond trial, experiment 4 (publication in preparation), was designed to compare a 35% protein "Ecosafe" diet containing no fish meal, or other protein derived from marine animal sources (see formulation in Table 4), to a standard commercial diet containing 35% marine animal protein (Both diets were manufactured by Zeigler Bros., Inc., Gardners, PA USA). In the Ecosafe diet, pet food grade poultry by-product meal and soybean meal were incorporated as the primary protein sources. The study was carried out in six 0.10 hectare ponds, 3 ponds per diet treatment. Ponds were stocked with nursery reared shrimp (~1.4 g) at a density of 80 shrimp/m^3 and were managed with minimal water exchange encouraging a rich microbial community to develop. A 20% exchange was carried out in all ponds to maintain adequate dissolved oxygen levels during a power outage that lasted for several hours. An additional 20% exchange was carried out late in the production cycle to reduce solids.

Temperature, salinity, dissolved oxygen, and pH were measured every morning and afternoon. Although comparisons among ponds for morning and afternoon salinity and pH indicate statistically significant differences between treatments, actual ranges are small and both fall well within the optimal range for shrimp pond culture. Inorganic nitrogen was determined for all ponds and was not significantly different between treatments for TA-N, NO_2-N, and NO_3-N (P=0.3116, 0.2811, and 0.8535, respectively). Only nitrate in one pond was significantly different from the others. Nitrate is not considered toxic to shrimp under normal production conditions. All values for potentially toxic inorganic nitrogen compounds were below concentrations of concern at the pH and salinity ranges maintained during this pond study.

Ponds were harvested after 13 weeks of growout. Table 6 provides harvest data comparing the diet treatments. There was no interaction between treatment (diet) and ponds. Shrimp at harvest were significantly different in sizes with shrimp in ponds fed the Ecosafe diet being significantly larger at harvest than shrimp in ponds fed the standard commercial diet. Survival to harvest was not significantly different between treatments averaging 72.4% ± 1.4% in ponds fed conventional diets and 85.6% ± 13.2% in Ecosafe ponds. Some problems were again experienced with power failures and heavy algal blooms but overall survival was reasonable in all ponds. Production levels of 10,919.7 ± 671.5 kg/ha were achieved in the ponds fed the Ecosafe feeds versus 11,722.7 ± 1,556.8 kg/ha in the standard diet fed ponds. There was no significant difference in harvested biomass between diet treatments. These production rates are quite high for earthen pond culture with very little water exchange demonstrating the potential of these feeds even at relatively high stocking densities typical for intensive culture of this species. The feed conversion ratio (FCR) was not significantly different at harvest and was reasonable for all treatments averaging 1.7.

Nutritional quality

Shrimp, like most seafood, are a good source of long-chain n-3 and n-6 fatty acids. Previous studies have demonstrated that fatty acid 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 fatty acids essential to the health of the shrimp and beneficial to the humans who consume the shrimp. As detailed above the current studies have demonstrated an alternative nutritional technology which replaces marine products including oils with microbial meals rich in DHA and ARA. A complete analysis was conducted post-harvest for the pond production experiment 3 (in which the organically certifiable plant based diets were compared with conventional diets) to determine effect of these replacement strategies on shrimp composition in terms of human health benefits. These results have been published by Browdy et al. (2006) and additional details on methods and results can be found in that publication.

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 to 80% phospholipids (membrane lipid), 6 to 10% sterols, and the remaining 10 to 24% neutral lipids (storage lipid).

Seventy one (71) fatty acids were identified in the feed or shrimp. The 18 fatty acids that had mean percentages ≥ 0.5% for either the feeds or shrimp and together account for >90% of the total fatty acids in both the feeds and the edible shrimp tissue are shown in Table 7. The plant based diet contained approximately 16% saturates, 20% monoenes and 62% polyunsaturated fatty acids (PUFAs); the fish meal based diet 30% saturates, 26% monoenes and 43% PUFAs. PUFAs in the plant based feed were 41.1% linoleic acid (LA) and 19.0% linolenic acid (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). ARA was relatively low in both feeds, at 0.22% of fatty acids in the plant based feed and 0.64% in the fish meal based feed.

The major fatty acids 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 7). Major differences in the fatty acid profiles between the two groups were in the percentages of the PUFAs. LA ($F_{1,35} = 1055$, $P = <0.0001$) and LnA ($F_{1,35} = 1069$, $P = <0.0001$) were significantly higher in shrimp fed the plant based diet while ARA

($F_{1,35}$ = 64, P = <0.0001), EPA ($F_{1,35}$ = 519, P = <0.0001) and DHA ($F_{1,35}$ = 182, P = <0.0001) were significantly lower (Table 7; Fig. 1, Panel B). The ratio of total n-6/n-3 fatty acids 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 fatty acids. This was not surprising since LA acid comprised over 40% of the fatty acids 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.

Conclusions

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 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 on to the consumer through aquacultured seafood fed fish meal based diets. On these two counts the experimental diets employed in these studies were successful.

There were no significant reductions in the average weight of shrimp at harvest, the total production, the growth rate, survival, or feed conversion ratio of the treatment ponds fed the fish meal and fish oil free diets versus those receiving the conventional diets. This demonstrates that under the conditions of the presently described trials, the alternative nutritional technologies described above can provide for fully equivalent or superior shrimp production grow-out feeds compared to conventional fish meal based diets.

The results of the post-harvest analysis in experiment 3 indicate that fatty acid 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 omega-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 to 1.1 g/100 g) reported by Bragagnolo and Rodriguez-Amaya (2001) for several species of wild marine shrimp and farmed freshwater prawns.

The predominant difference in the FA composition of the two feed groups in experiment 3 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. While the mean percentages of LA and LnA were lower in the tissue of shrimp than in their respective diets, those of ARA, EPA, and DHA were considerably higher. ARA, EPA and DHA were significantly lower in tissue of shrimp fed the plant based diet when compared with those fed the fish meal based diet. However, this difference was substantially less than might be expected based on the differences in levels of these fatty acids in their respective diets. Although the amounts of ARA, EPA, and DHA were ≤ 1.08% each in the plant based diet, they accounted for 3.0%, 10.8%, and 8.8% respectively of fatty acids found in shrimp fed this diet; 14, 20, and 8 times that found in their diet. The ARA, 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 7). Previous studies have shown that shrimp have very limited ability to convert LA to ARA (Lilly and Bottino 1981) and LnA to EPA and DHA (Kanazawa et al. 1977). Therefore ARA, EPA, and DHA must be acquired through diet, whether natural food sources or commercial feeds. It is becoming generally recognized that natural pond productivity may contribute significantly to *L. vannamei* nutrition, particularly in culture systems with low to no exchange in which microbial biofloc based communities develop (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 % ARA, 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 fatty acids equally to both feed groups in this study.

Ongoing research funded by the USDA Integrated Organic Program in a grant to Browdy, Leffler and Seaborn is focusing on development of a more holistic approach to culture system management and diet formulation to maximize contributions of natural productivity to cultured shrimp performance and nutritional quality. The project will develop protocols for managing the functional dynamics of the microbial floc communities in zero exchange shrimp aquaculture ponds to optimize the efficiency of growth and production of organically certifiable marine shrimp in a number of ways: 1) by maximizing contributions from natural productivity and improving formulation of organically certifiable feeds for improvement of feed conversion efficiency supplementation of prepared feeds, 2) by significantly reducing export of wastes to the environment through enhanced nutrient cycling, and 3) by reducing both economic risk and livestock stress by stabilizing water chemistry. The project is employing an ecosystem perspective of the dynamics of shrimp production ponds, specifically through defining and budgeting carbon and nitrogen inputs and outputs from the system relative to management of the timing and nature of system inputs including organic fertilizer, organic shrimp feed composition, stocking density, and community biological recruitment.

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 marine oils 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. 1A). This difference in FA profile clearly distinguishes between the shrimp raised on the two diets (Fig. 1B). 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; 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 formulations tested in this study, the human health significance of this difference may be relatively small compared to other popular protein sources. We compared the amount of EPA and DHA available from the shrimp raised on the two diets with values from the on-line United States Department of Agriculture Nutritional Database (United States Department of Agriculture 2005) for beef, pork, chicken, salmon, tuna, and mixed species shrimp. In order to make these comparisons the relative amounts of fatty acids determined for the study shrimp were converted to g FA/100 g tissue using equations published by Weihrauch et al. (1977). Since 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 new diets without fish meals and fish oils to meet organic standards must be cautious about maintaining a fatty acid 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 due to 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. In the future, fatty acid levels in shrimp tissues could be modified by increasing present inclusion of microbial meals to adjust dietary lipid profiles or by changing the level and type of natural foods. Future research into the use of finishing diets at the end of grow-out to restore human-health-optimal fatty acid ratios to shrimp raised on fish meal free formulations might also prove productive in addressing this issue.

Recommendations

Based on the results of this work under the conditions tested, fish meal and fish oil can be removed from diets for marine shrimp *L. vannamei* without significantly reducing growth, survival or feed conversion efficiency. Both organic terrestrial vegetable and terrestrial animal proteins can be viable alternatives for meeting marine protein requirements. However, with all plant diets, balancing amino acid requirements is somewhat more complex. Production performance on the diets incorporating poultry meals suggest that use of organically certifiable animal proteins in combination with the vegetable based ingredients should be considered as an allowable alternative by NOSB. Replacement of marine oils will require the addition of an alternative HUFA source to assure production performance and nutritional quality of the product. Microbial meals such as those tested in the present studies can fulfill this role providing an organically certifiable and environmentally sustainable alternative.

Research team

The research reported here was conducted by a team of scientists with funding from multiple sources. Craig Browdy, John Leffler, Tzachi Samocha and Allen Davis have conducted research on tank and pond production of marine shrimp using fish meal and fish oil free diets with funding from a NOAA SBIR grant to Advanced BioNutrition Corporation (ABN) led by Robert A. Bullis. ABN has commercialized a series of heterotrophically grown algal meal products which can provide an alternative source of DHA and ARA for aquaculture feeds. The purpose of the NOAA SBIR grant was to develop feed formulations which reduce dependence on marine protein and oil sources by utilizing terrestrial plant and animal protein alternatives. Facilities and operations for shrimp culture in Texas and South Carolina have been supported by the USDA CSREES US Marine Shrimp Farming Program. Fatty acid analyses were conducted by Gloria Seaborn. Support for fatty acid analyses of diets and shrimp tissues was partially provided by a grant from the NOAA Center of Excellence in Oceans and Human Health at the Hollings Marine Laboratory. Ongoing research on enhancing and incorporating contributions of natural productivity into new holistic organically certifiable shrimp diet formulations is being carried out at the SCDNR Marine Resources Research Institute, Waddell Mariculture Center by John Leffler, Craig Browdy and Gloria Seaborn with funding from the USDA Integrated Organic Program.

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Table 1. Diet formulations for tank scale experiments 1 and 2 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 1			Experiment 2		
	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 [2]	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, organic[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. Richmond 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 Corp., Muscatine, IA. 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.

Table 2. 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 (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.

Table 3. 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 (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)

Table 4. Diet formulations for pond scale experiments 3 and 4 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 3 Plant-Organic	Experiment 4 Poultry
Ingredients	Percent by Weight	Percent by Weight
Expelled soybean meal, 42/7, organic	58.10	
Soybean meal solvent extracted		39.44
Whole soft wheat, organic	12.00	
Feed wheat		30.17
Pet food grade poultry by-product meal		12.00
Canadian feed pea meal, organic	10.00	
Non-GM corn gluten meal, 60% protein	9.00	8.00
Flaxseed oil	2.00	
Di-Calcium phosphate	2.00	1.92
Aqua-Bond-CM		1.38
Federal vitamin premix #30 w/o choline	1.80	0.50
UF premix- CO		2.00
Flax seed		
Squid liver meal	1.00	
Liquid fish solubles	1.00	
AquaGrow-schizochytrium-DHA®	0.50	
USFW #3 Mineral Mix	0.50	
Non-GM lecithin	0.50	0.50
BetaFin BT-Danisco	0.50	
Kelp meal, Acadian Seaplants Limited	0.50	
Wheat starch		
Soy oil, no additives, organic	0.20	3.68
Choline chloride, 70%	0.20	0.20
Cholesterol		
AquaGrow ARA®	0.13	
Aqua Min		0.15
Stay C 35%	0.07	0.07

Table 5. FCR, survival, mean weight at harvest (± SD), and total production for *L. vannamei* fed fish meal based and plant based diets in experiment 3. Letters indicate significant difference (P<0.05) among ponds (rows) for average shrimp weight at harvest.

Diet	FCR	Survival (%)	Harvest Weight (g)	Production (kg/ha)
Fish meal based	1.4	91	18.5 ± 3.5^{b}	4,404
Diet	1.4	100	17.3 ± 2.7^{c}	4,549
(3 ponds)	1.3	87	20.3 ± 2.9^{a}	4,829
Treatment Mean	1.4	93	18.7	4,594
Plant based	1.4	86	18.6 ± 3.0^{b}	4,223
Diet	1.3	92	18.7 ± 2.8^{b}	4,782
(3 ponds)	1.3	85	20.3 ± 2.8^{a}	4,772
Treatment Mean	1.3	88	19.2	4,592

Table 6. FCR, survival, mean weight at harvest (± SD), and total production for *L. vannamei* fed fish meal based and poultry meal based diets in experiment 4.

	Standard Diet	Ecosafe Diet	*P* Value
Harvest Weight Mean (g) N = 300	14.0±2.9	15.3±2.7	<0.0001
Total Production (kg/ha) N = 3	10,919.7 ± 671.5	11,722.7 ± 1,556.8	0.4454
FCR N=3	1.6 ± 0.2	1.7 ± 0.1	0.3486
Survival (%) N = 3	72.4 ± 1.4	85.6 ± 13.2	0.1617

Table 7 Mean percentages (± SE) of selected fatty acids (mean values > 0.5%) found in shrimp (*L. vannamei*) raised on a plant based diet and shrimp raised on a fish meal based diet (Experiment 3).

	Plant based Feed				Fish meal based Feed			
	Feed	Shrimp (n=18)			Feed	Shrimp (n=18)		
		Mean		SE		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			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		

[a] for feed "as is"; for shrimp - wet weight.

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).