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Alternative diets for the Pacific white shrimp *Litopenaeus vannamei*

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Abstract

Future use of animal protein sources in shrimp feeds is expected to be considerably reduced as a consequence of increasing economical, environmental and safety issues. Of main concern has been the use of expensive marine protein sources, such as fish meal. Hence, shrimp research has recently focused on the development of practical feeds with minimal levels of fish meal and alternative, lower cost protein sources. To determine shrimp capacity to use practical feeds with plant proteins as replacement ingredients to animal protein sources, an 81-day growth trial was conducted in an outdoor tanks system, using juvenile (0.74 g) *Litopenaeus vannamei*. Experimental treatments included four diets with varying levels of fish meal in the diet (9, 6, 3 and 0%) in combination with 16% poultry by-product meal, a plant based feed containing 1% squid meal, and a commercial reference feed. Feeds were commercially extruded and offered as sinking pellets designed to contain 35% crude protein and 8% lipids. Mean final weight, percent weight gain, final net yield, feed conversion ratio and survival were evaluated. Final values for these parameters ranged from 17.4 to 19.5 g, 2249–2465%, 564.4–639.0 g m^{-3}, 1.07–1.20 and 83.3–89.2%, respectively. Evaluation of production parameters at the end of the study demonstrated no significant differences ($P \geq 0.05$) among any of the experimental treatments. These results indicate that fish meal can be replaced with plant protein sources in diets including 16% poultry by-product meal without affecting shrimp growth and production. In addition, results demonstrate that good performance can obtained by shrimp fed a plant protein based feed (solvent extracted soybean meal, corn gluten meal and corn fermented solubles) in combination with 1% squid meal. Although results with the primarily plant based diet are encouraging, further evaluations are recommended to allow the removal of the remaining marine ingredients.
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Keywords: *Litopenaeus vannamei*; Soybean meal; Fish meal; Plant proteins

1. Introduction

Animal origin ingredients such as fish meal, poultry by-product meal and meat and bone meal are considered among the most suitable protein sources for shrimp feeds. In spite of their importance, a considerable reduction in the use of these animal origin ingredients is expected in coming years. Limited availability, variable supply and safety issues are primary concerns. Given the growing demand by animal production industries for fish meal and its limited supply, prices are likely to continue to increase, therefore, restraining future use as the main protein source in shrimp feeds. Likewise, emerging environmental and safety issues associated to the use of potentially contaminated animal by-products in animal feeds and the effect of fish meal production from natural fish stocks have also been viewed negatively. It has been suggested that one way to

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address all these issues is through the development of all plant-protein feeds. Such a tactic could also provide an economical opportunity for shrimp producers, as some segments of the market would pay a higher price for a premium shrimp fed and produced under environmentally sound conditions (Josupeit, 2004; Davis et al., 2004; Samocha et al., 2004).

Facing these scenarios, various studies have focused on the development of alternatives that effectively replace or minimize the inclusion of animal protein sources in commercial shrimp formulations using plant proteins (Colvin and Brand, 1977; Lim and Dominy, 1990; Piedad-Pascual et al., 1990; Lim et al., 1998; Hardy, 1999; Divakaran et al., 2000; Davis and Arnold, 2000; Tacon, 2000; Mendoza et al., 2001; Dersjant-Li, 2002; Conklin, 2003; Fox et al., 2004; Gonzalez-Rodriguez and Abdo de la Parra, 2004; Samocha et al., 2004). According to Davis et al. (2004), the use of an all-plant protein feed can be limited due to a variety of factors including deficiency or imbalance of essential amino acids, reduced levels of minerals, limited levels of highly unsaturated fatty acids (HUFA), presence of anti-nutritional factors or toxins and decreased palatability. In spite of these limitations, and that nutritional information on shrimp is far from complete, the understanding of primary nutrient requirements for shrimp is adequate to allow the replacement of animal protein sources with alternative ingredients.

It is clear then, that the complete substitution of fish meal and animal by-product meals with other protein sources can be effectively achieved only when certain basic conditions are provided. Of primary concern is the proper formulation and supplementation of feeds with adequate lipids, phosphorus and amino-acids sources (i.e. lysine and methionine), to overcome the nutritional imbalances that arise when marine animal meals are removed from the formulation. (Akiyama, 1988; Akiyama et al., 1991; Lim et al., 1998; Tacon and Barg, 1998; Hertrampf and Piedad-Pascual, 2000; Fox et al., 2004). Furthermore, proper feed manufacture and feedstuffs processing have been found to improve the overall nutritional quality and water stability of replacement feeds (Swick et al., 1995; Davis and Arnold, 2000; Hernandez et al., 2004; Samocha et al., 2004). According to Carver et al. (1989), extrusion has the advantage of inactivating and/or destroying some of the heat-sensitive anti-nutritional factors found in plant protein sources, such as soybean meal, while also gelatinizing starch granules.

Just as the nutritional component of the feed is of primary importance in shrimp nutrition, feeding habits also play an important role when considering the implementation of feeds with minimal animal origin ingredients. Shrimp, for example, have the capacity to utilize naturally available foods in green water systems where they are commonly cultured. This means that shrimp growth in ponds is achieved through the simultaneous consumption of feed and endogenously produced food organisms such as micro-algae and microbial–detrital aggregates (Moss and Pruder, 1995; Moss et al., 2001; Forster et al., 2002; Tacon et al., 2002).

Studies evaluating the ability of shrimp to utilize diets with low levels of animal protein sources have been commonly carried out in clear water systems, where the length of the culture period is limited and environmental conditions greatly differ from those found in commercial ponds (D'Abramo and Castell, 1997). In addition, these studies have generally used laboratory made feeds that are appropriate for laboratory-scale studies, but lack the manufacturing and extrusion advantages provided by commercial feeds. There is limited information with regards to shrimp performance when fed extruded feeds with low levels of animal protein sources and reared in green water systems. Hence, the aim of this study was to evaluate the productive performance of the Pacific white shrimp (*Litopenaeus vannamei*) fed a commercially manufactured feed with varying levels of animal and vegetable protein sources and reared in an outdoor, semi-closed, recirculating system in which the shrimp had access to natural food.

2. Materials and methods

2.1. Shrimp and experimental units

Juvenile Pacific white shrimp (*Litopenaeus vannamei*) were obtained from 16 shrimp production ponds located at the Claude Peteet Mariculture Center, in Gulf Shores, Alabama. Shrimp (mean weight ± SD, 0.743 ± 0.031 g) were stocked at a density of 37.5 shrimp m^{-3} (30 shrimp/tank) in an outdoor, semi-closed recirculating system. The system was composed by 24 circular polyethylene tanks (0.85 m height × 1.22 m upper diameter, 1.04 m lower diameter) designed to contain 800-l and a reservoir tank (800-l) with a biological filter. Before stocking, the system was filled with algae rich (green water) brackish water (9.50 ppt) from a shrimp production pond. Daily water exchanges were performed during the experimental period between 04:00 in the morning and noon, when pond water was pumped into the central filter at a rate of 8 l per minute. This exchange rate allowed a 100% water exchange in the recirculating system every six days. Aeration was

Table 1
Ingredient composition of practical diets for *L. vannamei* used to evaluate the replacement of animal proteins by plant protein sources (values expressed on an as fed basis, g/100 g)

Ingredient	9% FM	6% FM	3% FM	0% FM	Plant based
Solvent extracted soybean meal 47% CP	32.48	34.82	37.17	39.52	56.46
Fish meal — Menhaden Select	9.00	6.00	3.00	–	–
Poultry by-product meal 65% CP	16.00	16.00	16.00	16.00	–
Milo (Sorghum)	35.47	33.82	32.33	30.68	14.99
Corn gluten meal	–	1.67	3.17	4.84	4.83
Fish oil	3.96	4.22	4.47	4.72	5.76
Di-calcium phosphate	1.50	1.88	2.27	2.65	3.38
Corn fermented solubles	–	–	–	–	9.99
Salt-potassium chloride	–	–	–	–	2.00
Squid meal	–	–	–	–	1.00
Bentonite	1.00	1.00	1.00	1.00	1.00
Mold inhibitor	0.15	0.15	0.15	0.15	0.15
Vitamin premix	0.34	0.34	0.34	0.34	0.34
Mineral premix	0.08	0.08	0.08	0.08	0.08
Stay-C (35% active vit. C)	0.02	0.02	0.02	0.02	0.02

Diets were commercially manufactured by Rangen® Inc. (Angleton, TX) using extrusion processing.

provided in the filter and in each tank by two air stones connected to a common air supply from a 1-hp regenerative blower.

During the experimental period, dissolved oxygen (DO), temperature, salinity and pH concentrations were measured in the reservoir tank and two of the system tanks at 06:00 in the morning and at 16:00 in the afternoon. Water quality parameters were measured using a YSI 556MPS meter (Yellow Spring Instrument Co., Yellow Springs, OH, USA). Total ammonia-nitrogen (TAN) was determined on a weekly basis, with water samples taken from the filter and two of the tanks and measured with a spectrophotometer (Spectronic Instrument Inc. Rochester, NY, USA) using the Nesslerization method (APHA, 1989).

2.2. Feeds and feed management

The six dietary treatments (Table 1) were randomly assigned to shrimp in four replicate tanks per treatment. The goal for the formulation of the first four diets, was to reduce the inclusion of menhaden fish meal (FM) as follows: 9%, 6%, 3% and 0% FM of the total diet, while increasing the inclusion of vegetable protein sources, mainly solvent extracted soybean meal and corn gluten meal. Poultry by-product meal (PBM) was fixed at 16% inclusion in the first four diets. The fifth diet was a second generation plant protein based feed, formulated using the profile of the diet previously reported by Davis et al. (2004), but including 1% squid meal as an attractant and source of essential nutrients. Because this plant protein feed was also formulated to be used in the low salinity conditions of west Alabama waters, it included an additional supplementation of 2% of potassium chloride. The sixth diet was a 35% protein commercial shrimp feed manufactured by Rangen Inc. and served as a high quality commercial reference.

The dietary treatments were formulated in order to provide equal protein and lipid levels while maintaining a minimum lysine and methionine+cystine content of 5% and 3% of the total protein, respectively (Table 2). Corn gluten meal was used as a natural source of methionine and no crystalline amino acids were supplemented. Corn fermented solubles were used as an additional protein source and only added to the plant protein based diet. Lipid levels were adjusted by adding menhaden fish oil, which is a rich source of highly unsaturated fatty acids (HUFA), such as decoeicosanoic acid (DHA) and arachidonic acid (AA). Calcium phosphate was added to ensure an adequate phosphorus supply. Feeds were produced by Rangen Inc. (Angleton, TX, USA) under commercial manufacturing conditions and offered to the shrimp as a sinking extruded pellet.

Daily feed inputs were back-calculated, based upon an expected shrimp weight gain of 1 g per week and an expected feed conversion ratio of 1.8:1. Feed was provided twice per day. In order to readjust daily feed

Table 2
Nutritional composition of practical diets for *L. vannamei*, used to evaluate the replacement of animal proteins with plant protein sources [a]

Nutrient	9% FM	6% FM	3% FM	0% FM	Plant based	Reference
Crude protein	35.7	35.9	36.2	36.6	36.1	37.6
Crude fat	8.4	8.3	8.6	8.4	8.4	10.6
Crude fiber	2.4	1.8	2.1	1.9	2.2	1.7
Ash	8.2	7.9	7.9	8.1	9.5	8.9
Calcium [b]	1.3	1.3	1.2	1.1	0.9	–
Total phosphorus [b]	1.2	1.2	1.2	1.3	1.2	–
Lysine [b]	2.0	2.0	1.9	1.8	2.0	–
Met+Cys [b]	1.1	1.1	1.1	1.1	1.1	–
% Protein from plant [c]	54.9	60.4	66.0	71.5	97.7	–
% Protein from animal [d]	45.1	39.6	34.0	28.5	2.3	–

[a] Analyzed value — New Jersey Feed Laboratory, Inc. Trenton, NJ. Methods of analyses used the following AOAC methods: Protein (990.03), Fat (920.39), Fiber (978.10), Ash (942.05), Moisture (930.15).
[b] Formulated value.
[c] Soybean meal, corn gluten meal, milo, corn fermented solubles.
[d] Fish meal, poultry by-product meal and squid meal.

Table 3
Summary of water quality parameters observed over an 81-day experimental period for *L. vannamei* fed practical diets with varying levels of animal and plant protein sources and cultured in an outdoor semi-closed recirculating culture system

	Temperature (°C)	DO[a] (mg l^{-1})	pH	Salinity (ppt)	TAN (mg l^{-1})[b]
am	27.4±1.3 (24.1, 29.7)	6.70±0.56 (3.79, 7.67)	7.30±0.28 (6.24, 8.18)	8.3±0.8 (7.0, 9.5)	0.31±0.35 (0.00, 0.88)
pm	29.6±1.8 (23.9, 32.8)	6.66±0.54 (4.70, 7.85)	7.66±0.25 (7.17, 8.29)		

Values are mean±standard deviation of daily and weekly determinations. Values in parentheses represent minimum and maximum readings throughout the study.
[a] Dissolved Oxygen.
[b] Total Ammonium Nitrogen. Weekly values.

inputs, shrimp survival was determined by counting all shrimp in each tank on a bi-weekly basis. Overall, each shrimp in each tank was offered a total of 20 g of feed throughout the 81-day experimental period. Feed inputs were stopped one day before harvesting.

Shrimp production was evaluated at the end of the growth trial considering the following parameters: mean final weight, weight gain (%), weekly weight gain, final net yield, feed conversion ratio (FCR) and survival. In order to evaluate the economic feasibility of using alternative shrimp diets, the following parameters were also determined: total value of shrimp production, feed price, cost for total feed inputs, partial “gross” returns and feed cost per kilogram of shrimp produced.

2.3. Statistical analyses

Experimental units were distributed on a completely randomized design, with six dietary treatments and four replicates per treatment. Data were analyzed by using one way analysis of variance (ANOVA), to determine if significant ($p < 0.05$) differences existed among treatment means. The Student–Neuman–Keuls multiple comparison test was used to determine whether significant differences existed between treatment means (Steel and Torrie, 1980). All statistical analyses were conducted using SAS (V9.1., SAS Institute, Cary, NC, USA).

3. Results

Water quality parameters throughout the 81-day growth trial were maintained at suitable levels for adequate growth and survival of shrimp (Table 3). Mortality was not considered when calculating final FCR and although cannibalism was not reported it was likely to happen to a certain extent as tanks were only sampled every two weeks. Mean final weight, weight gain (%), final net yield, FCR and survival were evaluated at the end of the 81-day culture period. Final values for these parameters ranged from 17.4 to 19.5 g, 2249–2465%, 564.4–639.0 g m^{-3}, 1.07–1.20 and 83.3–89.2%, respectively. Complete data of productivity and economic parameters of *L. vannamei* fed the six dietary treatments are presented in Tables 4 and 5. Results indicate that none of the parameters evaluated were significantly affected by the inclusion of plant protein sources as a replacement for fish meal and poultry by-product meal for any of the dietary treatments.

Although not statistically significant differences were found among any of the treatments and productive parameters evaluated, shrimp fed the 0% FM diet numerically showed the poorest performance for most parameters evaluated. Experimental units offered the plant protein based diet showed the lowest shrimp survival. Final mean weight of shrimp fed the reference feed (19.5 g) was numerically higher than those of

Table 4
Mean production parameters at the end of an 81-day culture period for *L. vannamei* reared in an outdoor semi-closed recirculating system and fed practical diets with varying levels of fish meal (FM), a plant based diet and a commercial reference diet[a]

Parameter	9% FM	6% FM	3% FM	0% FM	Plant	Ref.	PSE[b]	*P*-value
Initial weight (g)	0.72	0.73	0.75	0.74	0.72	0.78	0.014	0.056
Final mean weight (g)	18.5	18.6	18.8	17.4	18.4	19.5	0.439	0.072
Weight gain (%)[c]	2443	2439	2391	2249	2465	2401	80.742	0.486
Weekly weight gain (g)	1.53	1.55	1.56	1.44	1.53	1.61	0.038	0.096
Final net yield (g m^{-3})	586.2	624.0	622.5	564.4	574	639	18.752	0.055
FCR[d]	1.13	1.12	1.11	1.20	1.13	1.07	0.03	0.108
Survival (%)	85.0	89.2	88.3	86.7	83.3	87.5	2.766	0.687

[a] Based on analysis of variance (ANOVA), no significant differences ($P > 0.05$) were found among treatment means ($n = 4$).
[b] Pooled standard error of treatment means = $\sqrt{mse/n}$.
[c] Weight gain (%) = 100 × (final weight − initial weight) / initial weight.
[d] FCR, feed conversion ratio = Feed offered per shrimp / weight gain per shrimp.

Table 5
Mean economic parameters of *L. vannamei* cultured in an outdoor semi-closed recirculating system over an 81-day experimental period and fed practical diets with varying levels of fish meal (FM) and a plant protein based diet — estimates are based on production per cubic meter of culture system[a]

Parameter	9% FM	6% FM	3% FM	0% FM	Plant	*P*-value	PSE
Shrimp production (g m^{-3})	586.2	624.0	622.5	564.4	574.0	0.150	19.6
Production value (USD)[b]	2.12	2.26	2.26	2.05	2.08	0.149	0.07
Total feed inputs (g m^{-3})	637.5	668.7	662.5	650.0	625.0	0.630	22.1
Feed price (USD/kg)[c]	0.531	0.526	0.520	0.515	0.532	–	–
Total feed inputs cost (USD)[d]	0.34	0.35	0.34	0.33	0.33	0.778	0.01
Partial gross returns (USD)[e]	1.79	1.91	1.92	1.71	1.75	0.120	0.06
Feed cost per kg of shrimp produced (USD)[f]	0.60	0.59	0.58	0.62	0.60	0.308	0.015

[a] Based on analysis of variance (ANOVA) no significant differences ($P>0.05$) were found among treatment means ($n=4$). Commercial reference feed excluded from the economic analysis.
[b] Production value = Shrimp production (g m^{-3}) × \$3.63 USD/kg of shrimp. Shrimp price is based on Gulf fresh shrimp prices (head-on) between the 13 and 19 of October, 2005, as reported by the National Marine Fisheries Service, Fisheries Statistics Division, Silver Spring, MD, USA.
[c] Prices were based on those at the time of the research (May 2005) and are subject to change based on varying ingredients price. As fish meal has gone up in price and prices vary from region to region the cost effectiveness of the feed will change. Hence, conclusion with regards to cost effectiveness should be made on a case by case basis.
[d] Total feed inputs cost = Total feed inputs (g m^{-3}) × Feed price (USD/kg).
[e] Partial gross returns = Production value (USD) − Total feed inputs cost (USD/kg).
[f] Feed cost per kg of shrimp produced = FCR × Feed price (USD/kg).

shrimp fed the other treatments, with the lowest mean value for shrimp fed the 0% FM feed (17.4 g). Lowest mean weight gain (%) was found in the 0% FM (2249%) treatment. Mean final net yield ranged from 0.56 kg m^{-3} (0% FM) to 0.64 kg m^{-3} (Reference diet) and was within the range of yields given for intensive shrimp production systems. Feed conversion ratio (FCR) and survival values from this study ranged between 1.11 (3% FM) to 1.20 (0% FM) and 83.3% (0% FM) to 89.2% (6% FM), respectively. Low *P*-values of 0.056 and 0.055 were found for initial weight at stocking and final shrimp production (g m^{-3}) analyses, respectively, whereas percent weight gain, FCR and survival analyses resulted in larger *P*-values of 0.486, 0.108 and 0.687 respectively. No statistical significant differences were found among treatment means for any of these parameters.

Feed prices showed a slight decrease of nearly sixteen dollars between the 9% FM feed (\$531/mt feed) and the 0% FM feed (\$515/mt feed). There was a general trend toward reduced price as more plant protein sources were included in the formulation. Conversely, the plant protein diet showed a higher price than all of the other treatments (\$532/mt feed). Higher cost of the plant protein diet was the consequence of extra costs associated with the purchase of small quantities of select ingredients (e.g. corn fermented solubles), which is not a typical ingredient at this mill.

4. Discussion

Results from this study provide important information regarding the potential of Pacific white shrimp (*L. vannamei*) to utilize alternative feed formulations with low levels of animal protein sources under commercial production conditions. This research was conducted in replicated outdoor tanks using green water from a production pond and shrimp were fed commercially extruded diets. Because of these production characteristics, the rearing conditions used in this study closely resembled those found by shrimp in commercial semi-intensive production systems. Therefore, shrimp utilization of the diets used in this study can be considered as indicative of the important potential of marine shrimp to use similar diets under commercial pond conditions. The good production results observed in this study confirm that *Litopenaeus vannamei* can be fed commercially manufactured feeds including plant proteins as replacements of animal protein sources, without adverse effects on shrimp performance or production economics.

Shrimp performance was excellent across all treatments with no statistically significant differences among the diets. Production parameters were similar to those of shrimp produced using similar management procedures and reared in outdoor recirculating systems in tanks (Venero, 2006; Davis et al., 2004; Samocha et al., 2004). The numerically lower growth of shrimp fed the 0% FM feed, may indicate that further optimization of the diet may be possible. The plant diet which included 1% squid meal and resulted in the highest shrimp weight gain among all treatments (2465%) also showed the lowest survival. This increased growth could have been influenced by the reduced density. Although these results are not significantly different, further evaluations and refining of plant-based feed formulations for shrimp are recommended.

Findings from this study are in agreement with those of Davis et al. (2004), who using a similar rearing system, reported the successful replacement of animal protein sources with plant proteins in shrimp feeds containing fish oil. These authors also claimed that both fish meal and marine oil sources could 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.

Besides the nutritional component of the feed, part of the success of the replacement of animal proteins by alternative sources is supported on shrimp capacity to utilize natural productivity. Different authors have indicated that the enhanced growth and performance of animals reared under 'green-water' culture conditions is due to their ability to obtain additional nutrients from natural food organisms present in green water systems and/or pond ecosystems (Leber and Pruder, 1988; Moss and Pruder, 1995; Tacon, 1996; Moriarty, 1997). Shrimp benefit from a wide range of organisms within the green water culture environment, including bacteria, phytoplankton, protists, rotifers and nematodes (Moss and Pruder, 1995; Velasco and Lawrence, 2000; Schuur, 2003; Decamp et al., 2003). For example, among the natural food organisms some flagellates and ciliates, have been reported to be rich in highly unsaturated fatty acids (i.e. 16–25% of the total) which have a good growth promoting effect on juvenile *L. vannamei* (Lim et al., 1997). The importance of this endogenous community in intensive shrimp production systems, was reported by Gomez-Jimenez et al. (2005), who did not find differences in weight gain, final weight or survival of *L. vannamei* offered commercial diets with varying levels of protein (25%, 30%, 35% or 40% CP) in a zero water exchange system.

Even when some productive advantages are acquired with the use of these kind of systems, to capitalize on potential markets for shrimp grown under organic or environmentally sustainable conditions, one must also utilize organic ingredients from more sustainable sources (Davis et al., 2004). Since one of the premises of sustainable aquaculture is to minimize the use of resources of limited availability, further studies evaluating the replacement of the fish oil from these alternative diets are recommended.

5. Conclusion

This study demonstrates that fish meal can be removed from commercially manufactured shrimp diets including 16% poultry by-product meal using vegetable protein sources, with no adverse effect on the productive performance of *L. vannamei* reared in green water environments. Furthermore this study provided important evidence that animal protein sources can be completely removed from shrimp feeds without negatively affecting shrimp growth. Shrimp feeds in this study included varying levels of soybean meal, corn gluten meal and corn fermented solubles as alternative protein sources to fish meal and poultry by-product meal. Although the all-plant diet produced good shrimp performance, this diet may be marginal and further studies are recommended to evaluate potential limiting nutrients as well as the efficacy of squid meal. Additional studies with plant based diets at a larger scale under commercial pond conditions are also suggested.

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