REPLACEMENT OF POULTRY BY-PRODUCT MEAL IN PRODUCTION DIETS

FOR THE PACIFIC WHITE SHRIMP (*Litopenaeus vannamei*)

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REPLACEMENT OF POULTRY BY-PRODUCT MEAL IN PRODUCTION DIETS FOR THE PACIFIC WHITE SHRIMP (*Litopenaeus vannamei*)

Justin C. Markey

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THESIS ABSTRACT

REPLACEMENT OF POULTRY BY-PRODUCT MEAL IN PRODUCTION DIETS

FOR THE PACIFIC WHITE SHRIMP (*Litopenaeus vannamei*)

Justin Charles Markey

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In recent years there has been increasing interest in the replacement of animal proteins with plant proteins in commercial feeds for the Pacific white shrimp, *Litopenaeus vannamei*. Primary reasons for this interest are to reduce cost by using a high quality but lower cost ingredient and to move towards ingredients that are from renewable resources for which production can be expanded. As with oil prices, the cost for many of our protein sources such as fish meal, poultry by-product meal, and other animal protein meals have increased over the years driving up feed costs. The idea of replacing animal protein meals with plant proteins in diets reduces the dependence on

the animal protein industry and provides alternative choices when formulating feeds.

Consequently, two growth trials were conducted to evaluate the potential of replacing poultry by-product meal with soybean meal in production diets for *L. vannamei*. For those trials, four iso-nitrogenous diets were formulated to evaluate the replacement of poultry by-product meal with a combination of soybean meal and corn gluten meal. Juvenile shrimp were stocked at 34 shrimp/m^2 and were cultured in 0.1 hectare ponds under standardized production conditions. Feed inputs were pre-scheduled using feeding protocols that have a maximum feed input of 7.10 kg/ha/d. Additionally, these four diets, a fifth diet consisting of high plant protein content with substitution of fish oil with soybean oil, and a commercial reference diet were evaluated in a semi-enclosed recirculating system stocked with 30 juvenile *L. vannamei* per tank. Culture water was pumped from a production pond for six hours per day and was recirculated at a rate of 3 L/min within the tanks to mimic production pond conditions. Each diet had four replicates. Feed rates were predetermined by using a feed conversion ratio (FCR) of 1:1.2 and an average weekly growth of 1.5 g/wk.

At the conclusion of these studies, shrimp were harvested, final weights, population distribution, net yield, and feed utilization was determined. Final yields in the pond study averaged between 6,093-6,943 kg/ha. Average final weights varied between 22-24 g, survival was 80% and the FCR was 1.0. The 79-d tank culture produced average final weights between 19.4-20.5 g, survival varied between 94.2-96.7 %, and the FCR was 1.1. There were no significant differences found between any of the poultry by-product meal replacement diets in either the pond or tank study.

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REPLACEMENT OF POULTRY BY-PRODUCT MEAL IN PRODUCTION DIETS FOR THE PACIFIC WHITE SHRIMP (*Litopenaeus vannamei*)

RESEARCH OBJECTIVE

The objective of this study was to evaluate the replacement of poultry by-product meal using a combination of protein sources, primarily of plant origin, in feeds formulated for *Litopenaeus vannamei*.

INTRODUCTION

Global farm-raised shrimp production is increasing at an average annual rate of 12 percent, totaling 2 million metric tons in 2005 and nearly 2.3 million metric tons in 2006 (Hedlund, 2007). Farmers prefer raising the Pacific white shrimp, *Litopenaeus vannamei*, because the species is easier, quicker and more profitable to grow than other commonly farm-raised shrimp (Hedlund, 2007). Expansion of world production in combination with other economic factors, have led to a reduction of shrimp prices. Market experts predict these trends to continue. Consequently, the less efficient producers will not be able to compete with more efficient producers or producers that are able to sell their product to premium markets (FAO, 2006).

The need to become more competitive has sparked interest to develop better feed management practices and lower cost diets. If diets can be formulated with high quality but lower cost ingredients, preferably from renewable resources, feed costs can be reduced (Amaya et al., 2007a). Feed costs contribute 40-60% of the production cost of intensely reared shrimp (Tan and Dominy, 1997; Tan et al., 2005). Therefore, reductions in feed costs will decrease production costs. The first step in reducing feed costs is identifying the most expensive components, typically protein (Akiyama, 1992; Forster and Dominy, 2006).

One possible venue to reduce feed costs is reducing the cost of protein sources. In August 2007, market reports for the United States quoted fish meal at \$860/metric ton (m/t) (60% protein), meat and bone meal at \$215-280 m/t, feather meal at \$275 m/t, soybean meal at \$211-263 m/t (48% protein), and poultry by-product meal at \$300 m/t (57% protein) (United States Department of Agriculture, 2007; Hammersmith Marketing

LTD, 2007). Using these market estimates to compare cost per metric ton of protein between fish meal, poultry by-product meal, and soybean meal, fish meal was $1,433, poultry by-product meal $526, and soybean meal $494. Fish meal is a relatively expensive protein source commonly used in shrimp feeds (Josupeit, 2007a). In recent years, the replacement of fish meal in the diets has been gaining momentum due to increased demand and limited supply, which is driving up the cost of feed (Forster and Dominy, 2006). The idea of replacing fish meal with animal by-product meals, such as meat and bone meal, blood meal, feather meal, and poultry by-product meal in practical diets for *Litopenaeus vannamei* has been widely investigated (Davis and Arnold, 2000; Mendoza et al., 2001; Forster et al., 2003; Samocha et al., 2004; Tan et al., 2005). One of the more promising alternative ingredients is poultry by-product meal (Dong et al., 1993), which is a waste material of the poultry industry (Davis and Arnold, 2000).

Significant reduction of ingredients from animal sources is expected in the near future because of availability limitations, renewability, as well as environmental and safety constraints. Increasing demand and decreasing availability will cause the prices for these animal based meals to continue to increase and will restrict the use of animal based meals as the main protein source in commercial shrimp feeds (Amaya et al., 2007b). Fish meal produced from wild fish stocks has been and will continue to be an over-exploited resource, causing environmental concern in regard to fish meal production. The disease management spectrum of animal feeds has also become of great concern when formulating diets. After incidents such as bovine spongiform encephalopathy (BSE), feeding animals animal proteins has become a more speculated issue (Amaya et al., 2006a; Caparella, 2006).

The use of primarily plant-based meals has been suggested as one possible solution to these issues. Using plant-based meals may also offer an economic opportunity for shrimp producers, as some markets would pay a higher price for shrimp fed and raised under environmentally sound conditions (Josupeit, 2004b; Samocha et al., 2004; Davis et al., 2006; Amaya, 2007a). Plant proteins could broaden the spectrum of available feedstuffs and may even be a cheaper resource depending on local market value. Plant protein sources are also a more renewable resource than marine animal protein and animal by-product protein sources, such as menhaden fish meal and poultry by-product meal. Soybean meal, is much more cost efficient than animal by-product meals and is a source of highly digestible protein and moderate energy (United States Department of Agriculture, 2007).

Solvent extracted de-hulled soybean meal (soybean meal) is highly digestible by shrimp and is effective in diets for many aquatic species (Divakaran et al., 2000; Forster and Dominy, 2006). Typical analysis of soybean meal would yield 48% protein, 89% dry matter, 7% crude fiber, 0.5% fat, 0.65% phosphorus. (National Grain and Feed Association, 2007).

Replacing animal protein meals with plant proteins in well formulated diets may reduce the dependence on the animal protein industry and provides alternative choices when formulating feeds. Amaya et al. (2007a) demonstrated that a practical diet with complete replacement of fish meal with poultry by-product meal had no significant statistical differences in any production parameters. This study was designed as an extension of previous research, with the objective of evaluating the replacement of poultry by-product meal using a combination of protein sources in feeds formulated for *Litopenaeus vannamei*.

MATERIALS AND METHODS

The study was conducted at the Claude Peteet Mariculture Center in Gulf Shores, Alabama, and consisted of two parallel growth trials utilizing outdoor tanks and production ponds. Four diets were formulated to focus on the replacement of poultry by-product meal with soybean meal and produced under commercial conditions by Rangen Incorporated (Angleton, Texas) as steam extruded sinking pellets.

Shrimp nursery

Reception and Acclimation

Litopenaeus vannamei post-larvae were obtained from Ocean Boy Hatcheries of Clearwater, Florida. Eight day old post larvae (PL_8) were received with an initial mean weight of 0.003 g ± 0.001 g. The cargo was transported by truck in 20 L plastic transport bags. Upon arrival, temperature, dissolved oxygen (DO), pH, total ammonia-nitrogen (TAN), and salinity were measured in four randomly selected transport bags. The PL were then acclimated in an acclimation tank so that the water in the plastic transport bags slowly acclimated to the temperature and salinity of the nursery system. Average temperature in the plastic transport bags was 21.2 C and the nursery temperature average was 22.4 C. Temperature acclimation did not exceed a change of 4 C per hour. Once the temperature difference was less than one C, then water from the acclimation tank was slowly added into each transportation bag to allow for salinity acclimation. Average salinity in the plastic transport bags was 30.2 ppt and the average salinity in the nursery system was 30.1 ppt. Salinity acclimation did not exceed a change of four ppt salinity per hour.

Nursery System

The nursery system was a semi-closed recirculating system containing six culture tanks (3,600 L), biological filter (3,600 L), sand filter, and a one-horsepower (hp) Purex Triton II 140 circulation pump (Aquatic Eco-systems, Apopka, Florida, USA). Supplemental aeration was provided by a Sweetwater® common regenerative blower through six airlifts installed along the sides of each tank.

Determining PL numbers and weights

Once acclimated, the PL were drained from the acclimation tank and concentrated to a 57 L tank. Continuous hand mixing provided a homogenous mixture of the PL for sub-sampling. Six sub-samples for each concentration of the PL were taken using a 70 mL beaker (total volume) and PL from each sample were hand counted to estimate the number of PL per unit volume. Coefficient of variation was determined by multiplying 100 to the quotient of the standard deviation and mean of the samples. The sub-sample was considered adequate as long as the coefficient of variation was less than 15%. After determining the number of PL per unit volume, the PL were stocked at equal densities into six nursery tanks (3.0 x 1.5 x 0.8 m; 3,600 L total volume) at a density of 52 PL/L. The average weight and weight distribution of shrimp at stocking was determined by a composite sample of 60 PL. Each PL was individually dried on a paper towel and weighed using an analytical balance. Average weights of the shrimp thereafter were determined every three days by random sampling of least 30 PL from each nursery tank. Prior to weighing, the PL were dried on a paper towel and weighed to the nearest 0.1 mg.

Nursery Feeding

PL were fed four times a day (Table1) based on the biomass and estimated population; feeding rates were adjusted every three days until the end of the nursery phase (21-d). Biomass was calculated by the product of expected survival and average weight of the individuals in the population. Feeding rates were initially set at 25% of the body weight and gradually reduced to 10% at the end of the nursery phase.

Nursery system management and water quality

One month before stocking of the culture tanks, the biological filter was seeded and fed urea so that there was a population of nitrous-feeding bacteria. A week prior to the reception of the PL shipment, culture tanks were filled with filtered seawater which was disinfected by chlorination and de-chlorination methods. Utensils needed in handling the PL were also immersed into the chlorinated water for disinfection. The biological filter was not included in the chlorination and de-chlorination process.

Water volumes were maintained by an internal 12.7 cm diameter pipe, 80 cm in length, and nested within a screen (250 or 450 μm mesh) that was cleaned daily in order to avoid clogging. The biological filter was composed of four partitions of filter media (1.3 x 0.7 m) placed perpendicular in the tank. Biological filter material, 2.54 cm diameter bio-spheres, from Aquatic Eco-Systems of Apopka, Florida, was placed in between the filter media. The sand filter was cleaned by backwashing every three days during the nursery period. The nursery system was on a minimal water exchange policy. Accumulated waste and feed were siphoned as needed. During the last four days of the nursery phase, freshwater was added to the system to acclimate the juveniles to the average salinity of the production ponds.

Temperature, dissolved oxygen, salinity and pH and were measured twice a day (Table 2) in each tank using an YSI 556 MPS meter (Yellow Spring Instruments Co., Yellow Springs, OH, USA). Water samples were taken for total ammonia-nitrogen (TAN) readings every three days and measured with a spectrophotometer (Spectronic Instrument Inc., Rochester, NY, USA) by the Nesslerization method (APHA, 1989).

Nursery harvest

At the end of the 21-d nursery phase, the water level was reduced to approximately 20 cm and PL were captured in hand nets. To calculate average weight, five sub-samples of 300-500 shrimp were taken from each tank. Each sub-sample was weighed (0.01 g) using an Ohaus Scout Pro balance (Ohaus Balances and Scales, Pine Brook, NJ, USA) and then hand counted. After the number of shrimp per unit of weight was determined, shrimp were distributed homogeneously into 16 holding buckets, each bucket representing a growout pond. Final average weight, survival, feed conversion ratio (FCR= feed input / yield), and total biomass were determined for each nursery tank (Table 3).

Table 1. Feeding rates as percent biomass and feed type utilized through a 21-d nursery period for *L. vannamei*, post-larvae. Feed inputs were based on mean shrimp weight determined every three days and an assumed 78% survival.

Day	Mean Weight (mg)	Feed (% body weight)	Feed Type	Feed Ratio
1	2.98	25	Artemia / PL redi[a]	1 / 1
2	2.98	25	Artemia / PL redi	1 / 1
3	2.98	25	Artemia / PL redi	1 / 1
4	4.77	25	PL redi / Crumble #0[b]	1 / 1
5	4.77	25	PL redi / Crumble #0	1 / 1
6	4.77	25	PL redi / Crumble #0	1 / 1
7	7.55	25	PL redi / Crumble #0	1 / 3
8	7.55	25	Crumble #0	1
9	7.55	25	Crumble #0	1
10	14.05	15	Crumble #0 / Crumble #1[b]	1 / 1
11	14.05	15	Crumble #0 / Crumble #1	1 / 1
12	14.05	15	Crumble #0 / Crumble #1	1 / 2
13	15.71	15	Crumble #1	1
14	15.71	15	Crumble #1	1
15	15.71	15	Crumble #1	1
16	26.80	15	Crumble #1 / Crumble #3[b]	3 / 1
17	26.80	15	Crumble #1 / Crumble #3	3 / 1
18	26.80	15	Crumble #1 / Crumble #3	3 / 1
19	43.41	15	Crumble #1 / Crumble #3	3 / 1
20	43.41	15	Crumble #1 / Crumble #3	3 / 1

[a] PL Redi-reserve 400-600 microns. 50% Protein, produced by Zeigler Bros, Inc., Gardners, PA, USA

[b] Crumble feed, 45% protein, produced by Rangen Inc., Buhl, Idaho, USA

Table 2. Water quality parameters observed over a 21-d nursery period for *L. vannamei*, stocked at a density of 52 PL/L in a recirculating system composed of six, 3600-L nursery tanks.

	Average	Minimum	Maximum	Standard Deviation	CV[a]
Temperature (°C)	27.58	22.4	30.5	1.95	7.06
DO (mg/L)[b]	5.97	4.01	7.19	0.59	9.79
Salinity (ppt)	25.85	12.74	31.5	7.16	27.70
pH	7.24	6.46	8.14	0.45	6.16
TAN (mg/L)[c]	0.72	<0.10	1.42	0.50	69.09

[a] CV = Standard deviation / mean x 100

[b] Dissolved Oxygen.

[c] Total Ammonium Nitrogen

Table 3. Production parameters of *L. vannamei,* nursed for 21-d and stocked at a density of 52 PL/L in a recirculating system composed of six, 3600-L nursery tanks.

	Mean	Minimum	Maximum	Standard Deviation	CV[a]
Final Weight (mg)	29.6	24.2	35.0	4.7	15.85
Weight Gain [b] (mg)	26.6	21.2	32.0	4.7	17.63
Survival (%)	71.9	56.9	83.3	8.66	12.04
FCR[c]	1.28	1.06	1.82	0.28	21.64
Yield (g)	2,679	1,872	3,095	435.39	16.25
Standing Crop (kg/m^3)[d]	0.74	0.52	0.86	0.12	16.25

[a] CV = Standard deviation / mean x 100

[b] Weight Gain = Final Weight - Initial Weight

[c] Food conversion ratio = Feed offered per shrimp / weight gain per shrimp.

[d] Standing Crop = (Yield / 1,000) / 3.6 m^3

Pond Growout

At the conclusion of the nursery phase, juveniles were pooled and stocked into 16 grow-out ponds at a density of 34 shrimp/m^2.

Pond preparation

Each pond was 0.1 ha in surface area, with an average depth of one meter. Ponds were equipped with a 20 cm diameter standpipe, a concrete catch basin (3.0 x 2.0 x 0.5 m), and lined with 1.52 mm thick high-density polyethylene lining (HDPE) covered with a 25 cm deep layer of sandy-loam soil. Prior to filling, pond soils were dried and tilled to allow for oxidation and mineralization of organic matter.

Three weeks prior to stocking, the ponds were filled with brackish water (15-20 ppt) from the intra-coastal canal between Mobile and Perdido Bay. Intake water was filtered through a three foot 250 µm-mesh nylon filter sock to prevent the introduction of large predators and minimize the introduction of larval fish, shrimp and crabs, while allowing the introduction of small planktonic organisms. All ponds were fertilized with a combination of inorganic liquid fertilizers (10-34-0 / 32-0-0) applied at a ratio of 1:2 ($N:P_2O_5$) to provide four kg/ha nitrogen. A mixture of 1.68 L of 10-34-0 and 402 mL of 32-0-0 with pond water was prepared and slowly dripped into the ponds. No water exchanges occurred within one week prior to stocking.

Pond management and water quality

In each pond, temperature, dissolved oxygen, salinity, and pH was measured three times a day, at sunrise (5:00), noon, and at night (20:00) using an YSI 556MPS meter. Secchi disk readings were taken on a weekly basis. Water samples for TAN readings were taken at 40 cm from the pond surface and measured with a spectrophotometer according to previously described methods. A zero water exchange was applied except in cases of severe drought. In order to maintain a minimum DO level of three mg/L, each pond was provided with a base aeration capacity of 10 hp/ha (one hp/pond) and 10 to 20 additional hp/ha for emergency aeration throughout the grow-out phase. Paddlewheel and Aire-O_2 aerators (Aeration Industries International Incorporated of Minneapolis, Minnesota, USA) were used for this purpose. Each pond had a Big John paddlewheel (Big John Aerators, Quinton, Alabama, USA) powered by a one hp Weg motor (model D56C). The D56C was wired for a voltage of 220 V and had the following specs: 1,750 RPM, 115 V, 60 Hz, 14 A, and a capacitance of 540-648 μF. David Brown gearboxes with a gear ratio of 14/1 were utilized for the paddlewheels. Big John paddlewheels, Weg motors and David Brown gearboxes were purchased from Big John Aerators of Quinton, Alabama. The Aire-O_2 aerators were used for additional aeration and were applied only as needed. The Aire-O_2 aerators were powered by a Reliance Electric two hp Duty Master A-C motor. Its specs included: 3,450 RPM, 115 V, 20 A, and 60 Hz (Reliance Electric Industrial Company, Cleveland, Ohio, USA, 2007). All other Aire-O_2 parts, such as bearings, impellers, floats, shafts, and mounting plates were purchased from Aeration Industries International Incorporated of Minneapolis, Minnesota, USA. Each paddlewheel and Aire-O_2 was secured into position with quarter-inch, single braided nylon rope (Delta Net and Twine Co., Greenville, MI, USA) secured to 0.635 cm (¼ in. diameter) rebar stakes, 40 cm in length, placed along the sides each pond.

Grow-out Feeds and Feeding

Shrimp were fed twice daily, in the morning and late afternoon, following a predetermined feeding protocol. During the 1st, 2nd, 3rd, and 4th weeks the ponds were fed at fixed rates of 10, 15, 30, and 60 kg of feed/ha/d. Thereafter, feed amounts were calculated based on an expected weight gain of 1.5 g/wk, a feed conversion of 1.2:1, and a survival of 70% throughout the grow-out phase.

During the initial two weeks of pond culture, a commercial shrimp diet (Rangen Shrimp Grower, 35% protein, 8% lipid) was provided and the experimental diets were fed starting the third week of the grow-out phase when shrimp had reached an average weight of 2.3 g. Maximum feeding rates were set at 71.0 kg of feed/ha/d during the fifth week. Test diets (Table 4) were formulated to provide equal protein (35%) and lipid (8%) levels while maintaining required levels of lysine and total sulfur amino acids. Lipid levels were adjusted by adding menhaden fish oil. Soybean meal was used in combination with distilled corn to replace poultry by-product meal. Calcium phosphate was added to ensure an adequate supply of phosphorus. The objective of the four test diets was to reduce or eliminate the inclusion of poultry by-product meal in the basal formulation. As soybean meal levels were increased in the diet, additional squid liver was used to serve as an attractant. The diets were steam extruded sinking pellets produced by Rangen Incorporated (Angleton, Texas) under commercial feed manufacturing conditions.

Weekly sampling of the shrimp using seine or cast net was done for each pond in order to monitor weight. Sampling was done in the early morning to reduce stress on the shrimp.

Table 4. Ingredient composition of practical diets for *L. vannamei*, used to evaluate the replacement of animal proteins with plant protein sources (g /100 g as is).

Ingredient	15% PBM[a]	10 % PBM	5% PBM	0% PBM	Ecosafe[b]
Soybean meal	40.85	46.54	52.32	58.02	39.44
Sorghum	29.83	25.18	19.99	14.85	-
Poultry By-Product Meal	15.01	10.01	5.00	-	12.00
Feed Wheat	-	-	-	-	30.16
Corn Gluten	4.84	4.84	4.83	4.83	8.00
CFS Corn Distill	-	3.34	6.66	10.00	-
Soy Oil	-	-	-	-	3.68
Menhaden Fish Oil (Mixer)	2.22	2.59	2.97	3.32	-
Menhaden Fish Oil (Spray)	2.50	2.50	2.50	2.50	-
ABN Premix[c]	-	-	-	-	2.00
CaP-diebasic	2.65	2.90	3.13	3.38	1.92
Aqua-Bond	-	-	-	-	1.38
Bentonite	1.50	1.50	1.50	1.50	-
Squid Liver	-	-	0.50	1.00	-
Mold Inhibitor	0.15	0.15	0.15	0.15	-
Fed Vit #30: 2-95 (Ziegler)	-	-	-	-	0.50
Vitamin Premix	0.33	0.33	0.33	0.33	-
Mineral Premix	0.09	0.09	0.09	0.09	-
Aqua-Min (Zeigler)	-	-	-	-	0.15
Lecithin	-	-	-	-	0.50
Choline	-	-	-	-	0.20
Stay-C 35%	0.02	0.02	0.02	0.02	0.07
Copper Sulfate	0.01	0.01	0.01	0.01	-

[a] Percent poultry by-product meal in the diet.

[b] Plant-based diet in combination with PBM substituting menhaden fish oil with soy oil.

c ABN Premix designed to deliver essential fatty acids.

Table 5. Nutritional composition of practical diets and commercial reference formulated to contain 35% protein for *L. vannamei*, used to evaluate the replacement of animal proteins with plant protein sources[a].

Ingredient	15% PBM[b]	10% PBM	5% PBM	0% PBM	Ecosafe[c]	Reference[d]
Moisture	7.91	9.15	10.08	9.73	9.22	9.65
Protein	36.6	35.5	35.6	36.8	41.4[e]	36.9
Fat	8.39	8.66	8.04	8.12	6.22	7.65
Fiber	1.50	1.52	1.68	1.86	2.27	2.42
Ash	8.74	8.98	8.65	8.69	6.72	9.29

[a] Analyzed values from New Jersey Feed Laboratory Inc., Trenton, NJ (2006).

[b] Percent poultry by-product meal in the diet.

[c] Plant-based diet in combination with PBM substituting menhaden fish oil with soy oil.

[d] Rangen 35% protein shrimp production diet.

[e] Due to nitrogen in the binding agent of Ecosafe, protein content appears elevated 5%.

Harvesting

Feeding ceased 36 hours prior to harvest. Harvest took place September 27-29, 2006 (after 115-117 days of pond culture). Harvesting was accomplished by draining two thirds of the water from each pond the night prior to harvest. Aeration was provided throughout the night. On the day of harvest, the remaining water was drained and shrimp were pumped from the catch basin using a hydraulic fish pump (Aqualife-Life pump, Magic Valley Heli-arc and Mfg, Twin Falls, Idaho, USA), dewatered, and collected into a transport truck. Following harvest, the shrimp were cleaned and weighed. During the weighing, a random sample of 150 shrimp from each pond was collected to measure individual weights. These individual weights were used to further calculate mean individual weights, yields, survivals and size distributions of the shrimp.

Tank Trial

Shrimp and experimental units

Juvenile *L. vannamei* were obtained from 16 shrimp production ponds located at the Claude Peteet Mariculture Center in Gulf Shores, Alabama. Shrimp with a mean weight of 2.32 g $\pm$ 0.02 g were stocked at a density of 37.5 shrimp/m^2 (30 shrimp/tank) in an outdoor recirculating system. This system contained 24 circular polyethylene tanks (0.85 m height x 1.22 m upper diameter, 1.04 m lower diameter) designed to hold 800 L of water, as well as an 800 L reservoir tank, biological filter, circulation pump and supplemental aeration.

Before stocking, the system was filled with brackish green water (14-16 ppt) to mimic production pond settings. The system's make up water was exchanged daily between 4:00 AM and 12:00 PM. Pond water was pumped into the central filter at a rate of eight L/min. This exchange rate allowed a 100% water exchange of the system's make up water every six days. Aeration was provided in the filter and in each tank by two air stones connected to a common air supply from a one hp regenerative blower.

Water Quality

During the experimental period, DO, temperature, salinity and pH values were measured every morning and afternoon in the biological filter. Each week, water samples were taken from the biological filter and two randomly selected tanks for TAN readings measured as previously described.

Feeds and Feeding

Six test diets (Table 4) were fed amongst the 24 tanks (4 replicates). Four of these diets tested the replacement of poultry by-product meal with soybean meal. A fifth diet (Ecosafe) used soybean meal in combination with poultry by-product meal and feed wheat. The Ecosafe diet also substituted soy oil in combination with alternative oil meals for menhaden fish oil. The sixth diet was a commercial diet used as a reference (Rangen Shrimp Grower, 35% protein, 8% lipid). Nutrient composition for these diets is presented in Table 5. Feed amount was calculated using an expected growth (g/wk) of 1.5 g/wk and expected FCR of 1.2.

Data Analysis

All statistical analyses were performed using SAS (V9.1., SAS Institute, Cary, NC, USA). One-way analysis of variance was utilized to determine significant differences ($P<0.05$) in survival, final weight, yields, feed conversion ratio (FCR). Dunnett's t-test (Steel and Torrie, 1980) was used to compare the experimental treatments to the control.

RESULTS

Pond Growout

Prior to stocking juvenile shrimp into the grow-out ponds, Sechii disc readings varied between 40-110 cm. This is largely due to fertilizing two weeks prior to stocking, rather than one week as in the last year study (Amaya et al., 2007a). By the fourth week after stocking, plankton blooms were evident in every pond and the mean Sechii disc reading was 40 cm. Fluctuations in weekly Sechii disc readings were due to plankton bloom and die-offs in each pond. After a plankton die-off, it would take roughly two weeks for the pond to reach a Sechii disk reading less than 50 cm.

Daily feed inputs (Figure 1) changed on a weekly basis to meet the feed requirements necessary for optimum growth. On some days, ponds were fed half or none of the planned feeding due to severe thunderstorms, high winds, or heavy rain conditions. Weekly feed inputs are presented in Table 6.

Water quality parameters observed throughout the 17-wk grow-out phase were suitable for growth and survival of the shrimp (Table 7). Low DO readings (<2.5) were occasionally observed throughout the study. Dissolved oxygen and TAN levels were associated with plankton bloom and die-offs. As a plankton die-off occurred, oxygen demand increased, which decreased the amount of available oxygen, and TAN increased.

At the conclusion of the 17-wk culture period, there were no significant differences in the production parameters measured related to diet treatment (final weight, yield, weekly growth, FCR, and survival) (Table 9). Mean final weight of all treatments ranged between 21.9 - 24.2 g, with treatment 4 (0% PBM) having the numerically largest mean final weight and treatment 3 (5% PBM) with the lowest. Final yield of all treatments ranged between 6,093 - 6,943 kg/ha (Table 10). Feed conversion ratio (FCR) of all treatments ranged between 0.9 - 1.1. Survival of all treatments ranged between 78.9 - 82.2%. Treatment 1 (15% PBM) had the lowest survival of the four dietary treatments and treatment 2 (10% PBM) had the highest.

Percent distribution of shrimp by count size of head-on shrimp per pound for all treatments is also shown in Figure 2. In all treatments, about 50% of the population was 16-20 count and about 30% of the population was 21-25 count.

Tank Trial

Water quality parameters of the 79-d trial were suitable for growth and survival of shrimp (Table 11). There were no significant differences in production values between the four treatments and the Ecosafe diet (Table 12). Final weight, weekly weight gain, FCR, and survival among treatments ranged between 19.4-22.6 g, 1.5-1.8 g/wk, 1.0-1.2, and 90.8-96.7% respectively (Table 13). The reference diet had significantly higher values in mean final weight and weekly weight gain, and the lowest values for FCR and survival. The Ecosafe diet was lowest in final mean weight and weekly weight gain. Treatments 3 and 4, as well as the Ecosafe had the highest survival values. However, when compared to the productivity parameters of the reference diet, there were significant differences in final mean weight, weekly weight gain (g/wk), and FCR. There is generally a linear relationship between final weight and weekly weight gain. Therefore the largest shrimp will most likely have the greatest average weekly growth on average. The low survival percentage of the reference diet allowed the shrimp to gain more weight on average than the rest of the treatments because of the decreased stocking density in the tanks. The variability in survival amongst the tanks was greater for the reference diet than any other treatment, varying from 80–100%. There was no statistical difference in mean final yield among all treatments.

Figure 1. Daily feed inputs (kg/ha/d) for *L. vannamei*, reared in ponds at a density of 34 shrimp/m^2 over a 17-wk growing period and fed four practical diets with varying levels of PBM and plant protein sources.

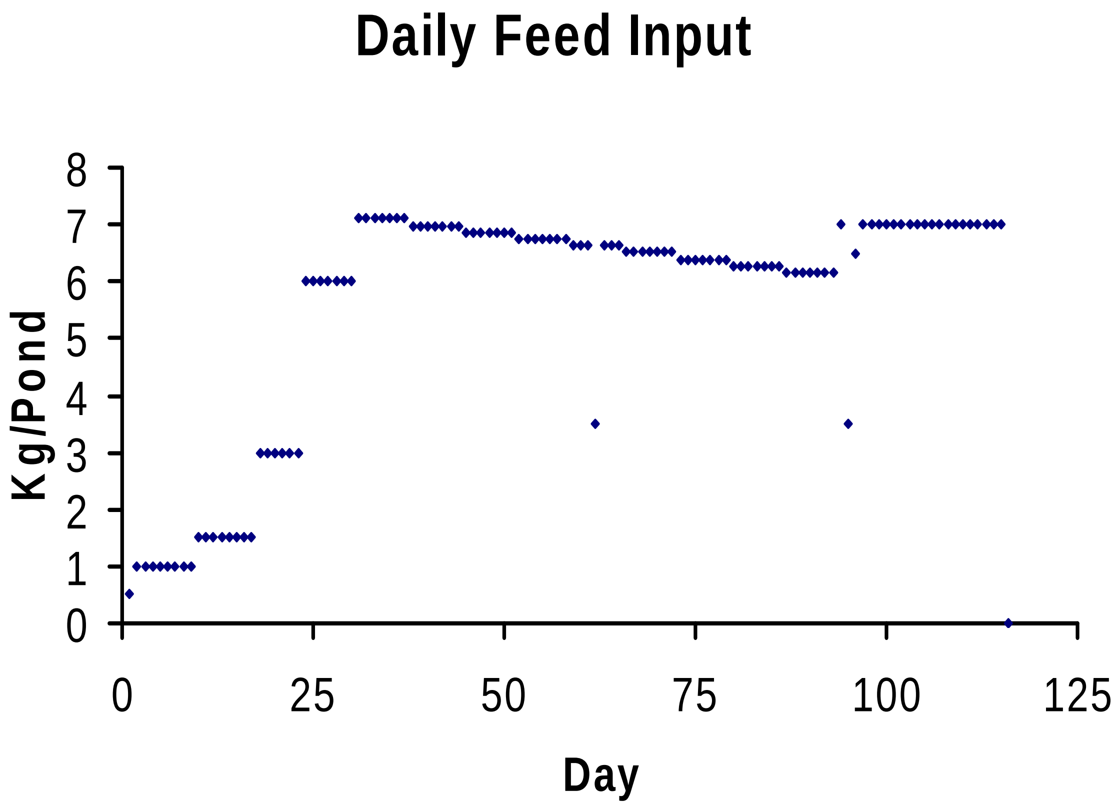


Table 6. Weekly feed inputs per pond for *L. vannamei* raised in 0.1 ha ponds at a density of 34 shrimp/m^2 for a 17-wk growing period and fed four practical diets with varying levels of PBM and plant protein sources.

			Shrimp		Feed Input-Kg	
Day	Date	Week	Population[a]	Weight (g)	Daily	Weekly
1	6/6/2006	0	34000	0.03	1.00	7.00
8	6/13/2006	1	33469	0.32	1.50	10.50
15	6/20/2006	2	32938	2.30	3.00	21.00
22	6/27/2006	3	32406	3.42	6.00	43.00
29	7/4/2006	4	31875	5.02	7.10	49.70
36	7/11/2006	5	31344	6.64	6.99	48.93
43	7/18/2006	6	30813	8.81	6.87	48.09
50	7/25/2006	7	30281	12.04	6.75	47.25
57	8/1/2006	8	29750	13.60	6.63	46.41
64	8/8/2006	9	29219	15.46	6.51	45.57
71	8/15/2006	10	28688	17.50	6.39	44.73
78	8/22/2006	11	28156	18.24	6.27	43.89
85	8/29/2006	12	27625	19.39	6.16	43.12
92	9/5/2006	13	27094	20.90	7.00	49.00
99	9/12/2006	14	26563	22.14	7.00	49.00
106	9/19/2006	15	26031	23.22	7.00	49.00

[a] Estimated population.

Table 7. Summary of water quality parameters observed over a 17-wk growing period for *L. vannamei,* fed four practical diets with varying levels of PBM and cultured in 0.1 ha ponds. Values are mean ± standard deviation of daily and weekly determinations. Values in parenthesis represent minimum and maximum readings.

Parameter	15% PBM	10% PBM	5% PBM	0% PBM	P Value
Temperature (C)					
am	28.49 ±1.72 (19.24, 32.14)	28.66 ±1.62 (22.08, 32.06)	28.53 ±1.67 (21.22, 32.12)	28.68 ±1.64 (22.12, 32.52)	0.7099
noon	30.95 ±1.64 (26.57, 34.41)	31.11 ±1.67 (26.66, 34.49)	30.92 ±1.63 (26.53, 34.34)	31.20 ±1.67 (26.87, 35.48)	0.5013
pm	30.34 ±1.64 (25.26, 34.09)	30.54 ±1.64 (26.19, 34.21)	30.30 ±1.63 (26.13, 33.81)	30.59 ±1.65 (26.01 35.00)	0.5275
DO (mg/L)[a]					
am	4.15 ±1.24 (0.55, 8.31)	4.12 ±1.25 (1.29, 8.39)	4.16 ±1.22 (1.09, 8.25)	4.24 ±1.23 (0.44, 9.15)	0.8488
noon	13.26 ±3.79 (4.53, 23.98)	13.59 ±3.81 (1.09, 24.77)	13.1 ±3.36 (1.68, 21.97)	13.50 ±4.0 (0.64, 26.32)	0.7693
pm	9.80 ±3.22 (0.94, 18.07)	9.93 ±3.12 (2.14, 18.04)	9.82 ±2.98 (0.23, 17.37)	9.82 ±3.38 (2.67, 21.74)	0.9753
Readings <2.5	31	24	19	15	
pH					
am	8.03 ±0.71 (5.66, 9.34)	7.92 ±0.68 (5.69, 9.42)	7.98 ±0.75 (5.62, 9.39)	7.93 ±0.65 (5.54, 9.35)	0.3741
noon	8.73 ±0.41 (7.71, 9.77)	8.69 ±0.34 (7.79, 9.53)	8.74 ±0.40 (7.73, 9.89)	8.64 ±0.37 (7.76, 9.73)	0.4260
pm	8.58 ±0.58 (6.10, 9.92)	8.54 ±0.54 (5.79, 10.72)	8.58 ±0.59 (5.78, 10.10)	8.49 ±0.58 (5.87, 9.99)	0.0980
Salinity (g/L)					
am	15.23 ±1.61 (12.02, 18.73)	15.31 ±1.93 (11.41, 19.21)	15.87 ±1.64 (12.15, 19.69)	16.20 ±1.62 (11.55, 18.75)	0.6370
noon	15.25 ±1.58 (12.18, 18.87)	15.40 ±1.92 (11.44, 19.12)	16.24 ±1.62 (12.28, 19.72)	15.92 ±1.59 (11.72, 18.76)	0.6285
pm	15.21 ±1.60 (11.90, 18.67)	15.33 ±1.93 (11.29, 19.15)	16.16 ±1.67 (11.77, 19.67)	15.87 ±1.80 (11.39,18.74)	0.6499
Sechii	41.190 ±32.69 (15, 110)	37.813 ±28.32 (15, 110)	40.234 ±31.88 (15, 110)	37.813 ±29.52 (10, 110)	0.4283
TAN (mg/L)[b]	1.0314 ±1.30 (0.00, 4.99)	1.1492 ±1.80 (0.00, 2.12)	0.9454 ±2.04 (0.00, 13.07)	1.2219 ±1.88 (0.00, 8.91)	0.8462

[a] Dissolved oxygen.

[b] Total ammonia nitrogen.

Table 8. Cast net sampling mean weekly weight gains (g) of *L. vannamei*, cultured in 0.1 ha ponds and fed four practical diets with varying levels of PBM and plant protein sources throughout the 17-wk culture period.

Week	15% PBM	10% PBM	5% PBM	0% PBM	Average
0	0.03	0.03	0.03	0.03	0.03
1	0.32	0.32	0.32	0.32	0.32
2	2.37	2.29	2.39	2.17	2.30
3	3.60	3.44	3.41	3.22	3.42
4	4.91	4.97	5.01	5.17	5.02
5	6.83	7.36	5.85	6.52	6.64
6	8.64	9.07	8.52	9.00	8.81
7	12.44	12.16	11.57	12.00	12.04
8	13.74	14.48	12.47	13.68	13.60
9	15.73	16.17	14.09	15.86	15.46
10	18.22	17.97	16.36	17.44	17.50
11	19.20	18.49	16.03	19.23	18.24
12	20.58	19.49	17.63	19.86	19.39
13	21.73	20.30	19.23	22.34	20.90
14	23.00	21.48	21.10	22.97	22.14
15	24.46	22.28	22.17	23.98	23.22
16	24.92	23.14	22.49	25.25	23.95
Final	23.92	21.99	21.91	23.13	22.74

Figure 2. Cast net sampling mean weekly weight gains (g) of *L. vannamei*, cultured in 0.1 ha ponds and fed four practical diets with varying levels of PBM and plant protein sources throughout the 17-wk culture period.

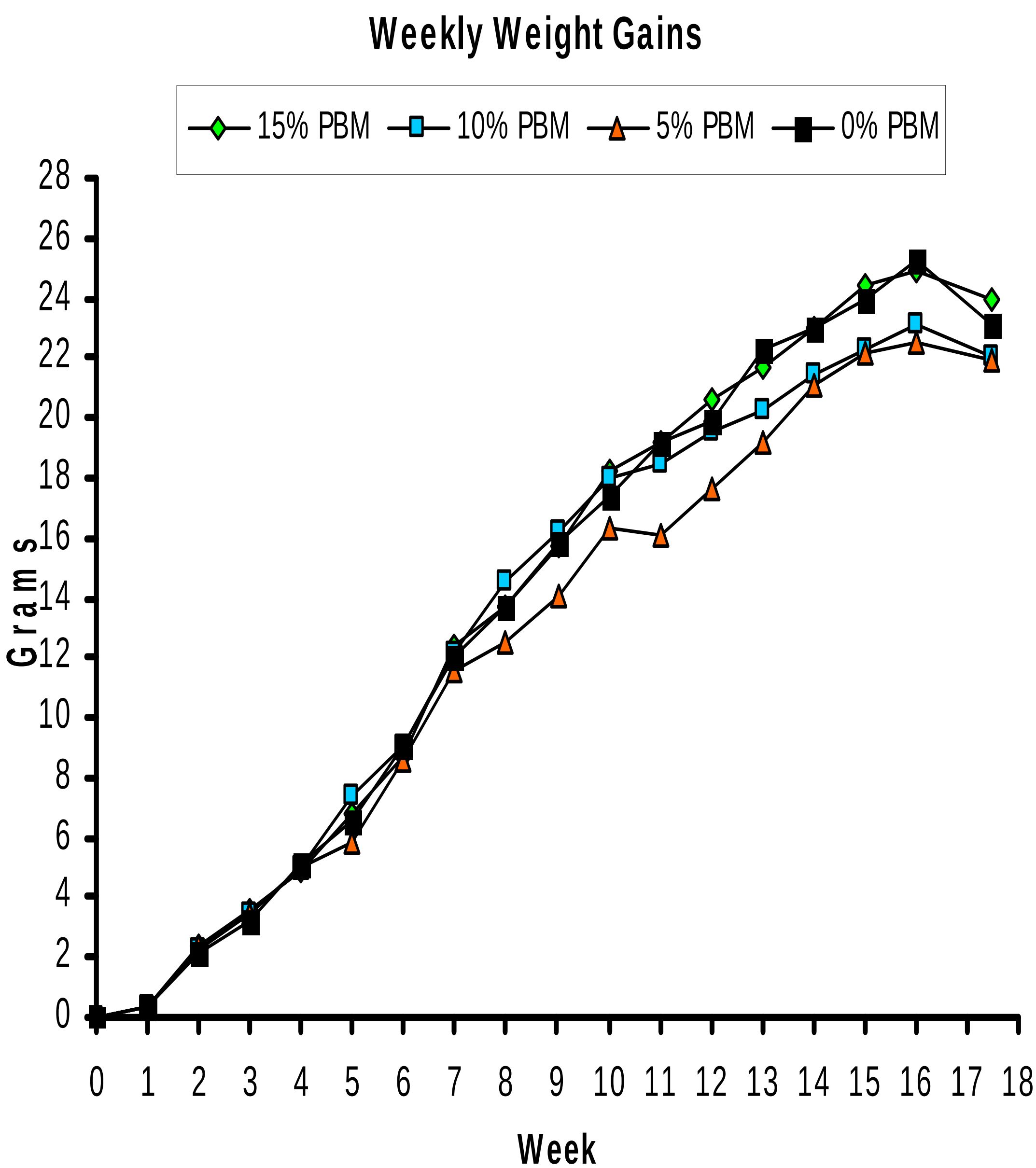


Table 9. Mean production parameters of *L. vannamei,* cultured in 0.1 ha ponds, at the end of a 17-wk culture period and fed four practical diets with varying levels of PBM and plant protein sources[a].

Parameter	Final Weight (g)	Final Yield (kg shrimp/ha)	Weight Gain (g/wk) [b]	FCR[c]	Survival (%)
15% PBM	23.9	6216	1.4	1.05	78.9
10% PBM	22.0	6451	1.3	1.00	82.2
5% PBM	21.9	6093	1.3	1.09	80.1
0% PBM	24.2	6943	1.5	0.94	80.9
PSE[d]	1.44	63.53	0.08	0.09	8.28
P Value	0.5605	0.7901	0.5605	0.7272	0.9337

[a] Based on analysis of variance (ANOVA), no significant differences ($P > 0.05$) were found among treatment means ($n = 4$).

[b] Weekly weight gain = (final weight - initial weight) / weeks of culture period.

[c] FCR, feed conversion ratio = Feed offered per shrimp / weight gain per shrimp.

[d] Pooled standard error of treatment means = $\sqrt{mse/n}$

Figure 3. Size distribution (shrimp count per pound) represented as percent population of *L. vannamei*, fed four practical diets with varying levels of PBM and raised in ponds for a 17-wk period at a density of 34 shrimp/m^2.

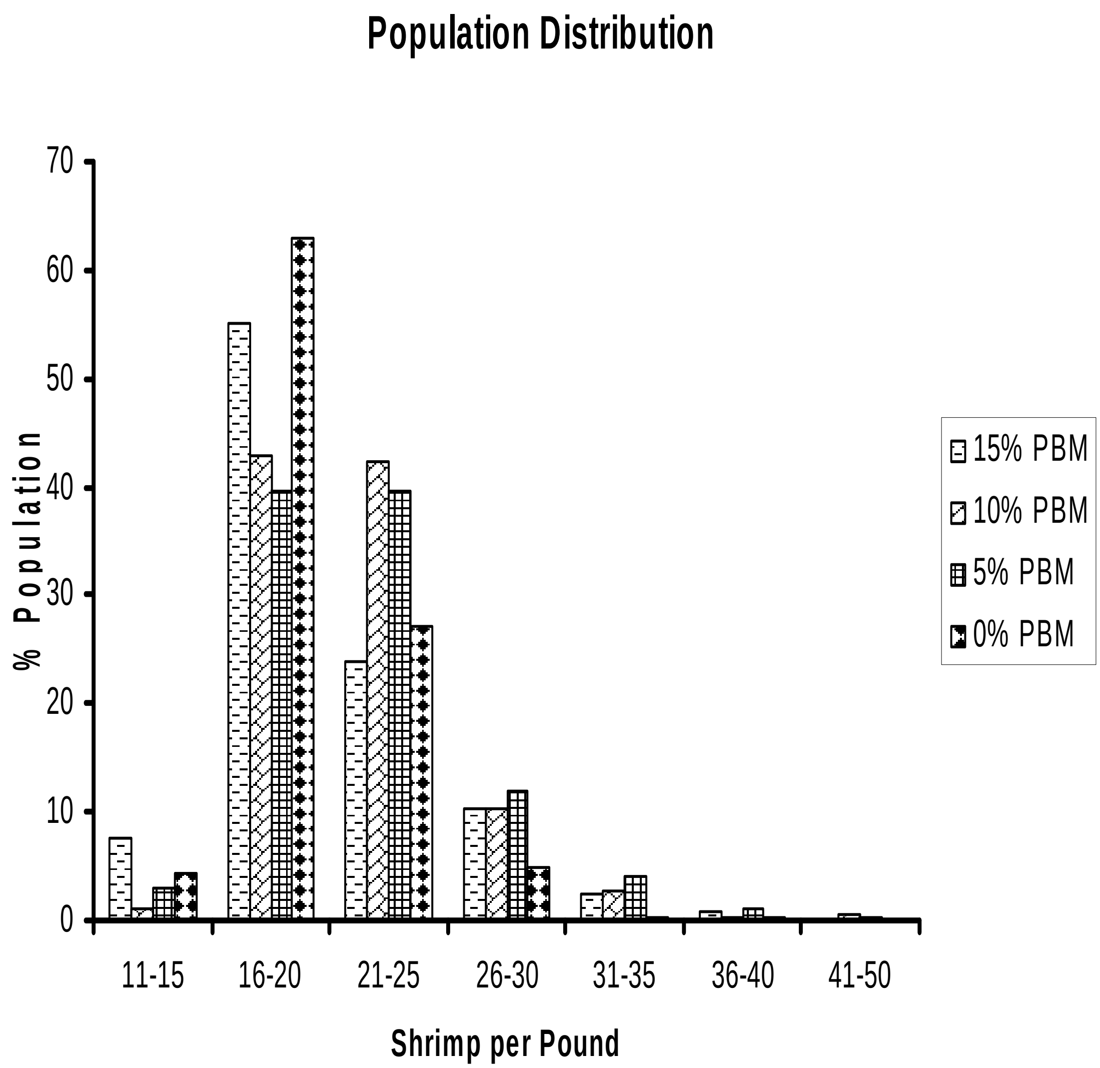


Table 10. Mean yields (kg/ha), by size class distribution of head-on shrimp per pound.

Class[a]	15% PBM	10% PBM	5% PBM	0% PBM
11-15	472	77	183	306
16-20	3,419	2,761	2,413	4,375
21-25	1,492	2,736	2,416	1,888
26-30	634	658	731	333
31-35	149	168	256	28
36-40	50	13	61	14
41-50	0	39	37	0
Gross Total	6,216	6,452	6,097	6,944

[a] Shrimp per pound.

Table 11. Summary of water quality parameters observed over a 79-d experimental period for *Litopenaeus vannamei,* fed practical diets with varying levels of animal and plant protein sources and cultured in an outdoor semi-closed recirculating culture system. Values are mean ± standard deviation of daily and weekly determinations. Values in parenthesis represent minimum and maximum readings.

	Temperature (C)	DO (mg/L)[a]	pH	Salinity (g/L)	TAN (mg/L)[b]
am	27.33 ±1.45 (24.27, 30.90)	6.26 ±0.79 (1.09[c], 7.64)	7.89 ±0.31 (6.00, 8.95)	15.76 ±1.06 (13.70, 18.14)	0.66 ±0.44 (0.00, 1.57)
pm	29.99 ±1.46 (25.85, 33.50)	6.27 ±0.52 (4.37, 7.55)	8.14 ±0.27 (6.23, 9.07)	15.74 ±1.06 (14.13, 18.10)	

[a] Dissolved oxygen.

[b] Total ammonia nitrogen.

[c] Value obtained due to system mechanical error; had no effect in regards of survival.

Table 12. Mean productive parameters at the end of a 79-d culture period for *L. vannamei,* reared in an outdoor semi-closed recirculating culture system and fed practical diets with varying levels of PBM, a plant based diet and a Rangen commercial reference diet[a].

Parameter	Initial Weight (g)	Final weight (g)	Final yield (kg shrimp/tank)	Weight gain (g/wk)[b]	FCR[c]	Survival (%)
15% PBM	2.30	19.9 [y]	0.562	1.56 [y]	1.13 [z]	94.2
10% PBM	2.30	20.3 [y]	0.574	1.60 [y]	1.11 [z]	94.2
5% PBM	2.32	20.1 [y]	0.582	1.58 [y]	1.12 [z]	96.7
0% PBM	2.32	20.5 [y]	0.595	1.61 [y]	1.10 [z]	96.7
Ecosafe	2.34	19.4 [y]	0.564	1.51 [y]	1.18 [z]	96.7
Reference	2.33	22.6 [z]	0.616	1.80 [z]	0.99 [y]	90.8
PSE[d]	0.01	0.24	0.02	0.02	0.02	2.23
P Value	0.3297	0.0001	0.4962	0.0001	0.0001	0.614

[a] Based on analysis of variance (ANOVA), significant differences ($P < 0.05$) were found among treatment means ($n = 4$).

[b] Weekly weight gain = (final weight - initial weight) / weeks of culture period.

[c] FCR, feed conversion ratio = Feed offered per shrimp / weight gain per shrimp.

[d] Pooled standard error of treatment means = $\sqrt{mse/n}$

DISCUSSION

Replacing animal protein meals with plant proteins in formulated diets may reduce the dependence on the animal protein industry and provides alternative choices when formulating feeds. The replacement of poultry by-product meal (PBM) using a combination of soybean meal and corn gluten meal in feeds formulated for *Litopenaeus vannamei* can expand the array of feedstuffs as evidenced by the results of this study. Depending on local market value, replacing PBM with plant proteins should reduce the cost of formulated feeds for *L. vannamei*.

Research conditions for the pond study were suitable for good survival and growth. The standardized methods utilized were similar to those of a commercial farm. Meteorological conditions were optimum, as there were no hurricanes or tropical storms. Results of the production pond study demonstrate that there are no significant differences in mean final weight, yield, weekly gain, FCR, and survival amongst the four experimental diets for the juvenile Pacific white shrimp (*L. vannamei*). Hence, there was no difference in performance as PBM was replaced with soybean meal and corn gluten meal in practical diets for juvenile Pacific white shrimp reared under pond production conditions. The FCR results were particularly interesting as they were noticeably quite low (0.9 – 1.1) when compared to the expected (1.2).

An FCR below one is of great interest because the shrimp are utilizing additional nutrients from natural productivity and not just the feed. Natural productivity is an excellent food source of nutrients and minimal requirements must be identified to maximize potential productive gains (Davis et al., 2006). Lawrence and Lee (1997) found that natural productivity accounted for more than 25% of the natural intake of shrimp (Venero et al., 2007). Anderson et al. (1987) estimated that the contribution of feed to production of *L. vannamei* at 5 mt/ha/crop was only between 23-47%, and at the same production level, Lawrence and Houston (1993) estimated that value to be between 24 to 31%. The low FCR observed is also a testament of a well managed feeding protocol as well as the ability of this species to utilize natural food items.

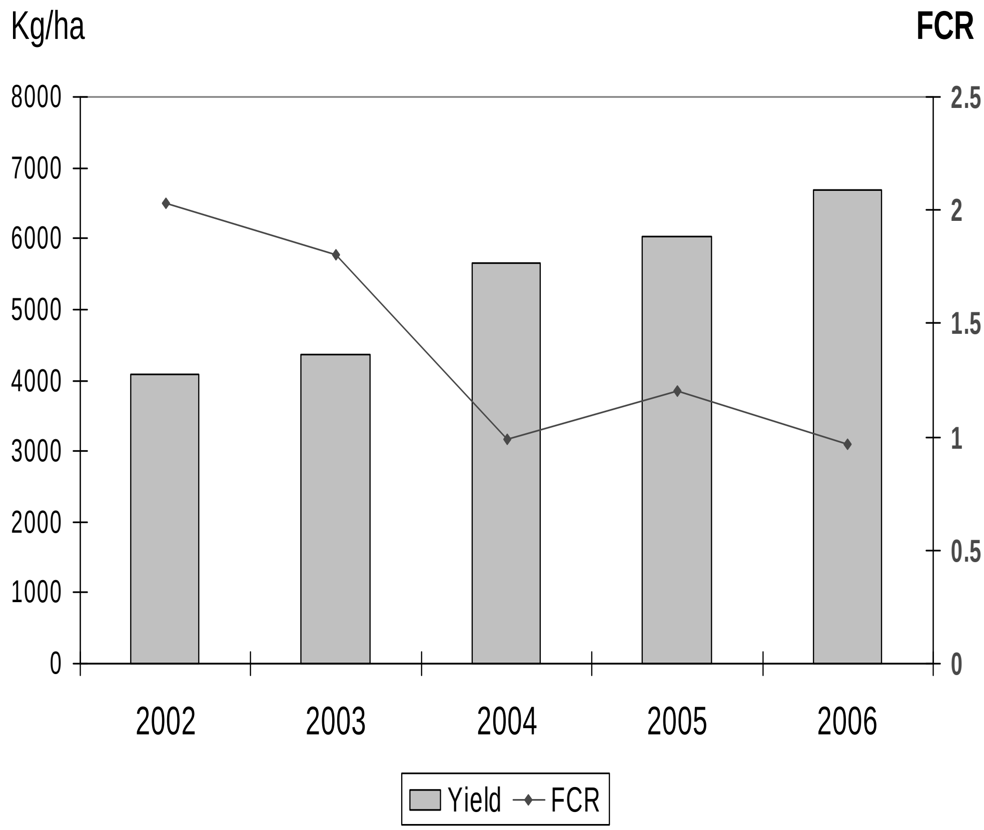
The use of historical data with the knowledge and understanding of the production ponds' nutrient availability aided in the development of a more conservative feeding protocol. Garza et al. (2001) compared juvenile *L. vannamei* growth between a traditional feeding table and a fixed FCR in 0.1 ha green water ponds over a 16-wk period stocked at 35 shrimp/m^2. There where no significant differences in growth or FCR. A FCR of 2.03 was obtained from both treatments. Unfortunately, most farmers encourage higher feed inputs to "load nutrients" into production ponds as they believe it will lead to higher yields. However, it is well known that over feeding leads to increased pollution loading of the system and the feed inputs must be matched to nutrient requirements. For example, it has been found that in semi-intensive systems, increasing the nutrient density of the diet in combination with reducing feed inputs, will allow for reductions in the feed inputs without affecting growth or feed efficiency (Venero et al., 2007). Zelaya et al. (2005) utilized a high protein diet (40% crude protein) to reduce feed inputs and FCR. For this type of system a maximum daily feed load of 100-120 kg/ha/d has been recommended when night-time mechanical aeration is provided (Boyd, 1989; Boyd and Tucker, 1998; Venero et al., 2007). The maximum feed input in this research was 71 kg/ha/d, which was below the maximum feed load capacity recommended for this type of system. Venero et al. (2007) suggests that lower feed levels in semi-intensive systems might improve water quality, thus improving survival and production. We have found that with well formulated feeds, proper pond ecosystem management, and the use of historical productive trends, a more conservative feeding protocol results in increased production parameters with systematic improvements in yield as well as reductions in FCR (Table 13) (Garza et al., 2004; Zelaya et al., 2007; Venero et al., 2007; Amaya et al., 2007a).

The pond study results were also supported by the tank trial results, as there were no significant differences among the four PBM replacement diets and the Ecosafe diet. However, under reported conditions, the commercial reference diet did result in significantly larger shrimp than those reared on other treatments. The significantly higher weight may be in part to the reduced survival which could have lead to enhanced growth due to reduced density. The mean final survival of the commercial diet had a range of ten percent throughout the 79 day growth trial. The other five diets had an average range of six percent. The lower survivals of the commercial diet offered opportunity for greater weekly growth (which increases final weight and reduces FCR) because these shrimp were introduced to a lower density. Larger shrimp are generally produced in lower population densities, whereas higher population densities are associated with smaller shrimp and slightly higher yields (Williams et al., 1996; Mena-Herrera et al., 2006). Regardless of the difference in survival, FCR, and mean final weight between the commercial diet and the experimental diets, the mean final yield in all six treatments was similar (0.6 kilograms per tank).

Supporting the results of the present study is work conducted by Samocha et al. (2004) who demonstrated, in outdoor tank systems with primary production, that complete replacement of menhaden fish meal was possible using a co-extruded poultry by-product meal with egg supplement. Patnaik et al. (2006) tested a complete replacement of fish meal and fish oil using plant protein and non-marine oil sources in practical diets for juvenile *L. vannamei* stocked at 30 shrimp per tank in high density polyethylene tanks under production water conditions. No significant differences were found in growth under these experimental conditions.

The results of this study make a strong case when evaluating the replacement of PBM with soybean meal in practical diets for the juvenile *L. vannamei* under green water production conditions. Under similar approach, Amaya et al. (2007a) completely removed fish meal using alternative vegetable protein sources in combination with PBM without negatively compromising the productive performance of *L. vannamei* in semi-intensive systems. Amaya et al. (2007b) further strengthened their case by obtaining similar results in a green water semi-enclosed recirculating system, providing evidence that animal protein sources can be completely replaced by varying levels of soybean meal, milo, corn gluten meal and corn fermented solubles in the diet.

Table 13. Annual growth improvement of *L. vannamei* raised under commercial pond conditions at the Claude Peteet Mariculture Center, Gulf Shores, Alabama, with the use of historical productive trends, conservative feeding protocol, and well formulated feeds.



Reducing the dependence of marine ingredients and animal by-products by the addition of plant meals in commercially manufactured feeds may provide some relief to over-harvested wild fish stocks and may provide more peace of mind to some environmentalists. The use of all plant ingredients in practical diets for juvenile shrimp may expand to the possibilities of organically raised shrimp, as the popular trend of organically manufactured food has been increasing rapidly. This niche market would also increase farmers' chances to out compete the rest of the market because the uniqueness and limited availability of their product would turnover a higher dollar value.

CONCLUSION

Results from this study demonstrate that in well formulated commercial feeds, poultry by-product meal can be successfully replaced with soybean meal and corn gluten meal as the primary protein sources without affecting productivity of *L. vannamei* reared in semi-intensive green water systems. These production responses were the result of a combination of a well formulated diet, accurately manufactured feed, correct feed inputs, favorable meteorological conditions and good pond management.

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