FISH MEAL REPLACEMENT IN PRACTICAL DIETS FOR PACIFIC WHITE

SHRIMP (*Litopenaeus vannamei*) REARED IN GREEN WATER SYSTEMS

Except where reference is made to the work of others, the work described in this thesis is my own or was done in collaboration with my advisory committee. This thesis not include proprietary or classified information

____

Elkin A. Amaya

Certificate of Approval:

William Daniels Associate Professor Fisheries and Allied Aquacultures	D. Allen Davis, Chair Associate Professor Fisheries and Allied Aquacultures
David B. Rouse Professor Fisheries and Allied Aquacultures	Stephen L. McFarland Acting Dean Graduate School

FISH MEAL REPLACEMENT IN PRACTICAL DIETS FOR PACIFIC WHITE SHRIMP (*Litopenaeus vannamei*) REARED IN GREEN WATER SYSTEMS

Elkin A. Amaya

A Thesis

Submitted to

the Graduate Faculty of

Auburn University

in Partial Fulfillment of the

Requirements for the

Degree of

Master of Science

Auburn, Alabama
Aug 7th, 2006

FISH MEAL REPLACEMENT IN PRACTICAL DIETS FOR PACIFIC WHITE

SHRIMP (*Litopenaeus vannamei*) REARED IN GREEN WATER SYSTEMS

Elkin A. Amaya

____
Signature of Author

____
Date of Graduation

VITA

Elkin A. Amaya, son of Nestor Amaya and Martha Rojas, and brother of Edwin, Lady and Jonathan, was born on February 24^{th}, 1978, in Bogota, Colombia. He was conferred his Bachelor of Science degree in Animal Science at the “Universidad Nacional de Colombia” in Bogota, Colombia in 2002. After working for two years in the areas of animal production with governmental agencies and animal nutrition with the feed industry, in 2004 he joined Auburn University to pursue a Master of Science degree in Aquaculture at the Department of Fisheries and Allied Aquacultures.

THESIS ABSTRACT

FISH MEAL REPLACEMENT IN PRACTICAL DIETS FOR PACIFIC WHITE SHRIMP (*Litopenaeus vannamei*) REARED IN GREEN WATER SYSTEMS

Elkin A. Amaya

Master of Science, Aug 7th, 2006
(B.S., Universidad Nacional de Colombia, Bogota, Colombia, 2002)

80 Typed Pages

Directed by D. Allen Davis

A series of experimental studies were conducted with Pacific white shrimp, *Litopenaeus vannamei*, to evaluate the use of plant protein sources as replacement ingredients for fish meal in commercially manufactured diets for shrimp reared in green water environments. In the first study, juvenile (mean weight ± S.D., 0.031 ± 0.0005g) shrimp were stocked in 16 0.1-ha low-water-exchange ponds and reared over an 18-week culture period. Four commercially extruded diets formulated to contain 35% crude protein and 8% lipids were evaluated. These diets included 16% poultry by-product meal and varying levels of fish meal (9, 6, 3, and 0%), which was replaced by a combination of soybean meal and corn gluten meal to replace the protein originating from fish meal. At

the conclusion of the culture period, there were no significant differences ($P \geq 0.05$) on any of the production parameters evaluated among the test diets, whereas feeding costs were significantly ($P \leq 0.05$) reduced as more plant proteins were included in the diets. Mean gross production, final weight, FCR and survival were evaluated at the end of the 18-week culture period, with final values ranging from 5,363 - 6,548 kg/ha, 18.4 - 20.7 g, 1.12 - 1.38 and 84.0 - 94.0 %, respectively. This study demonstrated that fish meal can be completely substituted by alternative vegetable protein sources in commercially manufactured shrimp feeds, without negatively compromising the productive and economic performance of *Litopenaeus vannamei* reared in ponds. In the second study, juvenile (mean weight $\pm$ S.D. 0.74 g ± 0.03, n=30) shrimp were stocked in an outdoor covered recirculating system composed of 24 800-L tanks with 30 shrimp per tank and four replicates per treatment. Experimental treatments included four diets with varying levels of fish meal in the diet (9%, 6%, 3% and 0%), a plant protein-based diet, and a commercial reference feed. Feeds were commercially extruded and offered as sinking, extruded pellets designed to contain 35% crude protein and 8% lipids. Production parameters at the end of the study demonstrated no significant differences ($P \geq 0.05$) among any of the treatments evaluated. Mean net production, final weight, weight gain (%), FCR and survival were evaluated at the end of the 81-day culture period. Final values for these parameters ranged from 564.4 - 639.0 g/m^3, 17.4 - 19.5 g, 2,249 - 2,465 %, 1.07 - 1.20 and 83.3 - 89.2 %, respectively. These results indicate that fish meal can be replaced with solvent extracted soybean meal when diets contain 16% poultry by-product meal. In addition, plant protein sources such as soybean meal, corn gluten meal and corn fermented solubles can be used without affecting shrimp performance in diets with no animal protein sources. Feed costs per unit of production indicate that feed costs can be reduced if fish meal is replaced with alternative protein sources. Findings from these two studies provide evidence that shrimp reared in green water systems can successfully use alternative plant based diets with no fish meal. In addition, it indicates that shrimp have the capacity to effectively use a plant protein based diet without negatively compromising shrimp performance. The use of alternative shrimp diets is therefore recommended as a way to reduce pressure and dependance on fish meal and other animal protein sources, with the subsequent reduction on feed production costs as cheaper, high quality plant ingredients are included. Provided that these reduced feed costs are effectively transferred from the feed companies to the shrimp producers, shrimp producers could gain from better profit margins as cheaper feeds are used. An additional reason for using alternative plant-based diets is that they open an opportunity for new markets that would be willing to pay a higher price, for shrimp fed and produced under more ecologically and sustainable conditions that do not represent a threat to the environment or the human health.

ACKNOWLEDGMENTS

I would like to thank my advisor Dr. Allen Davis for his support and guidance throughout my Auburn experience, same as my committee members Dr. William Daniels and Dr. David Rouse for their guidance. I am especially grateful to my parents Nestor and Martha and my siblings Edwin Lili and Jonathan for their constant company. I am also greatly thankful to Evi and her company and support throughout this time in Auburn.

This study was funded by the American Soybean Association (ASA, MASEA Grant) and the Department of Fisheries and Allied Aquacultures at Auburn University. Special thanks to all the students of the Nutrition and Technology Laboratory of the Department of Fisheries and Allied Aquacultures of Auburn University for their help and support during the development of this study. I also want to thank the Alabama Department of Conservation and Natural Resources, Marine Resources Division, for allowing the use of their facilities during the experimental part of this study and for their physical and logistic support.

Style manual of journal used: Aquaculture

Computer software used: Word Perfect 12, Microsoft Power Point, Microsoft Excel XP, and SAS v. 9.1

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

The commercial production of farmed shrimp has been expanding steadily. According to FAO (2005), 1,804,932 mt (metric tons) of shrimp were cultured in 2003, representing an increase of around 640,000 mt over the production of 2000. The contribution of aquaculture to world shrimp production reached 32.5% in the period between 2001 and 2003 compared to an average of 27.5% during the 1990's. Most of this increase was the result of the development of Pacific white shrimp (*Litopenaeus vannamei*) aquaculture in Asian countries, such as China and Thailand, and to the increased production from Brazil in the western hemisphere. Combined, these three countries produced around 510,000 mt out of the 723,858 mt of the *Litopenaeus vannamei* cultured in 2003 (FAO, 2005). This increased production has been accompanied by a decrease in shrimp value either due to depressed economies and/or abundant supplies. Expansion of this industry is expected to continue and shrimp market prices are likely to continue to fall, therefore, reducing the profitability of the industry. According to Josupeit (2004), shrimp trade has not grown in value in recent years and since 2000 all unit values of shrimp have gone down with record low prices in 2004. Facing this scenario, there has been a general interest in finding alternatives to generate an added value to the shrimp industry and to re-evaluate and improve the shrimp production systems in terms of yields, production costs and feed efficiencies.

When evaluating ways to reduce production costs, one consideration is to minimize the use of expensive marine animal ingredients in the feeds. Of particular concern is fish meal, for which high demand and limited supply makes it a costly ingredient. The interest for exploring alternative protein sources to fish meal in marine shrimp diets was initially led by the often variable nature of the price, supply and quality of the fish meal (Colvin and Brand, 1977; Divakaran *et al.*, 2000). Recently, however, growing concerns about the use of fish to feed fish, and the increasing demand and limited fish meal supply have heightened this interest. Different authors have also claimed that a better shrimp value could be achieved in alternative markets, such as organic markets, which among other characteristics, require limitations of specific ingredients such as those of animal origin (Davis *et al.*, 2004; Josupeit, 2004).

Fish and marine animal meals are used in aquatic feeds because they are excellent sources of essential nutrients such as protein and indispensable amino acids. Additionally, they contain essential fatty acids, cholesterol, vitamins, minerals, attractants and unidentified growth factors (Swick *et al.*, 1995; Samocha *et al.*, 2004). Because of these characteristics, fish meal is also the primary and most expensive ingredient in commercial shrimp feeds, with commercial formulations commonly including between 25% to 50% of the total diet. (Tacon and Barg, 1998; Dersjant-Li, 2002).

Protein ingredients that can be utilized to partially or completely substitute marine animal meals include terrestrial animal and plant by-products readily available on world markets. (Samocha *et al.*, 2004). Animal sources are primarily rendered by-products, such as blood meal, feather meal, meat and bone meal and poultry by-product meal, which usually contain 45–65% crude protein and are often good sources of indispensable amino acids. The nutritional composition of these meals, however, is highly variable depending on both the quality of raw ingredients and the type of processing. Additionally, due to events of BSE (mad cow disease) and contaminants such as PCBs, there is an increasing public concern in the use of potentially contaminated by-products in animal feeds (Dersjant-Li, 2002; Samocha *et al.*, 2004).

Because of their low price, relatively consistent nutrient composition and supply, plant proteins, such as oil seed cakes, are often economically and nutritionally valuable sources of protein. However, due to potential problems associated with insufficient levels of indispensable amino acids (e.g., lysine and methionine), anti-nutritional factors and poor palatability, their commercial use has been often limited. Among plant protein sources, soybean meal has received considerable attention as a replacement for fish meal in aquatic feeds because of its balanced amino acid profile, consistent composition, ample availability and price (Lim *et al.*, 1998; Hardy, 1999; Samocha *et al.*, 2004). Divakaran *et al.* (2000) showed that the apparent digestibility of protein in soybean meal is at least 89% in *Litopenaeus vannamei*.

Common concerns regarding the use of soybean meal are related to the presence of anti-nutritional factors, such as trypsin inhibitors, which interfere with protein and lipid digestion. Other anti-nutritional factors, such as antigens, lectins, saponins, and oligosaccharides negatively affect the palatability and nutrient absorption of feeds containing soybean meal (Dersjant-Li, 2002). According to Swick *et al.* (1995) the dietary limitation on soybean meal for shrimp is mostly due to negative effects on the physical stability of the pellets caused by fiber and soluble sugars. Additionally, most of the phosphorus in soybean meal is bound to phytic acid and only 30-40% of the total phosphorus content is considered to be available for *Litopenaeus vannamei*. (Akiyama *et al.* 1991; Hertrampf and Piedad-Pascual, 2000). According to Akiyama (1988), phosphorous is the most critical mineral when formulating fish feeds that contain a high level of soy protein. When compared to fish meal, soybean meal is also characterized by its lower composition of essential amino acids mainly methionine, lysine and threonine (NRC, 1993), and by its lack of n-3 marine fatty acids (eicopentaenoic acid, EPA and docosohexaenoic acid, DHA), which are essential for the growth and survival of marine shrimp (Fox *et al.*, 2004)

Studies evaluating soybean meal as the only replacement of marine animal proteins in diets for *Litopenaeus vannamei* have shown a moderate level of success. Lim and Dominy (1990) reported that up to 40% of a marine protein mix (53% menhaden fish meal, 34% shrimp waste meal and 13% squid meal) could be replaced by solvent-extracted soybean meal, without reducing the growth of *Litopenaeus vannamei*. At this substitution level, soybean meal represented 28% of the diet, similar to the 10% to 20% that Akiyama *et al.* (1991) reported as the percentage typically included in commercial shrimp feeds. A 25-30% dietary level of soybean meal as a replacement for 40-50% of the marine animal protein in shrimp feeds appears optimal (Swick *et al.*, 1995). According to Samocha *et al.* (2004), a promising alternative to using terrestrial plant or animal proteins independently would be to use a mixture of complementary ingredients to increase nutrient utilization and facilitate feed processing. Under this strategy, it is possible to obtain a more balanced nutrient profile in the feeds (i.e. essential amino acids, fatty acids) than when limited ingredients are used.

Recent studies of Davis and Arnold (2000) and Samocha *et al.* (2004) have provided evidence that co-extruded soybean and poultry by-product meal are adequate ingredients to substitute fish meal in practical diets for *Litopenaeus vannamei*. Davis and Arnold (2000) reported that up to 80% of the fish meal in diets for *Litopenaeus vannamei* can be substituted either by co-extruded soybean poultry by-product meal containing egg supplement or poultry by product meal without any apparent effect on shrimp survival or growth. Samocha *et al.* (2004) did not find significant differences in the weight gain, survival and feed efficiency values (FE) of *Litopenaeus vannamei* juveniles fed practical diets with 32% protein, where up to 100% of the fish meal was replaced with co-extruded soybean poultry by-product meal with egg supplement. The authors suggested that the favorable response of the shrimp to the diets was probably due to the high quality of the ingredients in terms of nutrient profile, digestibility and lack of palatability problems. They also reported that there were no indications of the feed being rejected. Furthermore, they recognized that co-extrusion was a valuable practice when processing the ingredients.

Based on these findings, it is clear that by using the single ingredient approach, the complete fish meal replacement with soybean meal is difficult to achieve. Yet, it also appears that fish meal and marine oil sources can be removed from shrimp diets if suitable alternative sources of protein and lipids are provided to meet the essential amino acid and fatty acid requirements of shrimp (Davis *et al.*, 2004). Under this scenario and considering recent advancements in additives and ingredients and feed processing technologies, it appears that soybean meal use can be further increased in shrimp feeds. According to Dersjant-Li (2002), properly processed plant ingredients containing high protein content with high digestibility of crude protein and low anti-nutritional components are potential protein sources for the replacement of fish meal in fish and shrimp diets. Today, for example, extrusion is widely used in manufacturing shrimp feeds, having the advantage of inactivating and/or destroying endogenous heat-sensitive anti-nutritional factors commonly found in soybean meal and gelatinizing starch granules (Carver *et al.*, 1989). Amino acid micro-encapsulation is another technology that may increase the use of synthetic amino acids to correct nutritional imbalances in shrimp feeds. According to Swick *et al.* (1995) micro-encapsulation prevents the rapid elevation of amino acid concentration in shrimp tissue and reduces the solubility problems of non-encapsulated sources.

Nutritional studies evaluating soybean meal have often been conducted in controlled, laboratory conditions to mitigate the effects of variable water quality variations found in shrimp ponds and remove external food sources (D'Abramo and Castell, 1997). The practical application of data from these studies is limited because laboratory conditions are different from the shrimp pond environment (Tacon, 1996). Growth enhancement of shrimp in ponds has commonly been attributed to their assimilation of micro-algae and microbial-detrital aggregates present in the water (Moss and Pruder, 1995). Furthermore, according to Moss *et al.* (2001), the culture environment may also significantly impact the digestive enzyme activity in shrimp, providing the shrimp with additional digestive capabilities, which may contribute to the growth-enhancing effect of shrimp in green water systems.

With increasing interest on the applicability of research results under commercial shrimp production conditions, the primary objective of this study was to evaluate the replacement of fish meal by using a combination of plant and animal protein sources in commercially-manufactured diets for *L vannamei* reared under production conditions in green water systems. To achieve this objective, two separate studies were carried out. In the first study, four diets with a fixed level of poultry by-product meal and varying levels of fish meal and plant protein sources were evaluated in shrimp reared in a pond production system with minimal water exchange. In the second study, shrimp reared in a green water recirculating system were used to evaluate the previous four diets in addition to a plant-based diet with no animal protein sources and a commercial reference diet. It is expected that because of the commercial and practical scale of this research, findings from this study will further increase the knowledge about the nutrition and feeding of shrimp fed diets with high levels of plant protein sources, and will provide the feed and shrimp industries with additional understanding of marine shrimp capacity to use these kind of ingredients in the feeds.

CHAPTER II

REPLACEMENT OF FISH MEAL IN PRACTICAL DIETS FOR THE PACIFIC WHITE SHRIMP (*Litopenaeus vannamei*) REARED UNDER POND CONDITIONS.

Abstract

Increasing economical and ecological concerns regarding the use of fish meal in diets for marine shrimp have led to the development of replacement strategies where soybean meal has received ample attention. Most studies evaluating these strategies have been carried out under laboratory conditions which greatly differ from production conditions in ponds. This study evaluated a fish meal replacement strategy using vegetable protein sources in practical feeds for marine shrimp reared in ponds. Juvenile Pacific white shrimp (*Litopenaeus vannamei*) (mean weight $\pm$ S.D., 0.031 $\pm$ 0.0005g) were stocked into 16 0.1-ha low-water-exchange ponds and reared over an 18-week period. Four commercially extruded diets formulated to contain 35% crude protein and 8% lipids were evaluated. These diets included varying levels of fish meal (9, 6, 3, and 0%) which was replaced by a combination of soybean meal (32.48, 34.82, 37.17 and 39.52% respectively) and corn gluten meal (0.00, 1.67, 3.17, and 4.84% respectively) to replace the protein originating from fish meal. At the conclusion of the experimental period, there were no significant differences ($P \geq 0.05$) in shrimp production among the test diets. Mean final production, final weight, FCR and survival were evaluated at the end of the 18-week culture period, with final values ranging from 5,363 - 6,548 kg/ha, 18.4 - 20.7 g, 1.38 - 1.12 and 84.0 - 94.0 %, respectively. Although not significant, the economic analysis numerically showed a general increase in the partial gross returns of shrimp production, as higher levels of plant proteins sources were included in diets fed to marine shrimp. Results from this study demonstrate that fish meal can be replaced using alternative vegetable protein sources in practical shrimp feeds without compromising productive and economic performance of *Litopenaeus vannamei* reared in ponds.

Keywords: *Litopenaeus vannamei*, Soybean meal, Fish meal, Plant proteins.

Introduction

The commercial production of farmed shrimp has been expanding steadily. According to FAO (2005), 1,804,932 mt (metric tons) of shrimp were cultured in 2003, representing an increase of around 640,000 mt over the production of 2000. This increased production has been accompanied by a decrease in shrimp price, either by depressed markets or overproduction. With shrimp aquaculture expected to continue to increase in coming years, shrimp prices are likely to continue to fall as production exceeds demand, therefore challenging the profitability of this industry.

An important issue considered to reduce shrimp production costs and increase producers profitability, is the use of feeds with low levels of fish meal and high levels of cheaper, high quality plant protein sources. Commercial shrimp formulations commonly include between 25% to 50% of fish meal, representing the primary and most expensive protein ingredient. (Dersjant-Li, 2002; Tacon and Barg, 1998). Fish meal is preferred among other protein sources because it is an excellent source of essential nutrients such as protein and indispensable amino, essential fatty acids, cholesterol, vitamins, minerals, attractants and unidentified growth factors (Swick *et al.*, 1995; Samocha *et al.*, 2004). However, limited availability and high demand make of fish meal a high cost ingredient.

Because of their low price and consistent nutrient composition and supply, plant proteins, such as oil seeds, are often economically and nutritionally valuable alternatives to fish meal. Among plant protein sources, soybean meal has received considerable attention in the replacement of fish meal in aquatic animal feeds because of its balanced amino acid profile, consistent composition, worldwide availability and lower price

(Colvin and Brand, 1977; Lim and Dominy,1990; Akiyama *et al.* (1991; Swick *et al.*, 1995; Lim *et al.*, 1998; Hardy, 1999; Divakaran *et al.*, 2000; Samocha *et al.*, 2004). However, when compared to fish meal, soybean meal is characterized by a lower composition of essential amino acids, mainly methionine, lysine and threonine (NRC, 1993), and by its lack of the n-3 marine fatty acids EPA (eicopentaenoic acid) and DHA (docosohexaenoic acid), which are essential for the growth and survival of marine shrimp (Fox *et al.*, 2004). Additionally, only 30-40% of the total phosphorus content is considered to be available for *Litopenaeus vannamei* (Hertrampf and Piedad-Pascual, 2000; Akiyama *et al.*, 1991). If the replacement strategy considers shifts in essential nutrients, it also appears that fish meal can be removed from shrimp formulations if suitable alternative sources of protein and lipids are provided to meet the nutritional requirements of the animal (Davis *et al.*, 2004). The use of complementary ingredients is a practice used to obtain a more balanced nutrient profile in the feeds (i.e. essential amino acids, fatty acids) and to increase nutrient utilization and facilitate feed processing (Mendoza *et al.*, 2001; Hernandez *et al.*, 2004; Samocha *et al.* 2004). Davis and Arnold (2000), reported that up to 80% of the fish meal in diets for *Litopenaeus vannamei* can be substituted by co-extruded soybean poultry by-product meal containing egg supplement or poultry by-product meal without any apparent effect on survival, growth and feed palatability. Samocha *et al.* (2004) did not find significant differences in weight gain, survival and feed efficiency of *Litopenaeus vannamei* fed 32% CP (crude protein) practical diets, where up to 100% of fish meal was replaced with co-extruded soybean poultry by-product meal with egg supplement.

Studies evaluating soybean meal and other alternative protein sources to fish meal have often been conducted in controlled laboratory conditions to mitigate the inherent effects of water quality and natural food sources found in shrimp ponds. Although these studies have provided valuable information regarding shrimp capacity to utilize these type of ingredients under controlled conditions, the practical application of data from these studies is limited. Main concerns are related to differences in the culture environment (e.g. green water ponds vs clear water tanks), length of the growth cycle, and feed processing (extruded vs pelleted) (Moss and Pruder, 1995; Tacon, 1996; D'Abramo and Castell, 1997; Moss *et al.*, 2001). As a normal transition to an effective process of technology transfer aimed to reach the shrimp and feed industries, the next step would be to carry out studies under conditions similar to those found commercially. This study evaluates fish meal replacement using soybean meal as the main protein source in commercially manufactured feeds for the Pacific white shrimp *Litopenaeus vannamei* reared under pond conditions.

Methods

Shrimp source

Pacific white shrimp *Litopenaeus vannamei* post-larvae (mean weight ± S.D., 1.48mg ± 0.39) were obtained from Shrimp Improvement Systems (Plantation Key, FL) and nursed for 19 days in a recirculation system at the Claude Peteet Mariculture Center located in Gulf Shores, Alabama, according to the procedures described by Garza *et al.* (2004). The nursery system was stocked at a density of 53 post-larvae per liter and the following water quality parameters maintained at adequate levels for the growth of shrimp post-larvae: temperature, dissolved oxygen, pH, salinity and total ammonia nitrogen. Feeding schedule for shrimp during the nursery period is presented in Table 1. At the conclusion of the nursery phase, juvenile shrimp (0.0312g $\pm$ 0.0005) were stocked into 16 ponds located in the same facilities, at a density of 35 shrimp per squared meter.

Pond management and water quality

Ponds used for the grow-out phase were 0.1 ha in surface area (46 x 20 m), 1.0 m average depth, and lined with a 1.52 mm thick, high-density polyethylene. Each pond bottom was covered with a 25-cm deep layer of sandy-loam soil and equipped with a 20-cm diameter screened standpipe and a concrete catch basin. Prior to use, pond soils were dried and tilled to allow oxidation and mineralization of organic matter. Two weeks before stocking, ponds were filled with brackish water (9-13 ppt) from the Intracostal Canal between Mobile and Perdido Bay, Alabama. Inlet water was filtered through a 250-μm nylon filter sock (Micron Domestic Lace Mfg., Inc) in order to prevent the introduction of predators and minimize the introduction of larval species. Inorganic liquid fertilizers were mixed and applied one week before stocking at a rate of 1,697 ml of 32-0-0 and 303 ml of 10-34-0 per pond, therefore, providing 5.73 kg of N and 1.03 kg of P_20_5/ha. Two weeks after the first fertilization, a second fertilizer application at half the initial rate was added to ponds with Sechii disk readings greater than 50 cm. Twenty-four hours before stocking, a 1:15 mixture of motor oil and diesel fuel was applied to the pond surface, at a rate of 900 ml per pond, to reduce the number of air breathing insects.

Table 1. Feeding rates as percentage biomass and feed type utilized through a 19-day nursery period for *Litopenaeus vannamei* post-larvae. Feed inputs were based on mean shrimp weight and an assumed 78% survival.

Day	Shrimp weight (mg)	Feed rate (%)	Feed type	Ratio (%)
1-3	1.5	25	Artemia / PL redi[a]	40 / 60
4-6	2.5	25	PL redi / Crumble #0[b]	40 / 60
7	4.1	25	PL redi / Crumble #0	20 / 80
8-9	4.1	25	Artemia / Crumble #0	5 / 95
10-12	5.7	15	Artemia / Crumble #0	15 / 85
13-15	11.9	15	Crumble #0 / Crumble #1[b]	50 / 50
16	20.1	15	Crumble #0 / Crumble #1	30 / 70
17-18	20.1	15	Crumble #1	100 / 0
19	28.9	15	Crumble #1 /Crumble #3[b]	50 / 50
Harvest	31.2			

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

[b] Rangen 45% protein, Rangen Inc., Buhl, Idaho, USA.

During the experimental period, dissolved oxygen, temperature, salinity and pH concentrations were measured at sunrise (05:00-05:30) and at night (20:00-22:00) using a YSI 556MPS meter (Yellow Spring Instrument Co., Yellow Springs, OH, USA). Total ammonium-nitrogen (TAN) and Sechii disk readings were determined on a weekly basis. Water samples for TAN analysis were taken at 40 cm from the pond surface and measured with a spectrophotometer (Spectronic Instrument Inc. Rochester, NY, USA) by the Nesslerization method (APHA 1989).

In order to maintain minimum dissolved oxygen levels of 3 mg/L, each pond was provided with a base aeration capacity of 30 hp/ha (7.5kW/ha). Paddle wheel aerators of 1-hp (Little John Aerator, Southern Machine Welding Inc. Quinton, AL) or propeller aspirators aerators of 1-hp (11.2 Ampers) or 2-hp (20 Ampers) (Aire-$O_{2,}$ Aeration Industries International, Inc. Minneapolis, Minnesota) were used for this purpose. When required, additional aeration (up to 30 hp/ha) was used to maintain adequate DO levels. Dissolved oxygen and temperature stratification within the water column after the 8^{th} week were managed by running the aerators for about 20 minutes in the late afternoon. Ponds were managed with a minimal water exchange strategy; therefore, there were no regular water exchanges. However, because of heavy rains associated with tropical storms, the water level of the pond had to be reduced by 20%, twice at different times of the crop to avoid the risk of flooding. Additionally, the week prior to harvest, 30% of the water from the ponds was replaced, in order to reduce the chance of algae crashes and to encourage shrimp molting before harvest (Davis, personal communication).

Feed formulation strategy and feed management

Shrimp ponds were randomly assigned to one of four dietary treatments, with four replicates per treatment. The basic guideline for the formulation of the four diets was to reduce the inclusion of menhaden fish meal (FM) as follows: 9%FM, 6%FM, 3%FM and 0%FM of the total diet, while increasing the inclusion of vegetable protein sources, mainly solvent extracted soybean meal (Table 2). The dietary treatments were formulated in order to provide equal protein and lipid levels while maintaining a minimum lysine and methionine plus cystine content of 5% and 3% of the total protein, respectively (Table 3). Corn gluten meal was used as a natural source of methionine. Lipid levels were adjusted by adding menhaden fish oil. Calcium phosphate was added to ensure adequate phosphorus supply as fish meal was removed. Feeds were produced by Rangen, Inc. (Angleton TX, USA) under commercial manufacturing conditions and offered twice a day to the shrimp, as a sinkable, extruded 3 mm pellet.

Feed inputs during the 1st, 2nd, 3rd and 4th weeks of pond culture were set at 10, 15, 30 and 60 kg of feed/ha/day, respectively. For the remainder of the growth trial, feed inputs were back-calculated, based on an expected weight gain of 1.5 g per week, a feed conversion of 1.2:1, and a total mortality of 30% (1.66%/wk) over an 18-weeks culture period. Feeding during the first 19 days after stocking was done using the same commercial 35% CP Rangen feed that had been used at the end of the nursery phase.

Table 2. Ingredient composition (g/100 g as is) of practical diets for *Litopenaeus vannamei* used for the replacement of fish meal (FM) with plant protein sources. Diets were commercially manufactured by Rangen® Inc.(Angleton, TX) using extrusion processing.

Ingredient	9% FM	6% FM	3% FM	0% FM
Soybean meal	32.48	34.82	37.17	39.52
Fish meal - Menhaden	9.00	6.00	3.00	-
Poultry By-Product meal	16.00	16.00	16.00	16.00
Milo	35.47	33.82	32.33	30.68
Corn Gluten meal	-	1.67	3.17	4.84
Fish Oil	3.96	4.22	4.47	4.72
Di-calcium Phosphate	1.50	1.88	2.27	2.65
Bentonite	1.00	1.00	1.00	1.00
Mold Inhibitor	0.15	0.15	0.15	0.15
Vitamin Premix	0.34	0.34	0.34	0.34
Mineral Premix	0.08	0.08	0.08	0.08
Stay-C 35%	0.02	0.02	0.02	0.02

Table 3. Nutritional composition of practical diets for *Litopenaeus vannamei* used for the replacement of fish meal (FM)[1].

Ingredient	9% FM	6% FM	3% FM	0% FM
Crude Protein	35.7	35.9	36.2	36.6
Crude Fat	8.4	8.3	8.6	8.4
Crude Fiber	2.4	1.8	2.1	1.9
Ash	8.2	7.9	7.9	8.1
Calcium[2]	1.3	1.3	1.2	1.1
Total Phosphorus[2]	1.2	1.2	1.2	1.3
Lysine[2]	2.0	2.0	1.9	1.8
Met + Cys[2]	1.1	1.1	1.1	1.1
% Protein from plant sources[2,3]	54.9	60.4	66.0	71.5
% Protein from animal sources[2,4]	45.1	39.6	34.0	28.5

[1] Analyzed value - New Jersey Feed Laboratory, Inc. Trenton, NJ.

[2] Calculated value.

[3] Soybean meal, corn gluten meal, milo.

[4] Fish meal, poultry by-product meal.

Treatment feeds were offered from day 20, when it was estimated that shrimp had reached 1 g of weight. Maximum feed inputs were set at 83.3 kg of feed/ha/day at the 5th week. Feed inputs throughout the study are shown in Figure 1. Shrimp growth was monitored on a weekly basis by determining the average weight in a sample of 70 to 100 animals per pond. Sampling was carried out by capturing shrimp by seine during first two weeks and cast net (monofilament net, 1.22 m radius and 0.95 cm opening) for the remaining of the culture period.

Harvest

Harvest took place over a three day period, after 18 weeks of pond culture. Feed inputs were stopped two days prior to harvesting a given pond. The night before harvest, two thirds of the water from each pond was drained and aeration was provided using paddlewheel aerators to keep shrimp alive and minimize erosion on pond bottoms. On harvest day, shrimp were pumped from the catch basin using a hydraulic fish pump equipped with a 25-cm diameter suction pipe (Aqualife-Life pump, Magic Valley Heli-arc and Mfg, Twin Falls, Idaho, USA). The pump was placed in the catch basin and shrimp were pumped, de-watered and collected in a hauling truck. Shrimp were then transferred to a laboratory located at the same facilities for washing, cleaning and weighing. During weighing a random sample of 125 shrimp was collected for individual weight determinations. Individual weights were used to calculate mean final production, mean final weight, survival and size distributions. After quantifying the biomass from each pond, mean final production (final biomass), feed conversion ration (total feed offered /

biomass increase), size distribution and survival were determined. Additional economic considerations about production and feeding costs were also calculated.

Data Analysis

Data were analyzed using an analysis of variance to determine significant ($p<0.05$) differences among treatment means. The Student–Neuman–Keuls multiple comparison test was used to determine significant differences between treatment means (Steel and Torrie, 1980). All statistical analyses were conducted using SAS (V9.1., SAS Institute, Cary, NC, USA).

Results and Discussion

Water quality parameters throughout the 19 days nursery phase maintained suitable levels for the adequate growth and survival of juvenile shrimp (Tables 4 and 5). Prior to stocking of the nursed shrimp in the ponds, Sechii disk readings were measured, with values ranging from 70-110 cm. These values were considerably low as compared to readings at stocking from previous research at the same facilities (Venero, 2006; Zelaya, 2005) where they obtained readings between 25-85 cm. Low readings at stocking were the result of only having one week from fertilization to stocking, as compared to four weeks in the other studies. By the 5th week after stocking, it was clear that plankton blooms had completely developed with Sechii disk readings ranging from 25-35 cm. Weekly fluctuations in Sechii disk readings were the results of plankton bloom and die-off cycles in the ponds.

Table 4. Water quality parameters observed over a 19-day nursery period for *Litopenaeus vannamei* stocked at a density of 53 post-larvae/L in a water recirculating system composed of six, 4000-L nursery tanks.

Parameter	Mean	Minimum	Maximum	Standard Deviation	CV[a]
Temperature (°C)	28.0	24.3	30.3	1.38	4.93
Dissolved Oxygen (mg/L)	6.18	4.76	7.82	0.45	7.30
pH	7.50	6.97	7.83	0.21	2.80
Salinity (ppt)	28.0	13.8	31	4.54	16.19
TAN [b] (mg/L)	0.63	0.00	1.78	0.56	89.50

[a] CV = Standard deviation / mean * 100.

[b] Total Ammonium-Nitrogen.

Table 5. Mean production parameters of *Litopenaeus vannamei* nursed for 19 days and stocked at a density of 53 post-larvae/L (mean weight ± S.D., 1.48 ± 0.39mg) in a water recirculating system composed of six, 4000-L nursery tanks.

Parameter	Mean	Minimum	Maximum	Standard Deviation	CV[a]
Final weight (mg/shrimp)	31.2	27.2	37.2	3.6	11.61
Weight Gain (%)	1,950	1,740	2,415	249	12.78
Survival (%)	74.2	61.5	79.6	7.1	9.56
FCR[b]	1.04	1.00	1.09	0.03	2.85
Final biomass (kg/m^3)	1.18	1.13	1.22	0.03	2.41

[a] Coefficient of variation = Standard deviation / mean * 100.

[b] Apparent feed conversion ratio = Total weight of feed given / Biomass increase.

Water quality parameters throughout the 18-week experimental period maintained suitable levels for adequate growth and survival of shrimp (Table 6). Analysis of mean values for water quality parameters showed no statistically significant differences among treatments. Specific events of quick reduction in DO concentrations were observed during the second half of the study. These were principally associated to algae die-offs, which subsequently increased TAN concentrations and oxygen demand. Whenever an algae die-off was noticed, two aerators were set to operate continuously for as long as it was required (typically 3-4 days), until a new bloom could develop.

The mean number of dissolved oxygen readings that fell below a critical value of 2.5 mg/L, which is considered to initiate stressful conditions in shrimp (Venero 2006; Zelaya 2005), are presented in Table 6. Potentially stressful pH values below 6 units were not identified, whereas pH readings greater than 10 units were identified only four times. Analysis of extreme dissolved oxygen and pH values demonstrated no statistically significant differences among any of the treatments evaluated.

Feed inputs were modified from the original feeding program when conditions such as low dissolved oxygen levels, risk of storm or lack of electric power to run the aerators were present. Final daily feed inputs throughout the study are shown in Figure 1. Mean productive parameters of *Litopenaeus vannamei* fed the four dietary treatments at the end of the experimental period are presented in Table 7. Mean final productions (kg/ha) divided into production per count size of head-on shrimp are presented in Table 8. Distribution percentages of shrimp divided by count size of head-on shrimp for all treatments are presented in Figure 2.

Table 6. Summary of water quality parameters observed over an 18-week growing period for *Litopenaeus vannamei* fed four practical diets with varying levels of fish meal (FM) 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 of the whole data set.[1]

Parameter	9% FM	6% FM	3% FM	0% FM
DO (mg/l)[2]				
am	4.12 ± 1.64 (0.06, 13.28)	4.09 ± 1.59 (0.13, 11.56)	4.22 ± 1.48 (0.59, 12.83)	4.01 ± 1.58 (0.78, 12.62)
pm	7.25 ± 2.43 (0.97, 16.57)	6.92 ± 2.48 (0.08, 16.80)	7.11 ± 2.42 (0.66, 17.15)	7.31 ± 2.39 (0.82, 16.98)
Readings < 2.5	16.00 ± 7.87	20.00 ± 10.64	11.00 ± 1.82	16.75 ± 6.29
Temperature (°C)				
am	29.1 ± 1.6 (24.5, 32.9)	29.2 ± 1.6 (24.5, 33.0)	29.1 ± 1.7 (24.5, 32.7)	29.2 ± 1.6 (24.4, 32.7)
pm	30.6 ± 1.6 (25.3, 34.0)	30.7 ± 1.6 (25.1, 34.4)	30.6 ± 1.6 (25.2, 34.0)	30.8 ± 1.7 (24.7, 34.8)
pH				
am	7.61 ± 0.87 (6.16, 9.69)	7.40 ± 0.83 (6.17, 9.62)	7.60 ± 0.87 (6.10, 9.47)	7.41 ± 0.85 (6.12, 10.27)
pm	8.15 ± 0.74 (6.54, 10.04)	8.25 ± 0.77 (6.50, 10.73)	8.41 ± 0.72 (6.87, 9.84)	8.30 ± 0.73 (6.85, 10.02)
Readings > 10	0.25 ± 0.50	0.25 ± 0.50	-	0.50 ± 0.57
Salinity (g/l)				
	9.79 ± 1.2 (7.4, 13.3)	9.62 ± 1.2 (7.4, 13.8)	9.57 ± 1.2 (7.3, 12.9)	8.93 ± 1.3 (5.6, 11.9)
Turbidity (cm)				
	43.7 ± 32.6 (14, 110)	46.1 ± 34.2 (10, 110)	43.6 ± 33.2 (14, 110)	44.5 ±35.5 (10, 110)
TAN (mg/l)[3]				
	0.77 ± 1.50 (0, 9.33)	1.05 ± 1.68 (0, 7.65)	0.98 ± 1.74 (0, 9.01)	1.15 ±1.86 (0, 9.88)

[1] Based on analysis of variance (ANOVA) no significant differences ($P > 0.05$) were found among treatment means ($n = 4$).
[2] Dissolved Oxygen.
[3] Total Ammonium Nitrogen. Measured once per week.

Figure 1. Daily feed inputs (kg/ha/day) for *Litopenaeus vannamei* raised in ponds at a density of 35 shrimp/m^2 over an 18-week growing period and fed four practical diets with varying levels of fish meal and plant protein sources.

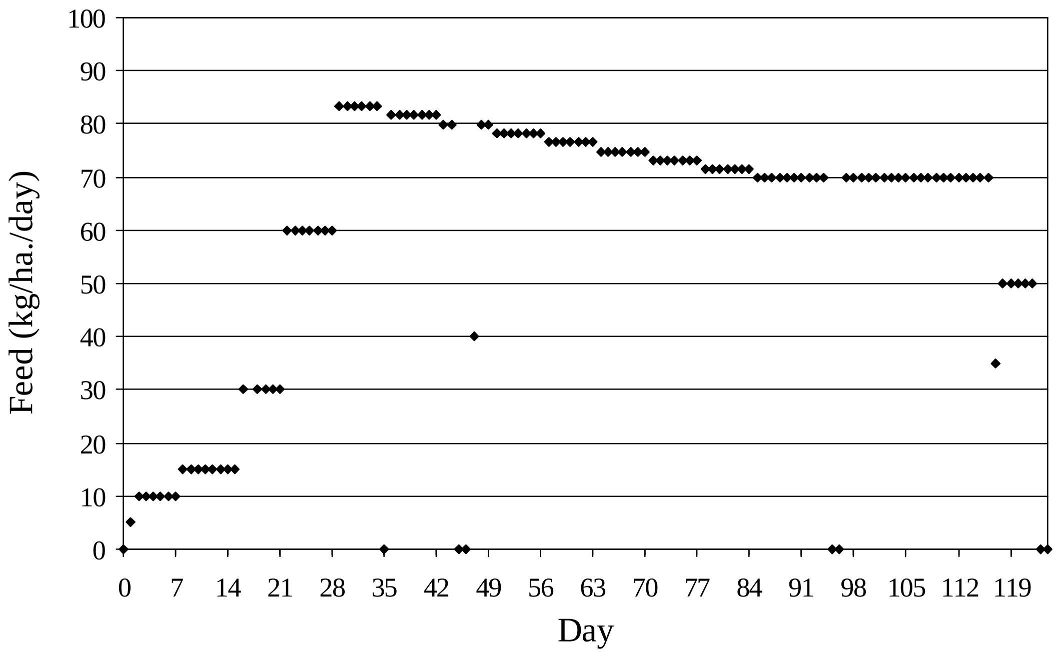


Table 7. Mean productive parameters of *Litopenaeus vannamei* cultured in 0.1-ha ponds, at the end of an 18-week culture period and fed four practical diets with varying levels of fish meal (FM) and plant protein sources[1].

Parameter	9% FM	6% FM	3% FM	0% FM	PSE[2]	P Value
Final weight (g)	19.6	18.4	19.8	20.7	1.39	0.695
Weight gain (%)[3]	62,603	58,635	62,708	67,219	4,691	0.652
Production (Kg/pond)	584.7	536.3	654.8	634.7	36.1	0.145
Weight gain (g/week)	1.11	1.04	1.13	1.19	0.079	0.695
FCR[4]	1.24	1.38	1.12	1.14	0.078	0.130
Survival (%)	87.2	84.0	94.0	87.4	5.72	0.661

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

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

[3] Weight gain (%) = 100 x (final weight - initial weight) / initial weight.

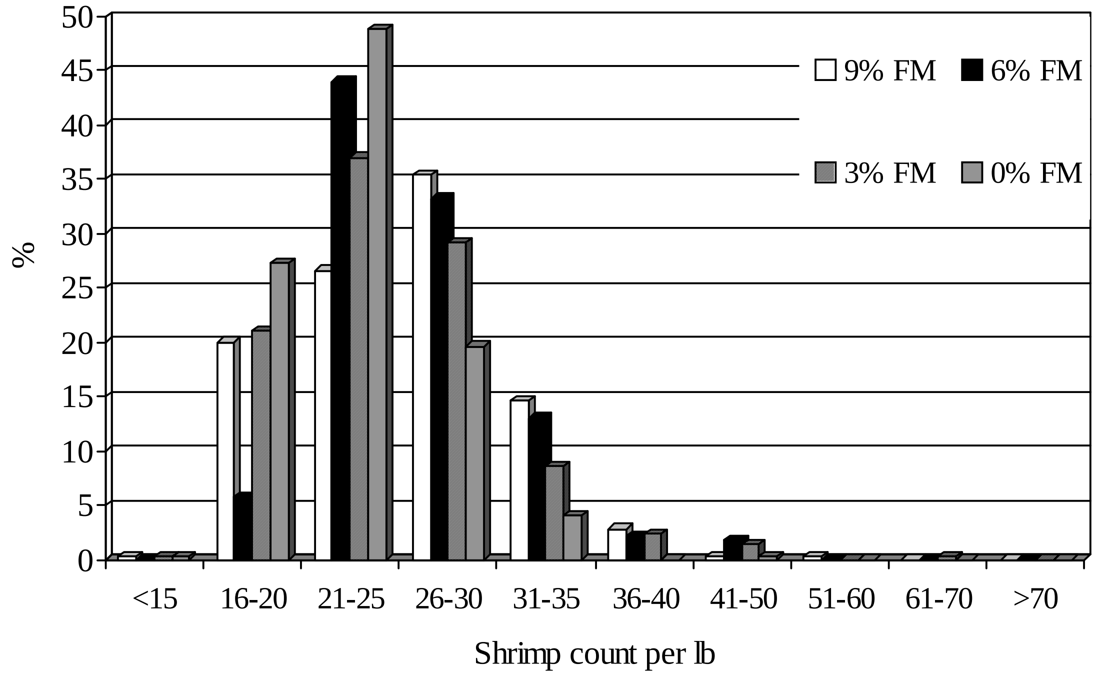
[4] Apparent feed conversion ratio = Total feed offered / biomass increase.

Table 8. Mean production (kg/ha) of *Litopenaeus vannamei* fed four practical diets with varying levels of fish meal (FM) and plant protein sources. Production is divided in the number of head-on shrimp per lb (454g). Prices are based on Gulf fresh shrimp prices between the 13 and 19 of October, 2005[1].

Count of head-on shrimp/Lb	9% FM	6% FM	3% FM	0% FM	USD/Lb
< 15	12	-	13	13	2.00
15-20	1,169	311	1,375	1,726	1.80
21-25	1,555	2,360	2,423	3,097	1.65
26-30	2,070	1,781	1,912	1,244	1.60
31-35	854	697	563	254	1.55
36-40	164	118	157	-	1.50
41-50	12	96	92	13	1.40
51-60	11	-	-	-	1.20
61-70	-	-	13	-	1.10
> 70	-	-	-	-	0.95
Total Kg/ha	5,847	5,363	6,548	6,347	

[1] Reported by the National Marine Fisheries Service, Fisheries Statistics Division, Silver Spring, MD, USA.

Figure 2. Population percentages of *Litopenaeus vannamei*, divided on the size distribution of head on shrimp count per lb. Shrimp was fed four practical diets with varying levels of fish meal and raised in ponds for an 18-week period at a density of 35 shrimp/m^2.



No significant differences were found for any of the production parameters evaluated. Final weights ranged from 18.4 - 20.7 g and were numerically higher in treatment 4 (0% FM); whereas mean final production, FCR and survival were higher in treatment 3 (3% FM) with values ranging for all treatments between 5,363 - 6,548 kg/ha, 1.12 - 1.38 and 84% - 94%, respectively. High mean survival rates suggest appropriate use of the available food and healthy water quality. These results also indicate that feed inputs were adequately assimilated, allowing the shrimp to achieve consistent productions and low feed conversion ratios. Production results obtained in this study are within the production range of commercial shrimp production in semi-intensive production systems. At the same facilities and with similar pond management, these production results were similar to those reported by Venero (2006) and better than those obtained by Zelaya (2005) and Garza (2004). Commercial shrimp feeds are commonly reported to include fish meal levels between 25% and 50% of the total diet (Dersjant-Li, 2002; Tacon and Barg, 1998). However, recent studies have shown that commercial shrimp feeds containing 30-35% crude protein can include levels as low as 7.5-12.5% fish meal without compromising shrimp performance (Fox *et al.*, 2004). The inclusion of soybean and corn gluten meals for the partial and total replacement of fish meal protein in the present study resulted in no adverse affects on the performance of shrimp. Considering that all the test diets had a fixed level of poultry by-product meal (16%), it can be concluded that up to 71.5 % of the dietary protein can be provided by high quality plant protein sources (0% FM feed) in shrimp diets (Table 3). One of the options proposed to significantly reduce the production costs of the shrimp industry is to replace fish meal with vegetable protein sources in the formulation, therefore reducing the cost of the feeds. In this study, feed costs were slightly reduced as more plant proteins were included in the formulation. When evaluated in terms of the total cost of the feed inputs per treatment, significant differences were found among the treatments, indicating that under the conditions of this study, feeding cost were effectively reduced by increasing the level of plant proteins in the diet (Table 9). On the other hand, there were no significant differences in the partial gross returns obtained when subtracting the total costs of the feed inputs from the total value of the final production. Thus, at current ingredient prices and at the substitution levels evaluated in this study, plant protein substituted for fish meal in a properly formulated and manufactured feed is a cost effective practice. If production costs are to be reduced, shrimp producers must not only have well balanced diets but they must also feed them properly. This means that overfeeding must be minimized and avoid nutrient loading that goes beyond the assimilation capacity of the culture system. In addition, in cases where the animal has the potential of using natural productivity, the producer must take advantage of this capacity as a way to improve FCR. It is generally accepted then, that besides feed inputs, growth and feed utilization by shrimp reared in minimal water exchange pond systems are influenced by the shrimp ability to consume the microbial organisms produced within the culture system (Tacon *et al.*, 2002). Thus, it is clear that the nutrition and feeding of shrimp must be studied under conditions which mimic as closely as possible those of the intended farm production unit and environment (Tacon 1996). Likewise, technologies aimed to reach feed and shrimp producers require the delivery of information produced under conditions comparable to those used commercially.

Table 9. Mean economic parameters of *Litopenaeus vannamei* reared in ponds at the end of an 18-week culture period and fed four practical diets with varying levels of fish meal (FM) and plant protein sources[1]. Estimates are based on shrimp production per hectare[1].

Parameter	9% FM	6% FM	3% FM	0% FM	PSE	P Value
Final production (Kg/ha)	5,847	5,363	6,548	6,347	360.8	0.145
Production value (USD/ha)[2]	21,134[a]	19,131[a]	23,889[a]	23,445[a]	1,562	0.169
Total feed inputs (kg/ha)	7,244[a]	7,232[a]	7,244[a]	7,232[a]	13.5	0.835
Feed price (USD/kg)[3]	0.531	0.526	0.52	0.515	-	-
Total cost of feed inputs (USD)[4]	3,849[a]	3,802[b]	3,769[c]	3,723[d]	7.2	<0.0001
Partial gross returns (USD)[5]	17,285[a]	15,328[a]	20,119[a]	19,722[a]	1559	0.159

[1] Means (n = 4) not sharing a common supercript within a row are significantly different ($P < 0.05$) based on Student Newman-Keuls multiple range test.

[2] Calculated based on prices and size distribution from Table 8.

[3] Price on May 2005. Subject to change based on varying ingredients price. Prices were based on those at the time of the research. 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.

[4] Total feed inputs cost = Total Feed Inputs (kg/ha) * Feed price (USD/kg).

[5] Partial Gross Returns = Production value (USD) - Total Feed Cost (USD/kg).

In recent years, co-extrusion of feed ingredients has been considered as a reasonable option to improve the nutritional quality of soybean meal when used as a replacement ingredient in laboratory manufactured shrimp feeds (Davis and Arnold, 2000; Mendoza *et al.*, 2001; Samocha *et al.*, 2004). Results from this study are in agreement with findings from these authors, in terms that commercial extrusion may have an important role in improving the overall nutritional quality of shrimp diets including high levels of plant protein sources. However, further studies are still required to evaluate the real benefits of using previously co-extruded products as ingredients of shrimp diets that will be posteriorly extruded.

Conclusion

Results from this study demonstrate that in commercially manufactured shrimp feeds, fish meal can be completely removed from the formulation using alternative vegetable protein sources in combination with poultry by-product meal without compromising the production performance and economic returns of *Litopenaeus vannamei* reared in pond systems. It is likely that the positive response of shrimp fed the replacement feeds, was the result of a combination of adequate feed formulation and manufacture, accurate feed inputs and management of the pond ecosystem and the contribution of natural food organisms to the total feed intake. Further pond studies evaluating the potential of *Litopenaeus vannamei* to utilize feeds without any animal proteins are suggested.

Aknowledgements

The authors would like to extend their thanks to those who have taken the time to critically review this manuscripts as well as those who helped in supporting this research at the Claude Peteet Mariculture Center and in Auburn University. This research was supported in part by the United Soybean Board. Mention of a trademark or proprietary product does not constitute an endorsement of the product by Auburn University and does not imply its approval to the exclusion of other products that may also be suitable.

References

Akiyama, D.M., 1988. Soybean meal utilization by marine shrimp. AOCS world congress on vegetable protein utilization in human food and animal feedstuffs, Singapore, October 2-7. American Soybean Association, Singapore.

Akiyama, D.M., Dominy, W.G., Lawrence, A.L., 1991. Penaeid shrimp nutrition for the commercial feed industry revised. In: Proceedings of the Aquaculture Feed Processing and Nutrition Workshop. Thailand and Indonesia,(ed. by D.M. Akiyama and R.KH. Tan) Sept. 19-25. American Soybean Association, Singapore. pp. 80-90.

APHA (American Public Health Association), American Water Works Association, and Water Pollution Control Association. 1989. Standard Methods for the Examination of Water and Waste Water, 17th edition. American Public Health Association, Washington, D.C., USA.

Carver, L.A., Akiyama, D.M., Dominy, W.G., 1989. Processing of wet shrimp heads and squid viscera with soy meal by a dry extrusion process. American Soybean Association Technical Bulletin. 3, 777 Craig Road, St. Louis, MO, USA. AQ16, 89-4.

Colvin, L.V., Brand, C.W., 1977. The protein requirement of penaeid shrimp at various life-cycle stages in controlled environment systems. Proc. World Maric. Soc. 8, 821-840.

D'Abramo, L.R., Castell, J.D., 1997. Research methodology. In: Crustacean Nutrition, Advances in World Aquaculture (ed. by L.R. D'Abramo D.E. Conklin and D.M. Akiyama), pp. 3-25. World Aquaculture Society, Louisiana.

Davis, D.A., Arnold, C.R., 2000. Replacement of fish meal in practical diets for the Pacific white shrimp, *Litopenaeus vannamei*. Aquaculture185, 291-298.

Davis, D.A.,Samocha, T.M., Bullis, R.A., Patnaik, S., Browdy, C., Stokes, A. and Atwood, H., 2004. Practical Diets for *Litopenaeus vannamei* (Boone. 1931): Working Towards Organic and/or All Plant Production Diets. Avances en Nutricion Acuicola VII. Memorias del VII Simposium Internacional de Nutricion Acuicola. 16-19 Noviembre, 2004. Hermosillo, Sonora, Mexico

Dersjant-Li, Y., 2002. The use of soy protein in aquafeeds. Avances en Nutricion Acuicola VI. Memorias del VI Simposium Internacional de Nutricion Acuicola. 3 al 6 de Septiembre del 2002. Cancun, Quintana Roo, Mexico.

Divakaran, S., Velasco, M., Beyer, E., Forster, I., Tacon, A.G.J., 2000. Soybean meal apparent digestibility for *Litopenaeus vannamei*, including a critique of methodology. Avances en Nutricion Acuicola V. Memorias del V Simposium Internacional de Nutricion Acuicola. 19-22 Noviembre, 2000. Merida, Yucatan, Mexico.

Food and Agriculture Organization FAO, 2005. FAO Fisheries Department, Fishery Information, Data and Statistics Unit. FISHSTAT Plus: Universal software for dtatistical time series. Version 2.3, 2000.

Fox, J.M., Lawrence, A.L., Smith, F., 2004. Development of a low-fish meal feed formulation for commercial production of *Litopenaeus vannamei*. Avances en Nutricion Acuicola VII. Memorias del VII Simposium Internacional de Nutricion Acuicola. 16-19 Noviembre, 2004. Hermosillo, Sonora, Mexico

Garza, A., Rouse, D.B., Davis, D.A., 2004. Influence of nursery period on the growth and survival of *Litopenaeus vannamei* under pond production conditions. J. World Aquac. Soc. 35 (3), 357-365.

Hardy, R.W., 1999. Alternate protein sources. Feed Management 50, 25-28.

Hernandez, C., Sarmiento-Pardo, J., Gonzalez-Rodriguez, B., Abdo de la Parra, I., 2004. Replacement of fish meal with co-extruded wet tuna viscera and corn meal in diets for white shrimp (*Litopenaeus vannamei*, Boone) Aquac. Res. 36, 834-840.

Hertrampf, J.W., Piedad-Pascual, F., 2000. Handbook on Ingredients for Aquaculture Feeds. Kluwer Academic Publishers (ed.). Dordrecht, The Netherlands. 573 p.

Josupeit, H., 2004. An overview of the world shrimp market. World Shrimp Markets 2004, 26-27 October 2004, Madrid, Spain. http://www.globefish.org/

Lim, C., Dominy, W., 1990. Evaluation of soybean meal as a replacement for marine animal protein in diets for shrimp (Penaeus vannamei). Aquaculture 87, 53-63.

Lim, C., Klesius, P.H., Dominy, W., 1998. Soyabean products. International Aqua Feeds 3, 17-23.

Mendoza, R., De Dios, A., Vazquez, C., Cruz, E., Ricque, D., Aguilera, C., Montemayor, J., 2001. Fishmeal replacement with feather-enzymatic hydrolyzates co-extruded with soya-bean meal in practical diets for the Pacific white shrimp (*Litopenaeus vannamei*). Aquac. Nutr. 7, 143-151.

Moss, S.M., Divakaran, S., Kim, B.G., 2001. Stimulating effects of pond water on digestive enzyme activity in the Pacific white shrimp, *Litopenaeus vannamei* (Boone). Aquac. Res. 32, 125-131.

Moss, S.M., Pruder, G.D., 1995. Characterization of organic particles associated with rapid growth in juvenile white shrimp, *Penaeus vannamei* (Boone), reared under intensive culture conditions. J. Exp. Mar. Biol. Ecol. 187, 175-191.

NRC, National Research Council. 1993. Nutrient requirements of fish.

Samocha, T., Davis, D.A., Saoud, I.P., DeBault, K., 2004. Substitution of fish meal by co-extruded soybean poultry by-product meal in practical diets for the Pacific white shrimp, *Litopenaeus vannamei*. Aquaculture 231, 197-203.

Steel, R.G.D., Torrie, J.H., 1980. Principles and Procedures of Statistics: A Biometrics Approach. McGraw-Hill, New York, NY, USA.

Swick, R.A., Akiyama, D.M., Boonyaratpalin, M., Creswell, D.C., 1995. Use of soybean meal and synthetic methionine in shrimp feed. American Soybean Association, Technical Bulletin (Vol. AQ43-1995).

Tacon A.G.J., 1996. Nutritional studies in crustaceans and the problems of applying research findings to practical farming systems. Aquac. Nutr. 1, 165-174.

Tacon, A.G.J., Barg, U.C., 1998. Major challenges to feed development for marine and diadromous finfish and crustacean species. In: De Silva, S.S. (Ed.), Tropical Mariculture. Academic Press, San Diego, CA, USA, 171 - 208.

Tacon, A.G.J., Cody, J.J., Conquest, L.D., Divakaran, S., Forster, I.P., Decamp, O.E., 2002. Effect of culture system on the nutrition and growth performance of Pacific white shrimp *Litopenaeus vannamei* (Boone), fed different diets. Aquac. Nutr. 8, 121-137.

Venero, J., 2006. Optimization of dietary nutrient inputs for Pacific white shrimp *Litopenaeus vannamei*. Doctoral dissertation. Auburn University, Auburn, Alabama, USA. .

Zelaya, O., 2005. An evaluation of nursery techniques and feed management during culture of marine shrimp *Litopenaeus vannamei*. Doctoral dissertation. Auburn University, Auburn, Alabama, USA.

CHAPTER III

ALTERNATIVE DIETS FOR THE PACIFIC WHITE SHRIMP *Litopenaeus vannamei*

Abstract

Future use of animal protein sources in aquatic animal feeds is expected to be considerably reduced as a consequence of increasing economical, environmental and safety issues. Shrimp research has recently focused on the development of alternative feeds, with minimal levels of marine protein sources. To determine shrimp capacity to use practical feeds with varying levels of plant proteins, an 81-day growth trial was conducted using juvenile (0.74 g) *Litopenaeus vannamei* stocked into replicated outdoor culture tanks. 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 at the end of the 81-day culture period. Final values for these parameters ranged from 17.4 - 19.5 g, 2,249 - 2,465 %, 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 shrimp diets including 16 % poultry by-product meal. Additionally, results demonstrate the good performance can be obtained by using a combination of plant protein sources (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.

Keywords: *Litopenaeus vannamei*, Soybean meal, Fish meal, Plant proteins.

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 its 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 a one way to 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; Hardy, 1999; Divakaran *et al.*, 2000; Davis and Arnold, 2000; Conklin, 2003; 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, 1991; Akiyama *et al.*, 1991). Furthermore, proper feed manufacture and feedstuffs processing have been found to improve the overall nutritional quality and water stability of replacement feeds (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 *et al.*, 1995; 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.

Materials and Methods

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 ± 0.031g) 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 (0.85m height x 1.22 m upper diameter, 1.04 m lower diameter) designed to contain 800-L each and a reservoir tank (800-L) with a biological filter. Before stocking, the system was filled with brackish green water (9.50 ppt) from a shrimp production pond. Daily water exchanges were performed during the experimental period, between 4:00 am and noon, when pond water was pumped into the central filter at a rate of 8 liters per minute. This exchange rate allowed a 100% water exchange in the recirculating system 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 1-hp regenerative blower.

During the experimental period, dissolved oxygen (DO), temperature, salinity and pH concentrations were measured in the central filter 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).

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.

Table 1. Ingredient composition of practical diets for *Litopenaeus vannamei* used to evaluate the replacement of animal proteins by plant protein sources (values expressed on an as fed basis, g/100 g). Diets were commercially manufactured by Ranger® Inc. (Angleton, TX) using extrusion processing.

Ingredient	9% FM	6% FM	3% FM	0% FM	Plant Based
Soybean Meal	32.48	34.82	37.17	39.52	56.46
Fish Meal-Menhaden	9.00	6.00	3.00	-	-
Poultry By-product meal	16.00	16.00	16.00	16.00	-
Milo	35.47	33.82	32.33	30.68	14.99
Corn Gluten	-	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

Table 2. Nutritional composition of practical diets for *Litopenaeus vannamei* used to evaluate the replacement of animal proteins with plant protein sources[1].

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[2]	1.3	1.3	1.2	1.1	0.9	-
Total Phosphorus[2]	1.2	1.2	1.2	1.3	1.2	-
Lysine[2]	2.0	2.0	1.9	1.8	2.0	-
Met + Cys[2]	1.1	1.1	1.1	1.1	1.1	-
% Protein from plant sources[2,3]	54.9	60.4	66.0	71.5	97.7	-
% Protein from animal sources[2,4]	45.1	39.6	34.0	28.5	2.3	-

[1] Analyzed value - New Jersey Feed Laboratory, Inc. Trenton, NJ.

[2] Formulated value.

[3] Soybean meal, corn gluten meal, milo, corn fermented solubles.

[4] Fish meal, poultry by-product meal.

[5] Squid meal.

Lipid levels were adjusted by adding menhaden fish oil, which is a rich source of highly unsaturated fatty acid (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 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 feasability 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.

Statistical Analyses

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).

Results

Water quality parameters throughout the 81-day growth trial were maintained at suitable levels for adequate growth and survival of shrimp (Table 3). 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 - 19.5 g, 2,249 - 2,465 %, 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 *Litopenaeus 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, except for survival. 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 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 (2,249%) 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.

Table 3. Summary of water quality parameters observed over an 81-day 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 throughout the study.

	Temperature (C)	DO[1] (mg/l)	pH	Salinity (ppt)	TAN (mg/l)[2]
am	27.4 ±1.3	6.70 ± 0.56	7.30 ± 0.28	8.3 ± 0.8	0.31 ± 0.35
	(24.1, 29.7)	(3.79, 7.67)	(6.24, 8.18)	(7.0, 9.5)	(0.00, 0.88)
pm	29.6 ± 1.8	6.66 ± 0.54	7.66 ± 0.25		
	(23.9, 32.8)	(4.70, 7.85)	(7.17, 8.29)		

[1] Dissolved Oxygen.

[2] Total Ammonium Nitrogen. Weekly values.

Table 4. Mean production parameters at the end of an 81-day culture period for *Litopenaeus vannamei* reared in an outdoor semi-closed recirculating culture system and fed practical diets with varying levels of fish meal (FM), a plant based diet and a commercial reference diet[1].

Parameter	9% FM	6% FM	3% FM	0% FM	Plant	Ref.	PSE [2]	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 (%)[3]	2,443	2,439	2,391	2,249	2,465	2,401	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	574.0	639.0	18.752	0.055
FCR[4]	1.13	1.12	1.11	1.20	1.13	1.07	0.030	0.108
Survival (%)	85.0	89.2	88.3	86.7	83.3	87.5	2.766	0.687

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

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

[3] Weight gain (%) = 100 x (final weight - initial weight) / initial weight.

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

Table 5. Mean economic parameters of *Litopenaeus 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[1].

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)[2]	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)[3]	0.531	0.526	0.520	0.515	0.532	-	-
Total feed inputs cost (USD)[4]	0.34	0.35	0.34	0.33	0.33	0.778	0.01
Partial gross returns (USD)[5]	1.79	1.91	1.92	1.71	1.75	0.120	0.06
Feed cost per kg of shrimp produced (USD)[6]	0.60	0.59	0.58	0.62	0.60	0.308	0.015

[1] Based on analysis of variance (ANOVA) no significant differences ($P > 0.05$) were found among treatment means ($n = 4$). Reference feed excluded from the economic analysis.

[2] Production Value = Shrimp production per m^3 x $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 .

[3] Price on May 2005. Subject to change based on varying ingredients price. Prices were based on those at the time of the research. 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.

[4] Total feed inputs cost = Total Feed Inputs (g/m^3) x Feed price (USD/kg).

[5] Partial Gross Returns = Production value (USD) - Total Feed Cost (USD/kg).

[6] Feed cost per kg of shrimp produced = FCR x Feed price (USD/kg).

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 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.

Discussion

Results from this study provide important information regarding the potential of *Litopenaeus vannamei* to utilize alternative feed formulations under commercial production conditions. This research was conducted in replicated outdoor tanks using green water from a production pond and the 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 are indicative of the important potential of

Litopenaeus vannamei 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 (2,465%) 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 the 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 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; 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 *Litopenaeus 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 *Litopenaeus 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 diets are recommended.

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 *Litopenaeus 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.

Aknowledgements

The authors would like to extend their thanks to those who have taken the time to critically review this manuscripts as well as those who helped support this research at the Claude Peteet Mariculture Center and at Auburn University. This research was supported in part by the United Soybean Board. Mention of a trademark or proprietary product does not constitute an endorsement of the product by Auburn University and does not imply its approval to the exclusion of other products that may also be suitable.

References

Akiyama, D.M., 1988. Soybean meal utilization by marine shrimp. AOCS world congress on vegetable protein utilization in human food and animal feedstuffs, Singapore, October 2-7. American Soybean Association, Singapore.

Akiyama, D.M., Dominy, W.G., Lawrence, A.L., 1991. Penaeid shrimp nutrition for the commercial feed industry revised. In: Proceedings of the Aquaculture Feed Processing and Nutrition Workshop. Thailand and Indonesia,(ed. by D.M. Akiyama and R.KH. Tan) Sept. 19-25. American Soybean Association, Singapore. pp. 80-90.

APHA (American Public Health Association), American Water Works Association, and Water Pollution Control Association. 1989. Standard Methods for the Examination of Water and Waste Water, 17^{th} edition. American Public Health Association, Washington, D.C., USA.

Carver, L.A., Akiyama, D.M., Dominy, W.G., 1989. Processing of wet shrimp heads and squid viscera with soy meal by a dry extrusion process. American Soybean Association Technical Bulletin. 3, 777 Craig Road, St. Louis, MO, USA. AQ16, 89-4.

Colvin, L.V., Brand, C.W., 1977. The protein requirement of penaeid shrimp at various life-cycle stages in controlled environment systems. Proc. World Maric. Soc. 8, 821-840.

Conklin, D.E., 2003. Use of Soybean Meal in the Diets of Marine Shrimp. Department of Animal Science, University of California, Davis, United Soybean Board and American Soybean Association. Technical review paper AQ 144-2003.

D'Abramo, L.R., Castell, J.D., 1997. Research methodology. In: Crustacean Nutrition, Advances in World Aquaculture (ed. by L.R. D'Abramo D.E. Conklin and D.M. Akiyama), pp. 3-25. World Aquaculture Society, Louisiana.

Davis, D.A., Arnold. C.R., 2000. Replacement of fish meal in practical diets for the Pacific white shrimp, *Litopenaeus vannamei*. Aquaculture185, 291-298.

Davis, D.A.,Samocha, T.M., Bullis, R.A., Patnaik, S., Browdy, C., Stokes, A. and Atwood, H., 2004. Practical Diets for *Litopenaeus vannamei*, (Boone. 1931): Working Towards Organic and/or All Plant Production Diets. Avances en Nutricion Acuicola VII. Memorias del VII Simposium Internacional de Nutricion Acuicola. 16-19 Noviembre, 2004. Hermosillo, Sonora, Mexico

Decamp, O., Cody, J., Conquest, L., Delanoy, G., Tacon, A.G.J., 2003. Effect of salinity on natural community and production of *Litopenaeus vannamei*, (Boone), within experimental zero-water exchange culture systems. Aquac. Res. 34, 345-355.

Dersjant-Li, Y., 2002. The use of soy protein in aquafeeds. Avances en Nutricion Acuicola VI. Memorias del VI Simposium Internacional de Nutricion Acuicola. 3-6 de Septiembre del2002. Cancun, Quintana Roo, Mexico.

Divakaran, S., Velasco, M., Beyer, E., Forster, I., Tacon, A.G.J., 2000. Soybean meal apparent digestibility for *Litopenaeus vannamei*, including a critique of methodology. Avances en Nutricion Acuicola V. Memorias del V Simposium Internacional de Nutricion Acuicola. 19-22 Noviembre, 2000. Merida, Yucatan, Mexico.

Forster, I.P., Dominy, W., Tacon, A.G.J., 2002. The use of concentrates and other soy products in shrimp feeds. Avances en Nutricion Acuicola VI. Memorias del VI Simposium Internacional de Nutricion Acuicola. 3-6 de Septiembre del 2002. Cancun, Quintana Roo, Mexico.

Fox. J.M., Lawrence, A.L., Smith, F., 2004. Development of a low-fish meal feed formulation for commercial production of *Litopenaeus vannamei*. Avances en Nutricion Acuicola VII. Memorias del VII Simposium Internacional de Nutricion Acuicola. 16-19 Noviembre, 2004. Hermosillo, Sonora, Mexico

Gomez-Jimenez, S., Gonzalez-Felix, M.L., Perez-Velazquez, M., Trujillo-Villalba, D.A., Esquerra-Brauer, I.R., Barraza-Guardado, R., 2005. Effect of dietary protein level on growth, survival and ammonia efflux rate of *Litopenaeus vannamei* (Boone) raised in a zero water exchange culture system. Aquac. Res. 36, 834-840.

Gonzalez-Rodriguez, B., Abdo de la Parra, I., 2004 Replacement of fish meal with co-extruded wet tuna viscera and corn meal in diets for white shrimp (*Litopenaeus vannamei*, Boone). Aquac. Res. 35, 1153-1157.

Hardy, R.W., 1999. Alternate protein sources. Feed Management 50, 25-28.

Hernandez, C., Sarmiento-Pardo, J., Gonzalez-Rodriguez, B., Abdo de la Parra, I., 2004. Replacement of fish meal with co-extruded wet tuna viscera and corn meal in diets for white shrimp (*Litopenaeus vannamei*, Boone) Aquac. Res. 36, 834-840.

Hertrampf, J.W., Piedad-Pascual, F., 2000. Handbook on Ingredients for Aquaculture Feeds. Kluwer Academic Publishers (ed.). Dordrecht, The Netherlands. 573 p.

Josupeit, H., 2004. An overview of the world shrimp market. World Shrimp Markets 2004, 26-27 October 2004, Madrid, Spain. http://www.globefish.org/

Leber, K.M., Pruder, G.D., 1988. Using experimental microcosms in shrimp research: the growth-enhancing effect of shrimp pond water. J. World Aquac. Soc. 19, 197-203.

Lim, C., Dominy, W., 1990. Evaluation of soybean meal as a replacement for marine animal protein in diets for shrimp (*Penaeus vannamei*). Aquaculture 87, 53-63.

Lim, C., Ako, H., Brown, C.L., Hahn, K. 1997. Growth response and fatty acid composition of juvenile *Pennaeus vannamei* fed different sources of dietary lipid. Aquaculture 151, 143-153.

Lim, C., Klesius, P.H., Dominy, W., 1998. Soyabean products. International Aqua Feeds 3, 17-23.

Mendoza, R., De Dios, A., Vazquez, C., Cruz, E., Ricque, D., Aguilera, C., Montemayor, J., 2001. Fishmeal replacement with feather-enzymatic hydrolyzates co-extruded with soya-bean meal in practical diets for the Pacific white shrimp (*Litopenaeus vannamei*). Aquac. Nutr. 7, 143-151

Moriarty, D.J.W., 1997. The role of microorganisms in aquaculture ponds. Aquaculture 151, 333-349.

Moss, S.M., Divakaran, S., Kim, B.G., 2001. Stimulating effects of pond water on digestive enzyme activity in the Pacific white shrimp, *Litopenaeus vannamei* (Boone). Aquac. Res. 32, 125-131

Moss, S.M., Pruder, G.D., 1995. Characterization of organic particles associated with rapid growth in juvenile white shrimp, *Penaeus vannamei* Boone, reared under intensive culture conditions. J. Exp. Mar. Biol. Ecol. 187, 175-191.

Piedad-Pascual, F., Cruz, E.M., Sumalangcay, A., 1990. Supplemental feeding on *Penaeus monodon* juveniles with diets containing various levels of defatted soybean meal. Aquaculture, 89: 183-191.

Samocha, T., Davis, D.A., Saoud, I.P., DeBault, K., 2004. Substitution of fish meal by co-extruded soybean poultry by-product meal in practical diets for the Pacific white shrimp, *Litopenaeus vannamei*. Aquaculture 231, 197-203.

Schuur A.M., 2003. Evaluation of biosecurity applications for intensive shrimp farming. Aquac. Eng. 28, 3-19.

Steel, R.G.D., Torrie, J.H., 1980. Principles and Procedures of Statistics: A Biometrics Approach. McGraw-Hill, New York, NY, USA.

Swick, R.A., Akiyama, D.M., Boonyaratpalin, M., Creswell, D.C., 1995. Use of soybean meal and synthetic methionine in shrimp feed. American Soybean Association, Technical Bulletin (Vol. AQ43-1995).

Tacon A.G.J., 1996. Nutritional studies in crustaceans and the problems of applying research findings to practical farming systems. Aquac. Nutr. 1, 165-174.

Tacon, A.G.J., Barg, U.C., 1998. Major challenges to feed development for marine and diadromous finfish and crustacean species. In: De Silva, S.S. (Ed.), Tropical Mariculture. Academic Press, San Diego, CA, USA, 171 - 208.

Tacon, A.G.J., Cody, J.J., Conquest, L.D., Divakaran, S., Forster, I.P., Decamp, O.E., 2002. Effect of culture system on the nutrition and growth performance of Pacific white shrimp *Litopenaeus vannamei* (Boone) fed different diets. Aquac. Nutr. 8, 121-137.

Venero, J., 2006. Optimization of dietary nutrient inputs for Pacific white shrimp *Litopenaeus vannamei*. Doctoral dissertation. Auburn University, Auburn, Alabama, USA.

IV. SUMMARY AND CONCLUSIONS

The primary objective of this study was to evaluate the replacement of fish meal and other animal proteins by using alternative plant protein sources in commercially manufactured diets for *Litopenaeus vannamei* reared under green water conditions. Currently there is pressure to minimize the use of animal protein sources, mainly fish meal, in shrimp feeds. Limited fish meal supply and high demand for this ingredient by other animal productions make fish meal one of the primary ingredient costs in shrimp formulations. Regardless of this phenomena, commercial formulations have been commonly reported to contain between 25 - 50 % of fish meal of the total diet. With shrimp market prices not growing in value in recent years and feed representing the main cost associated with the production of shrimp, the evaluation of nutritional strategies focusing on replacing fish meal with cheaper protein sources seems a reasonable option to contribute to the reduction of shrimp production costs

The use of plant protein sources as alternative ingredients to fish meal have important ecological and economical implications. For example, reduced fish meal levels in shrimp diets would help minimize pressure on limited fish meal resources with the added advantage of helping to reduce the cost of the feeds as higher levels of cheaper plant protein sources are incorporated into the diets. In addition, alternative feeds (e.g. using organically-grown ingredients) will allow producers to reach higher value markets where consumers may be willing to pay a premium price for shrimp fed diets with no animal origin ingredients and produced using technologies and practices that represent a minimal threat to the environment or the human health. Shrimp do not have a specific requirement for ingredients of animal origin in their diet, rather, shrimp need to be supplied a basic level of nutrients to effectively fulfill their biological demands for adequate maintenance, growth and/or reproduction. Consequently, the key to the effective use of plant-based feeds by shrimp is to have an adequate knowledge of the nutritional requirements of the animal and a good understanding of limiting factors associated with the use of plant ingredients in practical diets. Besides the nutritional component, adequate ingredient processing and feed manufacturing also play a key role to further increase the use of plant protein sources in shrimp feeds. Properly processed, plant ingredients are characterized by having a low content of anti-nutritional factors and high digestibility and protein content, making them more suitable as a replacement for fish meal.

Based on these principles for the use of alternative shrimp diets and considering that the practical application of studies conducted in controlled, clear water conditions is limited, two studies were carried out in semi-commercial shrimp culture conditions to evaluate *Litopenaeus vannamei* potential to use alternative plant based diets in production systems with access to natural food organisms. The first study evaluated the performance of shrimp fed four practical diets with varying levels of fish meal (9, 6, 3, 0 %) and plant protein sources (soybean meal and corn gluten meal). All of the feeds evaluated in this study included poultry by-product meal at a fixed level of 16% of the diet. In this study, a minimal water exchange ponds production system was used to raise shrimp to commercial size over an 18-week experimental period. In the second study, the same four diets from the first study were evaluated in addition to a practical plant protein-based diet with no poultry by-product meal, and an additional commercial reference diet. In this study, shrimp were reared to commercial size in a green water semi-closed recirculating system, over an 81-day culture period.

Findings from these two studies demonstrated that in commercially manufactured shrimp diets including poultry by-product meal, fish meal can be completely replaced using alternative protein sources, such as soybean meal and corn gluten meal, without negatively compromising the productive performance and economic returns of *Litopenaeus vannamei* reared in practical shrimp production systems. Results from these studies also provided evidence that the cost of commercial shrimp diets can be reduced as increasing levels of plant sources are included in the diets as replacement ingredients to fish meal.

In addition to these findings, the second study also provided interesting information about the important potential of *Litopenaeus vannamei* to effectively use alternative plant-based diets. In this study, shrimp fed the plant-based diet showed no significant differences in their productive performance as compared to shrimp fed the diets including fish meal and/or poultry by-product meal and shrimp fed the commercial reference diet. These are encouraging findings; however, further work refining and improving the plant based diet must be carried out in order to remove squid meal, and ensure the nutritional adequacy of this diet.

It is likely that the positive response of shrimp fed the replacement feeds was the result of a combination of different elements, such as adequate feed formulation and manufacture, accurate feed inputs and management of the pond and tanks ecosystem and the contribution of natural food organisms to the total nutrient intake. Further studies evaluating the potential of *Litopenaeus vannamei* to utilize plant-based diets without any poultry by-product meal and squid meal are suggested.

Results from this study demonstrate that *Litopenaeus vannamei* reared in green water environments can use plant protein sources as replacement ingredients to fish meal in commercially manufactured shrimp diets without negatively affecting production while reducing feed costs. Based on the findings from this study, it is recommended to use plant proteins, such as solvent extracted soybean meal and corn gluten meal as effective alternative ingredients to fish meal and poultry by-product meal in shrimp feeds. These kind of ingredients can be successfully used without affecting animal performance in commercial shrimp production.

The most important implications from these findings are associated to the fact that current fish meal inclusion levels can be considerably minimized, up to a 0% of inclusion, using alternative plant protein ingredients in commercial shrimp diets. Increasing use of cheaper plant proteins will allow for a important reduction on the cost of the feeds as costly marine ingredients are removed from the formulations, or at least used at more efficient levels. In addition, the optimization on the use of fish meal in shrimp feeds will also result in a reduction in the demand for this limited ingredient while contributing to reduce the pressure on wild fish stocks, used for the production of fish meal. Furthermore, the possibility of reaching higher value markets that may help in improving profitability for shrimp producers will also be favored by the use of organic feeds not including animal origin ingredients. Although the performance of shrimp fed the plant-based diet in this study was good, there may be limitations of specific nutrients as well as palatability problems that should be further evaluated.

V. LITERATURE CITED

Akiyama, D.M., 1988. Soybean meal utilization by marine shrimp. AOCS world congress on vegetable protein utilization in human food and animal feedstuffs, Singapore, October 2-7. American Soybean Association, Singapore.

Akiyama, D.M., Dominy, W.G., Lawrence, A.L., 1991. Penaeid shrimp nutrition for the commercial feed industry revised. In: Proceedings of the Aquaculture Feed Processing and Nutrition Workshop. Thailand and Indonesia,(ed. by D.M. Akiyama and R.KH. Tan) Sept. 19-25. American Soybean Association, Singapore. pp. 80-90.

APHA (American Public Health Association), American Water Works Association, and Water Pollution Control Association. 1989. Standard Methods for the Examination of Water and Waste Water, 17th edition. American Public Health Association, Washington, D.C., USA.

Carver, L.A., Akiyama, D.M., Dominy, W.G., 1989. Processing of wet shrimp heads and squid viscera with soy meal by a dry extrusion process. American Soybean Association Technical Bulletin. 3, 777 Craig Road, St. Louis, MO, USA. AQ16, 89-4.

Colvin, L.V., Brand, C.W., 1977. The protein requirement of penaeid shrimp at various life-cycle stages in controlled environment systems. Proc. World Maric. Soc. 8, 821-840.

Conklin, D.E., 2003. Use of Soybean Meal in the Diets of Marine Shrimp. Department of Animal Science, University of California, Davis, United Soybean Board and American Soybean Association. Technical review paper AQ 144-2003.

D'Abramo, L.R., Castell, J.D., 1997. Research methodology. In: Crustacean Nutrition, Advances in World Aquaculture (ed. by L.R. D'Abramo D.E. Conklin and D.M. Akiyama), pp. 3-25. World Aquaculture Society, Louisiana.

Davis, D.A., Arnold, C.R., 2000. Replacement of fish meal in practical diets for the Pacific white shrimp, *Litopenaeus vannamei*. Aquaculture185, 291-298.

Davis, D.A.,Samocha, T.M., Bullis, R.A., Patnaik, S., Browdy, C., Stokes, A. and Atwood, H., 2004. Practical Diets for *Litopenaeus vannamei* (Boone. 1931): Working Towards Organic and/or All Plant Production Diets. Avances en Nutricion Acuicola VII. Memorias del VII Simposium Internacional de Nutricion Acuicola. 16-19 Noviembre, 2004. Hermosillo, Sonora, Mexico

Decamp, O., Cody, J., Conquest, L., Delanoy, G., Tacon, A.G.J., 2003. Effect of salinity on natural community and production of *Litopenaeus vannamei* (Boone), within experimental zero-water exchange culture systems. Aquac. Res. 34, 345-355.

Dersjant-Li, Y., 2002. The use of soy protein in aquafeeds. Avances en Nutricion Acuicola VI. Memorias del VI Simposium Internacional de Nutricion Acuicola. 3 al 6 de Septiembre del 2002. Cancun, Quintana Roo, Mexico.

Divakaran, S., Velasco, M., Beyer, E., Forster, I., Tacon, A.G.J., 2000. Soybean meal apparent digestibility for *Litopenaeus vannamei*, including a critique of methodology. Avances en Nutricion Acuicola V. Memorias del V Simposium Internacional de Nutricion Acuicola. 19-22 Noviembre, 2000. Merida, Yucatan, Mexico.

Food and Agriculture Organization FAO, 2005. FAO Fisheries Department, Fishery Information, Data and Statistics Unit. FISHSTAT Plus: Universal software for dtatistical time series. Version 2.3, 2000.

Forster, I.P., Dominy, W., Tacon, A.G.J., 2002. The use of concentrates and other soy products in shrimp feeds. Avances en Nutricion Acuicola VI. Memorias del VI Simposium Internacional de Nutricion Acuicola. 3-6 de Septiembre del 2002. Cancun, Quintana Roo, Mexico.

Fox. J.M., Lawrence, A.L., Smith, F., 2004. Development of a low-fish meal feed formulation for commercial production of *Litopenaeus vannamei*. Avances en Nutricion Acuicola VII. Memorias del VII Simposium Internacional de Nutricion Acuicola. 16-19 Noviembre, 2004. Hermosillo, Sonora, Mexico

Garza, A., Rouse, D.B., Davis, D.A., 2004. Influence of nursery period on the growth and survival of *Litopenaeus vannamei* under pond production conditions. J. World Aquac. Soc. 35 (3), 357-365.

Gomez-Jimenez, S., Gonzalez-Felix, M.L., Perez-Velazquez, M., Trujillo-Villalba, D.A., Esquerra-Brauer, I.R., Barraza-Guardado, R., 2005. Effect of dietary protein level on growth, survival and ammonia efflux rate of *Litopenaeus vannamei* (Boone), raised in a zero water exchange culture system. Aquac. Res. 36, 834-840.

Gonzalez-Rodriguez, B., Abdo de la Parra, I., 2004 Replacement of fish meal with co-extruded wet tuna viscera and corn meal in diets for white shrimp (*Litopenaeus vannamei*, Boone). Aquac. Res. 35, 1153-1157.

Hardy, R.W., 1999. Alternate protein sources. Feed Management 50, 25-28.

Hernandez, C., Sarmiento-Pardo, J., Gonzalez-Rodriguez, B., Abdo de la Parra, I., 2004. Replacement of fish meal with co-extruded wet tuna viscera and corn meal in diets for white shrimp (*Litopenaeus vannamei*, Boone) Aquac. Res. 36, 834-840.

Hertrampf, J.W., Piedad-Pascual, F., 2000. Handbook on Ingredients for Aquaculture Feeds. Kluwer Academic Publishers (ed.). Dordrecht, The Netherlands. 573 p.

Josupeit, H., 2004. An overview of the world shrimp market. World Shrimp Markets 2004, 26-27 October 2004, Madrid, Spain. http://www.globefish.org/

Leber, K.M., Pruder, G.D., 1988. Using experimental microcosms in shrimp research: the growth-enhancing effect of shrimp pond water. J. World Aquac. Soc. 19, 197-203.

Lim, C., Dominy, W., 1990. Evaluation of soybean meal as a replacement for marine animal protein in diets for shrimp (*Penaeus vannamei*). Aquaculture 87, 53-63.

Lim, C., Ako, H., Brown, C.L., Hahn, K. 1997. Growth response and fatty acid composition of juvenile *Penaeus vannamei* fed different sources of dietary lipid. Aquaculture 151, 143-153.

Lim, C., Klesius, P.H., Dominy, W., 1998. Soyabean products. International Aqua Feeds 3, 17-23.

Mendoza, R., De Dios, A., Vazquez, C., Cruz, E., Ricque, D., Aguilera, C., Montemayor, J., 2001. Fishmeal replacement with feather-enzymatic hydrolyzates co-extruded with soya-bean meal in practical diets for the Pacific white shrimp (*Litopenaeus vannamei*). Aquac. Nutr. 7, 143-151

Moriarty, D.J.W., 1997. The role of microorganisms in aquaculture ponds. Aquaculture 151, 333-349.

Moss, S.M., Divakaran, S., Kim, B.G., 2001. Stimulating effects of pond water on digestive enzyme activity in the Pacific white shrimp, *Litopenaeus vannamei* (Boone). Aquac. Res. 32, 125-131

Moss, S.M., Pruder, G.D., 1995. Characterization of organic particles associated with rapid growth in juvenile white shrimp, *Penaeus vannamei* (Boone), reared under intensive culture conditions. J. Exp. Mar. Biol. Ecol. 187, 175-191.

NRC, National Research Council. 1993. Nutrient requirements of fish.

Piedad-Pascual, F., Cruz, E.M., Sumalangcay, A., 1990. Supplemental feeding on *Penaeus monodon* juveniles with diets containing various levels of defatted soybean meal. Aquaculture, 89: 183-191.

Samocha, T., Davis, D.A., Saoud, I.P., DeBault, K., 2004. Substitution of fish meal by co-extruded soybean poultry by-product meal in practical diets for the Pacific white shrimp, *Litopenaeus vannamei*. Aquaculture 231, 197-203.

Schuur A.M., 2003. Evaluation of biosecurity applications for intensive shrimp farming. Aquac. Eng. 28, 3-19.

Steel, R.G.D., Torrie, J.H., 1980. Principles and Procedures of Statistics: A Biometrics Approach. McGraw-Hill, New York, NY, USA.

Swick, R.A., Akiyama, D.M., Boonyaratpalin, M., Creswell, D.C., 1995. Use of soybean meal and synthetic methionine in shrimp feed. American Soybean Association, Technical Bulletin (Vol. AQ43-1995).

Tacon A.G.J., 1996. Nutritional studies in crustaceans and the problems of applying research findings to practical farming systems. Aquac. Nutr. 1, 165-174.

Tacon, A.G.J., Barg, U.C., 1998. Major challenges to feed development for marine and diadromous finfish and crustacean species. In: De Silva, S.S. (Ed.), Tropical Mariculture. Academic Press, San Diego, CA, USA, 171 - 208.

Tacon, A.G.J., Cody, J.J., Conquest, L.D., Divakaran, S., Forster, I.P., Decamp, O.E., 2002. Effect of culture system on the nutrition and growth performance of Pacific white shrimp *Litopenaeus vannamei* (Boone), fed different diets. Aquac. Nutr. 8, 121-137.

Venero, J., 2006. Optimization of dietary nutrient inputs for Pacific white shrimp *Litopenaeus vannamei*. Doctoral dissertation. Auburn University, Auburn, Alabama, USA. .

Zelaya, O., 2005. An evaluation of nursery techniques and feed management during culture of marine shrimp *Litopenaeus vannamei*. Doctoral dissertation. Auburn University, Auburn, Alabama, USA.