Development and Application of Soybean Based Diets for Pacific White Shrimp *Litopenaeus vannamei*

by

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Abstract

Soybean meal is a readily available feedstuff that can be used in shrimp feed formulations. Soybean meal and its products have been found to be an acceptable protein ingredient with good digestibility for shrimp. Compared to marine animal meal, soybean meal has lower nutrient content in terms of protein, essential amino acids, highly unsaturated fatty acids and minerals. However, these shortages can be adjusted with supplements or blending with other products. Various mixtures of feed ingredients (e.g., animal by-product, vegetable protein, and plant protein concentrate) in association with soybean meal are options that provide more balanced nutrients than using soybean meal as a sole ingredient. There is little information on the use of diets containing high levels of soybean meal in combination with other ingredients. Therefore, a series of feeding experiments were conducted at the Alabama Department of Conservation and Natural Resources Marine Resource Division, Claude Peteet Mariculture Center in Gulf Shores, Alabama between May 2007 and September 2009, to evaluate the use of high levels soybean meal as a main protein source in combination with other potential ingredients in formulated diets for *L. vannamei*.

Results from these studies demonstrated that formulated diets containing approximately 36% protein and 8% lipid with balanced amino acid profiles can be formulated using high levels of soybean meal as the primary protein source. Good

results were demonstrated when using high levels of soybean meal in combination with poultry by-product meal, distiller's dried grains with solubles, pea meal or soy protein concentrates (SPC). Although better results were obtained at lower levels of inclusion, using soy protein concentrate as a substitute for soybean meal at inclusion of 20% and greater resulted in a reduction in mean final weights and an increase in FCR. The supplementation of methionine or fish soluble to the diets containing high level of SPC (40%) did not enhance growth of *L. vannamei*.

Overall, results from these studies reveal that use of high levels of soybean meal as a main protein source in combination with other potential ingredients (poultry by-product meal, distiller's dried grains with solubles, pea meal or soy protein concentrates (SPC) in formulated diets for *L. vannamei* is viable as long as essential nutrients in diet are properly balanced to meet shrimp nutritional requirements.

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CHAPTER I

INTRODUCTION

Pacific white shrimp, *Litopenaeus vannamei* (Boone) is native to the eastern Pacific Ocean from Sonora, Mexico to Northern Peru. Currently, it is the most popular cultured shrimp species and has experienced a dramatic increase in aquaculture production from 186,113 tonnes in 1999 to 2,296,630 tonnes in 2007 (FAO, 2009). The industry growth has been paralleled by an increase in shrimp feed production. The increases in demand and limitations of supply have resulted in some ingredients becoming less available and more costly, especially fish meal. Fish meal and other marine ingredients, are considered desirable ingredients in shrimp feed because of their nutrient content and palatability. In commercial feeds, fishmeal typically accounts for 20 to 30% of shrimp feed formulation (Tacon and Metain, 2008). The cost of fish meal has generally increased over time as a result of the uncertainty of availability and large fluctuations in the price. Furthermore, there are growing social and environment concerns regarding the long term sustainability of the use of fishmeal. In addition to feed prices increasing, the market value for shrimp has declined because of increased production and limited demand. This has resulted in a reduction in the profit margin for shrimp farmers. When margins were good, feed manufacturers could afford to use expensive ingredients and over formulate a diet. However, as the margin decreased feeds must become more cost effective. Feed costs can account for as much as

40% to 60% of production costs (Hertrampf and Piedad-Pascual, 2000). Feed costs and feed management both influence the investment in feeds. Reducing or removing costly protein sources through the use of a combination of less expensive and more economical protein sources could result in substantial saving in feed cost. Practical diets using plant based protein sources to replace fish meal has become an interesting alternative which could reduce these problems.

Use of renewable plant protein sources have become the focus of protein substitution studies in shrimp feeds around the world because of their acceptable protein level, suitable amino acid content, economic opportunity, and consistent quality (Watanabe, 2002). Formulated diets are designed to contain sufficient levels of nutrients to meet requirements using plant based protein sources for which production can be expanded and are often more cost effective. Feeding plant-based proteins to shrimp requires that the ingredients possess certain nutritional characteristics, such as low levels of fiber, starch (especially insoluble carbohydrates), and antinutrients. They must also contain a relatively high protein content, favorable amino acid profile, high nutrient digestibility, and reasonable palatability (Gatlin et al., 2007; Naylor et al., 2009).

Ten indispensable amino acids which are required for growth and maintenance of shrimp are arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine (Guillaume, 1997; Kanazawa, 1990). These amino acids should satisfy shrimp requirements to support optimum growth performance. Fish meal is considered ideal fish and shrimp production because of its high level of essential amino acids. On the other hand, plant protein sources contain lower levels of some essential amino acids (Tacon, 1994). Thus, the essential amino acid balance must be considered when diets are formulated to contain plant protein sources to replace fish meal. In general, the, amino acid profile of soybean meal is comparable with that of fish meal, albeit is lower in sulfur amino acids, i.e., methionine and cystine (Peres and Lim, 2008).

Soybean meal is often considered as the most reliable ingredient and cost effective source in shrimp feed because of its high protein content, high digestibility, relatively well-balanced amino acid profile, reasonable price and steady supply (Amaya et al., 2007a, b; Davis and Arnold, 2000). The protein digestibility was found higher in soybean protein than that in the marine animal meals (Akiyama, 1989). However, the inclusion of soybean meal at high levels or as a sole protein source has resulted in reduced performance of the shrimp (Lim and Dominy, 1990). This could be the results of imbalanced amino acid profiles or deficiencies of other dietary nutrients that were not taken into account.

Fish meal is utilized as a protein source but it also provides lipids, essential fatty acids, minerals and vitamins to the diet. Consequently, there will be most likely a need to use a variety of feed ingredients in association with soybean meal to provide a better balanced nutrient profile. Utilization of various potential protein sources in shrimp feeds such as animal by-product and other plant sources have been evaluated under different rearing condition (Amaya et al., 2007a, b; Cruz-Suarez et al., 2001; Lim and Dominy, 1990; Piedad-Pascual et al., 1990; Ray et al., 2009; Sudaryono et al., 1995). One of those ingredients that are considered a promising alternative for the substitution of fish meal in shrimp feeds is poultry by-product meal (Amaya et al., 2007a; Davis and Arnold, 2000; Markey, 2007; Samocha et al., 2004). Distiller's dried grains with solubles (DDGS) is also a potential protein source for shrimp feed because of its low cost and consistent supply as a co-product of the bio-ethanol production which is expected to increase rapidly in the next decade. Several studies reported the successful use of DDGS as an alternative protein source in fish and crustacean feeds without causing negative impact on growth performance (Cheng and Hardy, 2004; Coyle et al., 2004; Lim et al. 2007, 2009; Robinson and Li, 2008; Stone et al., 2005; Thompson et al., 2008; Webster et al., 1991, 1992; Wu et al., 1994). Pea meal is also another widely used feed ingredient, mostly in livestock because of its high energy, moderate protein level (22 – 26% crude protein), amino acid profile, and low cost (Borlongan et al., 2003). Several studies indicated that feed pea is another potential ingredient in fish and shrimp feeds (Bautista-Teruel et al., 2003; Booth et al., 2001; Borlongan et al., 2003; Burel et al., 2000; Carter and Hauler, 2000; Cruz-Suarez et al., 2001; Davis et al., 2002; Gomes et al., 1995; Gouveia and Davies, 2000). Because of the limitation in nutrient component of most ingredients, more than one ingredient is required for balanced feed formulations. Therefore, shrimp diets containing soybean meal as a main protein source should be combined with other alternative protein ingredients, i.e., poultry by-product meal, DDGS, and pea meal.

Soybean and its products are acceptable protein sources with good digestibility for shrimp. However, soybean meal is deficient in the essential amino acids (EAAs) such as methionine, lysine, and tryptophan as well as essential fatty acids and minerals (Lim and Dominy, 1990). Methionine is one of the ten essential or indispensable amino acids that are dietary essential for shrimp (Millamena et al., 1996). Thus, supplementation of sulfur amino acids, i.e., methionine or cystine, in soybean based diet to meet the shrimp requirement is recommended to provide a good growth response (Akiyama, 1989). Low levels of methionine found in soybean meal can also be countered by mixing with other protein sources and or the supplementation of synthetic methionine. Several studies had reported successfully replacing fish meal with soybean meal with a methionine supplement in Milkfish (Davis et al., 1995; Shiau et al., 2007). Conversely, diet containing only soybean protein with a methionine supplement was poorly utilized by red drum (Reigh and Ellis, 1992). McGoogan and Gatlin (1997) suggested that diets containing soybean meal with low levels or no fish meal may have palatability problems. Thus, the inclusion of attractants or palatability enhancers, e.g., fish soluble, may be considered. A reduction in feed intake was reported in largemouth bass fed diets with increased soybean meal levels (Kubitza et al., 1997; Cho et al., 1974). Similar results were observed in red drum (Davis et al., 1995; Reigh and Ellis, 1992) and Pacific white shrimp (Lim and Dominy, 1990).

There are other issues of using soybean meal as an alternative to fishmeal besides nutritional factor such as the presence of nutrient inhibitors. Raw soybean contains anti-nutritional factors such as trypsin inhibitors, lectins, oligosaccharides, antigens, and saponins that may affect the digestion and reduce nutrient availability to shrimp (Dersjant-Li, 2002). However, the effect of some of these anti-nutrients can be reduced by heat process (New, 1987). The processes of producing solvent extracted soybean meal (SE-SBM), typically referred to as soybean meal, is initiated by removing the hulls then the beans are rolled into flakes to remove oil using a solvent, and toasted to inactivate trypsin inhibitors. Soybean meal, containing about 48% crude protein, is widely used in shrimp feeds. However, this product still contains some anti-nutritional factors such as trypsin inhibitors and lectins (Dersjant-Li, 2002; Venero et al., 2008).

Another disadvantage is that, due to low protein content (48%) of SE-SBM, its use in nutrient dense diets may be restricted as a result of insufficient "room" in the formulation for all necessary components. Soy protein concentrate is produced from high quality, dehulled soybean seeds by removing most of the oil, water soluble non-protein constituents, and many of the anti-nutritional factors. It contains approximately 65% crude protein in soy protein concentrate (SPC) which is similar to that found in fish meal, and considerably higher than that of SE-SBM (Liu, 1997; Paripatananont et al., 2001). Most feed manufacturers prefer a reasonable level of a high protein ingredient in their formulations to allow room to manipulate other ingredients. Hence, soy protein concentrate could be used as an alternative ingredient to replace fish meal in shrimp diets.

The uses of SE-SBM as a protein source in diets of *L. vannamei* has received considerable attention with good results (Alvarez et al., 2007; Lim and Dominy, 1990; Samocha et al., 2004). The use of soy protein concentrate in aquatic feed has been reported in many fish species and in shrimp (Forster et al., 2002; Kaushik et al., 1995; Mambrini et al., 1999; Paripatananont et al., 2001; Storebakken et al., 1998). However, there is limited information available on the utilization of SPC in *L. vannamei* feed.

Because of the interest in reducing feed costs, considerable effort is being made in evaluating and demonstrating the applicability of plant based diets and to identify limiting factors in these feeds. Therefore, information is needed for the practical use of high levels of soybean meal and the use of plant based diets with soybean meal as a main protein source in combination with other potential ingredients as well as the practical use of soy protein concentrate in formulated diets for *L. vannamei*.

The overall objective of this research is to demonstrate the feasibility of using soybean protein in practical feeds for the Pacific white shrimp (*Litopenaeus vannamei*) reared under both laboratory and field conditions. Three specific objectives were included to identify the response of *L. vannamei* to different treatments:

1. To evaluate the response of *L. vannamei* to different protein sources as substitutes for fishmeal.

2. To evaluate the response of *L. vannamei* to increasing levels of SPC reared under clear water condition.

3. To evaluate the response of *L. vannamei* to the use of SPC in commercial feed formulations and reared under various culture conditions.

CHAPTER II

POND PRODUCTION OF PACIFIC WHITE SHRIMP (*Litopenaeus vannamei*) FED HIGH LEVELS OF SOYBEAN MEAL IN COMBINATION WITH POULTRY BY-PRODUCT MEAL, FISH MEAL, DISTILLER'S DRIED GRAINS WITH SOLUBLES OR PEA MEAL

Abstract

The objective of this study was to demonstrate the feasibility of four diets formulated to contain high levels of soybean meal in combination with either poultry by-product, fish meal, distiller's dried grains with solubles, or pea meal in production diets for *L. vannamei*. Outdoor ponds and tanks were utilized to evaluate the response of the shrimp over the culture period. The pond production trial was conducted in sixteen, 0.1-ha ponds using four replicates per diet. Post larvae were obtained from commercial sources and nursed for three weeks. Juvenile shrimps (mean weight ± S.D., 0.038 ± 0.004 g) were stocked at 35 shrimp m^{-2} and were cultured under standardized pond production conditions for 18 weeks. Daily feeding rates were predetermined using expected weekly growth of 1.5 g wk^{-1}, a feed conversion ratio (FCR) of 1.2, and estimated survival rate of 70% over an 18-week period. The same dietary treatments were offered to shrimp maintained in outdoor tanks (800 L) over a 12-week period. A total of 24 tanks, five tanks for each of the four test diets and four tanks for a commercial reference diet, were stocked with juvenile shrimp (initial weight of 2.1 g) obtained from production ponds at a

density of 30 shrimp per tank. Daily feed inputs were calculated, based upon an expected weight gain of 1.5 g per week and an expected feed conversion ratio of 1.2. At the conclusion of these experiments, final production, final individual weight, survival, and FCR were evaluated. The results for the pond study demonstrated that final production ranged between 4,729 and 5,794 kg ha^{-1}, mean final weight varied from 14.27 to 17.76 g, survival ranging from 74.6 to 99.8%, and FCR was between 1.22 and 1.46. There were no differences ($P \geq 0.05$) or notable trends in shrimp production variables among different test diets. Shrimp reared in the outdoor tanks confirmed these findings. Average final weights ranged between 17.88 and 20.76 g, survival varied from 93.3 to 100.0%, and FCR was between 1.2 and 1.42. The results from the outdoor tank study also showed no differences ($P \geq 0.05$) in final weight, survival, or FCR among test diets. Diet containing partial fishmeal showed no benefit on growth performance of *L. vannamei* comparing with diets containing high levels of soybean meal in combinations with poultry by-product, distiller's dried grains with solubles, or pea meal. Therefore, these studies demonstrated that practical shrimp feeds contain no fish meal had no negative impact on final production, final weight, survival, and FCR of *L. vannamei*.

Introduction

World shrimp production has been rapidly increasing as aquaculture production has increased. Pacific white shrimp *Litopenaeus vannamei* is the major cultured shrimp species which accounts for over 2.3 million tonnes in 2007 compared to 186,113 tonnes in 1999 (FAO, 2009). The expanded production has resulted in an increasing demand for shrimp feed and the ingredients used to make the feeds. This same trend has occurred across aquaculture sectors which currently rely on fish meal as one of the primary protein sources.

According to Tacon and Metian (2008), estimated production of commercial shrimp feed will be increased from 0.9 million tonnes (Mt) in 1995 to 9.2 Mt in 2020. Fish meal has often been used as the protein source in commercial shrimp feed because of its excellent sources of nutrients, e.g., balanced amino acid profiles, essential fatty acids, and mineral content (Tacon and Akiyama, 1997). The inclusion of fish meal generally averages 20% of shrimp feed formulation. These estimates suggest that the global demand for fish meal in marine shrimp feed is expected to increase from 0.38 Mt to 1.97 Mt from the year 2000 to 2015. The combination of high price and fluctuated supply of fish meal during the past few years resulted in an increasing of feed cost. Feed costs can account for as much as 40 to 60% of production costs (Hertrampf and Piedad-Pascual, 2000), it is critical to minimize the cost of feeds relative to production output.

In order to reduce feed cost, finding alternative protein sources to replace costly proteins such as fishmeal is necessary. There is considerable interest in optimizing the protein sources in shrimp feed using terrestrial animal and plant protein sources. This is because of their steady supply, consistent quality, and low cost which could contribute to reduced feed costs (Samocha et al., 2004; Watanabe, 2002) as compared to fish meal. The amino acid profiles in fish meal are considered highly suitable for fish and shrimp. Therefore, alternative protein sources used to replace fish meal must contain optimum amino acids profiles that support the shrimp nutritional requirements for growth and survival.

Because of their acceptable protein level, suitable amino acid content, economic opportunity, and consistent quality, the use of renewable plant protein sources as the alternative protein sources to replace fish meal has become the focus of studies in shrimp feeds around the world. Plant protein sources and their products, such as soybean, pea, cotton seed, corn gluten, wheat gluten, and distiller's dried grains soluble, have been successfully used in aquatic animal feeds (Smith et al., 1999; Alvarez et al., 2007; Samocha et al., 2004; Lim and Dominy, 1990; Mambrini et al., 1999; Carter and Hauler, 1999; Kikuchi, 1999; Boonyaratpalin et al., 1998; McGoogan and Gatlin, 1997; Webster et al., 1991, 1993, 1995; Sudaryono et al., 1995, 1999; Kaushik et al., 1995; Allan et al., 2000; Fontaínhas-Fernandes, 1999; Watanabe et al., 1993). Among these plant protein sources, soybean meal is considered one of the most promising ingredients and widely used as a protein source in fish and shrimp feeds because of its acceptable amino acid profile, consistent composition, and consistent availability at a reasonable price (Forster et al., 2002). However, soybean meal has potential problems associated with insufficient levels of lysine and methionine, presence of anti-nutritional factors, and poor palatability (Dersjant-Li, 2002). Additionally, soybean meal contains lower levels of minerals such as phosphorus and lack of n-3 highly unsaturated fatty acids (HUFAs), e.g., eicopentaenoic acid (EPA) and docosohexaenoic acid (DHA) compared to fish meal (Fox et al., 2004).

To overcome these possible problems when fish meal is removed from feed, a balanced nutrient profile in shrimp feed must be considered. Samocha et al. (2004) indicated using mixtures of complementary ingredients as a substitute for fish meal in shrimp diets, e.g., a co-extruded soybean poultry by-product meal with egg supplement, can increase nutrient utilization and facilitate processing of shrimp feed. Furthermore, Suarez et al. (2009) reported high survival (84 - 86.5%) and weight gain (0.98 g wk^{-1}) of *L. vannamei* fed test diets containing a mixture of soybean meal and canola meal with combinations of fish meal ranging from 0 to 15% in a recirculation system under clear water conditions.

Considerable research has demonstrated that substitution of fishmeal with soybean meal in combination with other ingredients in shrimp diets resulted in acceptable growth, survival, and FCR (Amaya et al., 2007a,b; Cruz-Saurez et al., 2001; Ray et al., 2010; Lim and Dominy, 1990, 1992; Sudaryono et al., 1995; Samocha et al., 2004; Piedad-Pascaul et al., 1990; Smith et al., 2001). Amaya et al. (2007a) evaluated the diets containing 0 to 9% fish meal in combination with 16% of poultry by-product meal, a plant base diet containing 1% squid meal, and a commercial diet for *L. vannamei* reared under production pond conditions and an outdoor semi-closed recirculating system. The results from these studies demonstrated that shrimp growth and production were not different among shrimp feed diets with and without fish meal. Moreover, Roy et al. (2009) demonstrated that diets containing combinations of vegetable protein and a diet containing 10% fish meal resulted in similar weight gain, survival, and FCR of Pacific white shrimp reared in low salinity water under laboratory and outdoor tank conditions. Several studies demonstrated that diets containing partially substitution or no fish meal could be fed to shrimp without negative impact to growth and production when feeds were formulated to contain well balanced of nutrients for animal (Amaya et al., 2007a, b; Davis and Arnold, 1990; Davis, 2004). Conversely, Lim and Dominy (1990) and Molina-Poveda et al. (2004) reported the decline in shrimp weight gain when the animals were fed with diets containing soybean as a total replacement of fishmeal compared to diets with partially fish meal. However, it should be noted that not all studies are in agreement about the successful uses of non fish meal diets for *L. vannamei*.

Clearly the use of soybean meal in shrimp feed is feasible. However, there are several other plant protein sources that may be considered as alternative ingredients used in association with soybean meal to balance nutritional composition in feed formulation when non-fishmeal diets are formulated. Distiller's dried grains with solubles (DDGS) which is a co-product of the ethanol production from grain has been considered as a protein source for aquatic animals as a result of its inexpensive cost per unit protein basis. The nutrient composition of DDGS varies with the type of the process as well as quality of raw material; however, on a dry basis, corn DDGS usually contains approximately 30% protein and 10% fat (Bonnardeaux, 2007; Spiehs et al., 2002). Additionally, DDGS have become more available and less expensive ingredients as a result of an increasing in bio-ethanol production. Several studies have evaluated DDGS as another promising alternative protein sources in fish and crustacean diets such as rainbow trout (Cheng and Hardy, 2004,;Stone et al., 2005), sunshine bass (Thomson et al., 2008), tilapia (Wu et al., 1994; Coyle et al., 2004; Lim et al., 2007), and channel catfish (Webster et al., 1991, 1992, 1993; Robinson and Li, 2008; Lim et al., 2009), fresh water prawn (Tidwell et al., 1993), and Pacific white shrimp (Lemos et al., 2009).

Feed pea meal is also widely used as a feed ingredient, mostly in livestock feed, because of its nutrient profile with moderate protein level (22 – 26% crude protein), amino acid balance, high energy, and low cost (Borlongan et al., 2003; Gatlin et al., 2007). According to Gatlin et al. (2007), utilization of raw feed pea meal in fish feed is limited as a result of its high level of indigestible carbohydrate; however, heat processing or extrusion helps to break down starch and improve digestibility of feed pea. Several studies have reported the use of feed pea as a potential ingredient in feed for rainbow trout (Burel, 2000; Gomes et al., 1995), milkfish (Borlangan et al., 2003), salmon (Carter and Hauler, 1999), silver perch (Booth et al., 2001), sea bass (Gouveia and Davies, 2000), and shrimp (Bautista-Teruel et al., 2003; Davis et al., 2002; Cruz-Suarez et al., 2001).

Another viable ingredient for replacing fish meal in feed is rendered by-products of terrestrial animal protein which generally contain 45 to 65% crude protein and are often good sources of indispensable amino acids, essential fatty acids, vitamins, and minerals along with good palatability (Samocha et al., 2004; Meeker, 2009). Poultry by-product meal contains protein at a level similar to fish meal (ranges from 56.4 to 84.2%), but less expensive and slightly lower in some essential amino acids (Shiau, 2008; Yu, 2008). Earlier studies indicated that poultry by-product meal is a promising alternative for the substitution of fishmeal and soybean meal in aquatic animal feeds (Samocha et al., 2004; Webster et al., 1999, 2000; Fowler, 1991; Garza, 2009; Saoud et al., 2008; Davis and Arnold, 2000; Amaya et al., 2007a, b; Markey, 2007).

Many studies have been reported the use of alternative protein sources as replacements of fish meal in feeds. However, no one has yet evaluated the use of these potential sources together in practical diets for *L. vannamei* reared under outdoor conditions. Therefore, the objective of the present study was to evaluate the use of high levels of soybean meal in combination with pea meal, distiller's dried grains solubles, poultry by-product meal, or fish meal as a protein source in feed for Pacific white shrimp reared under pond conditions and outdoor semi-closed system.

Methods

A series of experiments were conducted at the Alabama Department of Conservation and Natural Resources Marine Resource Division, Claude Peteet Mariculture Center in Gulf Shores, Alabama. Two growth trials were carried out in parallel which included a pond trial and outdoor tank trial, during June through September 2007.

Experimental animals

Shrimp post larvae (PL) were received from a commercial hatchery, Shrimp Improvement System, Key West, FL. Dissolved oxygen (DO), temperature, salinity, and pH were randomly measured among the shipping bags using a YSI 556 MPS meter (Yellow Spring Instruments Co., Yellow springs, OH, USA). Two water samples were collected in order to determine the total ammonia-nitrogen (TAN) using a spectrophotometer (Spectronic 20 Genesys, Spectronic Instrument Inc. Rochester, NY, USA) following the Nesslerization method (APHA 1989). An oxygen tank with one air stone was operated to provide oxygen during the acclimation period. Newly hatched brine shrimp, *Artemia salina* (INVE Americas, Inc., Salt Lake City, UT, USA), were prepared one night before PL reception, and were fed to PL in the acclimation tank. PL were slowly acclimated and were released into the acclimation tank. Water quality variables were measured to control temperature and salinity change in the range of 4 C hr^{-1} and 2 ppm hr^{-1}, respectively. After acclimation, PL were concentrated by drain harvesting into a 57-L concentration tank and quantified volumetrically. Six sub-samples were taking using a 60 ml beaker while the water in the concentration tank was mixing vigorously. Average numbers of PL per ml were then determined. Sixty shrimp were randomly collected and individually weighed to determine the average weight and predict the biomass for determination of feed inputs.

The shrimp were divided and spread equally into six nursery tanks (6,000 L). The nursery system was stocked at a density of 33 postlarvae per liter. Each nursery tank was equipped with three air lifts and two air stones to help with water circulation and aeration which was provided by a common regenerative blower. The recirculating nursery system was composed of six culture tanks, a biological filter, a pressurized sand filter and a 1.5-hp circulation pump (Aquatic Eco-systems, Apopka, Florida, USA). Feeds were applied on daily basis four times per day. Uneaten feed was siphoned out to maintain the water quality in the nursery tanks (Table 1). PL were raised in nursery system for a 3-week period. Water quality was monitored two times per day at 800 h and 1600 h., and TAN was measured biweekly (Table 2). Every three days, 60 PL per tank were randomly collected and sub-samples of 10 shrimps each were pooled weighed in order to evaluate growth and adjust daily feed rate.

At the end of three weeks, each tank was partially drained to concentrate the shrimp. The shrimp were then collected by dip nets. Six sub-samples were taken from each tank to determine average weight of shrimp (g), Once the number of shrimp per unit weight was determined, shrimp were spread evenly into 16 buckets each representing one production pond. Shrimp from each nursery tank were distributed across all ponds to minimize any bias from variation in nursery tanks. The shrimp were then transferred to the corresponding pond and stocked. Final population, weight gain (g), percentage survival, final weight (g), and feed conversion ratio for each nursery tank was determined (Table 3).

Experimental diets

Four experimental diets were formulated to contain soybean meal as the primary protein source with the inclusion of either 10% of poultry by-product meal, fish meal, distiller's dried grains with solubles, or ground pea meal (Table 4). To further improve the amino acid balance of the plant-based diets, methionine at 1.7% of total protein and corn gluten meal at 4.8% of the diet were also included. The experimental diets were manufactured by Rangen Inc., under commercial feed manufacturing condition as a sinking extruded pellet. All experimental diets contain approximately 36% protein and 8% lipid with lysine and methionine plus cystine contents of 5% and 3% of the total protein, respectively (Table 5, Table 6). Dietary treatments were tested in production ponds and outdoor tank system.

Production pond Trial

Ponds used for the grow-out phase were approximately 0.1 ha in surface area, (rectangular 46 x 20 m) with a 1.0 m average depth. Each pond was equipped with a 20-

cm diameter standpipe, a concrete catch basin (3.0 x 2.0 x 0.5 m), and lined with 1.52 mm thick high-density polyethylene lining (HDPE). The bottom of each pond was covered with a 25-cm deep layer of sandy-loam soil. After each culture cycle, ponds were dried and tilled in order to allow oxidation and mineralization of organic matter. Approximately three weeks before stocking, ponds were filled with brackish water from the intra-costal canal between Mobile and Perdido Bay. Incoming water was filtered through a three foot 250 μm-mesh nylon filter sock (Micron Domestic Lace Mfg., Inc) to prevent the introduction of predators, as fish larvae, crab, or other planktonic organisms, but allow the introduction of primary productivity..

Two weeks before stocking juvenile pacific white shrimp, the combination of inorganic liquid fertilizers, 32-0-0 mixed with 10-34-0, were applied in a rate of 1,697 ml and 303 ml, respectively, per pond which provided 5.73 kg of N and 1.03 kg of P_20_5 ha^{-1} in order to promote natural productivity in all ponds, and the second fertilization was reapplied at half of the initial rate when the Secchi disk reading was greater than 40 cm. All ponds were provided aeration about 10 hp ha^{-1} using 1-hp paddlewheels aerators (Little John Aerator, Southern Machine Welding Inc. Quinton, AL) with either 1-hp or 2-hp Aire-O_2 aerators (Aire-$O_{2,}$ Aeration Industries International, Inc. Minneapolis, Minnesota) to maintain dissolved oxygen above 3 mg L^{-1}, additional aerators were provided as needed when dissolved oxygen fell below 3 mg L^{-1}. This study was a sustainable semi-intensive system with well-managed feeding, and minimal water exchanged.

Juvenile *L. vannamei* (initial weight of 0.04 g) were collected from nursery system and stocked in the production ponds at the rate of 35 shrimp m^{-2}. Four test diets were randomly assigned to 16 production ponds using four replicates per diet. Rations were divided to two feedings per day, early morning 800 h and late afternoon 1600 h. Feeding strategy for the first four weeks was based on previous studies which assumed that PL feed on primary productivity. Thus, during this period, a small amount of feed was applied to promote natural productivity in pond. Thereafter, feed input was calculated based upon an expected weight gain of 1.3 g wk^{-1}, a feed conversion of 1.2, and a survival of 75% during the pond culture period (Table 7).

Dissolved oxygen (DO), temperature, salinity, and pH were monitored three times a day, at sunrise (0500 h - 0530 h), during the day (1500 h - 1530 h), and at night (2000 h - 2200 h), using a YSI 556 MPS meter. Secchi disk reading and TAN were monitored once weekly. Water samples were taken in all ponds at the depth of 80 cm and TAN was determined using a spectrophotometer (Spectronic 20 Genesys, Spectronic Instrument Inc. Rochester, NY, USA) following the Nesslerization method (APHA 1989). Each week, sixty shrimps were sampling using two 5-foot cast nets (monofilament net, 1.22 m radius and 0.95 cm opening) to determine the average weight.

The shrimp were harvested at the end of the 18-week study period. Feed was withdrawn 36 h before harvest in order to clear the shrimp gut. To harvest, about two thirds of the water was drained from ponds the night before harvest. The following day the remaining water was drained and the shrimp were pump harvested from the catch basin using a fish pump equipped with a 25-cm diameter suction pipe (Aqualife-Life pump, Magic Valley Heli-arc and Mfg, Twin Falls, Idaho, USA), and collected into the container truck. The shrimp were rinsed and weighed then approximately 150 shrimps from each pond were randomly selected to measure individual weight. Mean final weight, yields, percent survival, and FCR were determined.

Outdoor tank system

The outdoor tank system was a recirculating system consisted of a central reservoir (800 L) with a biological filter, a 0.33-hp sump pump, and 24, circular polyethylene tanks (0.85 m height x 1.22 m upper diameter, 1.04 m lower diameter). Another sump pump was used to move water from one of the production ponds to the central reservoir at a rate of 8 L min^{-1}during the study period between 800 h and 1400 h to mimic a production pond setting.

Juvenile *L. vannamei* (2.13 g initial weight) were collected from the research ponds and were size-sorted by hand. Juvenile shrimps were randomly selected and stocked at a rate of 30 shrimp per tank. Each tank and central reservoir were provided with two air stones connected to a 0.5-hp regenerative blower (Sweetwater Aquaculture Inc. Lapwai, ID, USA) to supply aeration. All tanks were covered by netting to prevent the shrimps from jumping out.

Five test diets were randomly assigned amongst the 24 tanks (750 L). Four experimental diets used in production ponds were tested using five replicates per diet. The fifth diet, a reference commercial diet (Rangen Shrimp Grower, 35% protein, 8% lipid), was tested using four replicates. Juvenile shrimp were offered test diets twice per day at 0800 h and 1600 h. Daily ration of feed was calculated based upon an expected weekly growth of 1.5 g wk^{-1} and expected FCR of 1.2.

Dissolved oxygen concentration, temperature, salinity, and pH were monitored twice a day at 600 h and 1600 h using a YSI meter. Water samples were taken bi-weekly from central reservoir and two tanks to measure TAN with a spectrophotometer according to previously described methods.

At the conclusion of the 10-week growth trial shrimp were counted and weighed individually. Mean final weight, final biomass, percent survival, and feed conversion ratio were determined.

Statistical analysis

The data (mean final weight, yields, percent survival, and feed conversion ratio) (FCR) were statistically analyzed using one-way analysis of variance to determine significant differences ($P<0.05$) among treatments, followed by the Student–Neuman–Keuls multiple comparison test to determine significant differences among treatment means (Steel and Torrie, 1980). All statistical analyses were carried out using SAS (V9.1. SAS Institute, Cary, NC, USA).

Results

Nursery system

Average water quality variables throughout the 22-day nursery period were as follows: dissolved oxygen 6.02 ± 1.03 mg L^{-1}; temperature, 26.08 ± 1.05 ^{o}C; pH 7.60 ± 0.15; salinity 23.61 ± 1.71 ppt; and TAN 0.37 ± 0.04 mg L^{-1} (Table 2). Water quality conditions were maintained within acceptable limits for the culture of this species. At the conclusion of the shrimp nursery period, mean individual weight 0.038 ± 0.003 g, weight gain 0.036 ± 0.003 g, survival 57.94 ± 7.57%, and FCR 1.12 ± 0.06 were observed (Table 3).

Production pond trial

The overall mean, standard deviation, and range of morning, afternoon, and night water quality variables observed throughout the 18-week growth trial are displayed in Table 8. Water quality conditions were within a reasonable range for the culture of this species. However, high temperature readings (above 35.0 ^oC) and high salinity readings (above 32.0 ppt) were recorded related to high air temperature and drought condition Also, high dissolved oxygen readings (above 10 mg L^{-1}) were observed in the afternoon due to photosynthetic activity. On the other hand, low dissolved oxygen readings (below 2.5 mg L^{-1}) were observed during periods that the phytoplankton died off (Boyd, 2007, 2009).

Growth performance of *L. vannamei* reared under production pond conditions is summarized in Table 9. There were no differences ($P > 0.05$) in any of the production parameters. Mean final weight ranged from 16.86 g (fish meal treatment) to 15.98 g (PBM treatment). Shrimp survival ranged from 86.6% (fish meal treatment) to 93.74% (PBM treatment). Yield ranged from 5,055 kg ha^{-1} (fish meal treatment) to 5,266 kg ha^{-1} (DDGS treatment), and FCR ranged from 1.33 (DDGS treatment) to 1.38 (fish meal treatment).

Outdoor tank trial

The overall mean, standard deviation, and range of morning and afternoon water quality variables observed over the 10-week growth trial were within acceptable limits for the culture of this species (Table 10). The outdoor tanks system had similar temperature (from 22.56 to 33.98 ^{o}C) and salinity readings (from 22.18 to 31.98 ppt) as compared to those observed in the pond water that was used as the system water supply.

Production results from the outdoor tanks were similar to those observed in the ponds. There were no differences ($P > 0.05$) in growth performances of *L. vannamei* reared in the outdoor tank system. Biomass loading ranged from 548.3 g tank^{-1} (DDGS treatment) to 559.58 g tank^{-1}(PBM treatment), mean final weight ranged from 18.53 g (DDGS treatment) to 19.00 g (pea meal treatment), percent survival ranged from 97.33% (pea meal treatment) to 98.67% (PBM, fish meal, DDGS treatments), and FCR ranged from 1.30 (PBM treatment) to 1.33 (DDGS treatment). Shrimp maintained on the commercial reference diets were not significantly different from those maintained on the test diets, albeit reference treatment had slightly higher biomass (579.83 g tank^{-1}) and final weight (19.67 g), and lower FCR (1.24) (Table 11).

Discussion

Fish meal and other marine ingredients are excellent nutrient sources of essential nutrients for aquatic animals. These ingredients are from limited resources and will not support the continued expansion of aquaculture. The use of marine products will continue to be systematically replaced by terrestrial agriculture products for which production can be expanded. To reduce costs and improve the sustainability of shrimp feeds, fish meal must be replaced by other ingredients that are more cost effective and are from renewable resources. Soybean meal is the most widely used alternative ingredient to replace fish meal because of its lower cost per unit protein and capability to be produced in mass production. Moreover, the protein content and amino acid profile in soybean meal are easily balanced to make them comparable to those in fish meal. Several studies have demonstrated that soybean meal in combination with other ingredients is a viable alternative for partially or even totally replacement of fish meal from shrimp feeds (Davis and Arnold, 2000; Amaya et al, 2007a, b; Samocha et al., 2004; Lim and Dominy, 1990; Molina-Poveda et al., 2004; Hernandez et al., 2004). These studies used nutrient balanced feed formulation, without causing negative impacts on growth performance of *L. vannamei*

The results from the present study demonstrate that, *L. vannamei* fed formulated diets containing soybean meal as a basal ingredient in combination with either 10% poultry by-product meal, fish meal, distiller's dried grains with solubles or ground pea meal resulted in similar yield, growth, survival, and feed conversion ratio of shrimp. Furthermore, treatments receiving diets with no fish meal showed no differences in performances compared to fish meal treatment or commercial reference treatment in both pond production and outdoor tank trials. The results from the present study confirm that fish meal in soybean meal-based diets can be totally replaced with either 10% poultry by-product meal, DDGS or pea meal.

Similar results were observed by Roy et al. (2009) who tested these same dietary treatments with juvenile *L. vannamei* reared in inland low salinity waters of west Alabama. Similar growth, survival, weight gain (%) and FCR were observed among treatments in both laboratory and on farm outdoor tank trials. Shrimp fed diets containing either 10% poultry by-product meal, distiller's dried grains with solubles, or pea meal performed as well as shrimp fed the fish meal diet. Markey (2007) also reported no differences in growth when shrimp were offered a soybean based diet mixed with poultry by-product meal (PBM) at level of 0, 5, 10, and 15% PBM as a replacement for fish meal in diets for *L. vannamei* used in both production pond and an outdoor tank trials. They obtained excellent results with mean final weight ranging from 19.4 to 24 g, survival from 80 to 96.7%, and feed conversion ratio from 1.0 to 1.1. Amaya et al. (2007a,b) fed diets containing 16% PBM in combination with soybean meal as a major protein source and the inclusion of corn gluten meal to replace fish meal (FM) in different levels at 9%, 6%, 3%, and 0% fish meal for *L. vannamei* reared in production ponds and green water outdoor tanks. The results show no differences in mean net yield, mean final weight, survival, and FCR of the shrimp. Samocha et al. (2004) reported no differences on growth responses of Pacific white shrimp fed practical diets containing 0% to 100% co-extruded soybean poultry by-product meal with egg supplement as a substitute for fish meal. Steffens (1994) also reported a viable use of poultry by-product meal in combination with feather meal at 27% inclusion as a partial replacement or with lysine and methionine supplementation at the complete replacement to fish meal in diets for rainbow trout. The results from this study confirm the viable use of soybean meal in combination with poultry by-product to substitute fish meal without affecting shrimp growth and production.

Distiller's dried grains with soluble (DDGS) are also one of the potential ingredients which are co-product of ethanol production. DDGS up to 30% can be used in channel catfish diet when lysine is supplemented (Robinson and Li, 2008; Lim et al., 2009). The results from this study are similar to the results reported by Robinson and Li (2008) that fish fed diet containing DDGS mixed with soybean meal and corn gluten meal had similar or higher weight gain and lower FCR compared to fish fed soybean meal-fish meal control diet. Likewise, Webster et al. (1991) reported the feasibility of DDGS used as a partial replacement for soybean meal with the inclusion of 10% fish meal in juvenile channel catfish diet. Fish fed diets contain 35% DDGS performed as well as fish fed control diet (0% DDGS. They also reported that, with lysine supplementation, up to 70% DDGS can be used in juvenile channel catfish diet. Additionally, Webster et al. (1992) demonstrated that fish meal in juvenile channel catfish diet can be totally replaced by 35% DDGS and soybean meal with supplemental lysine without adversely affecting their growth performance. Therefore, diet containing 10% DDGS inclusion with soybean meal and corn gluten meal can be a potential alternative for *L. vannamei* diet.

Pea meal has been successfully used in aquaculture feeds. The results of the present study show that shrimp fed 10% pea meal inclusion with soybean meal and corn gluten meal have equal performance to shrimp fed the other test diets. Similar result was reported by Cruz-Suarez et al. (2001) who successfully replaced soybean meal and wheat with 30% pea meal in diets for blue shrimp, *L. stylirostris* without affecting shrimp performance. Gouveia and Davies (2000) also demonstrated the feasibility of 40% pea seed meal inclusion to substitute fish meal in European sea bass diet. Another successful research of pea meal inclusion to replace soybean meal and whole wheat in *L. vannamei* diets was reported by Davis et al. (2002). The results after two 7-week growth trials under the laboratory showed no significant effects on shrimp performance when fed diets containing pea meal.

Besides feed ingestion, growth, feed conversion ratio, and survival of shrimp can be affected by water condition, e.g., temperature, salinity, etc. Ponce-Palafox et al. (1997) indicated that juvenile *P. vannamei* had the best survival and growth between temperatures of 28 and 30 ^{o}C and salinities between 33 and 40 ppt. The mean temperature and salinity reading from this study were in acceptable range for the culture of *Litopenaeus vannamei*. However, vary temperature readings ranged from 22.56 to 33.98 °C, and 24.97 to 35.68 °C were observed in the outdoor tanks and production ponds, respectively. Additionally, obviously high salinity readings between 21.90 – 32.78 ppt were recorded in this study compared to those earlier reported for this site for the production ponds.

As demonstrated in Figure 1, weekly weight gain of shrimp from the pond study tended to increase as average temperatures moved from 28 C to 32 C then decrease when water temperature in ponds were greater than 32 °C. This finding is similar to the results reported by Wyban et al. (1995), who reported decreasing growth of *P. vannamei* when pond temperature was below 23 ^{o}C and at 30 ^{o}C or greater. Mean survival rates of this study are excellent, 98.3% under tank trial and 90.3% under pond trial. Similar results had been reported by Bray et al. (1994), who found no differences on survival of *L. vannamei* across different salinities from 5 to 49 ppt. However, mean final weights of shrimp cultured at low salinity were significantly greater than at high salinity.

Mean final weight of shrimp reared in a 10-week outdoor tank trial (15.98 – 16.86 g) are greater than shrimp reared in a 17-week production pond trial (18.5 – 19.7 g) at the similar salinity, but temperature in ponds was 2 °C greater than in tanks. These results suggested that the reduction of growth performance of shrimp observed from this study may affect by high temperature or the interaction of high water temperature. However, these factors showed no effect on survival of shrimp.

In conclusion, the result from this study indicated that diets containing soybean meal as a primary protein source in combination with 10% of poultry by-product meal, fish meal, distiller's dried grains solubles or pea meal are viable under a variety of research conditions. This is in agreement with Roy et al. (2009) who reported that high levels of soybean meal can be used for commercial shrimp feed formulations. Additionally, fish meal and animal protein can be replaced by plant protein via well balanced feed formulation.

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Table 1. Feeding regime for *Litopenaeus vannamei* post larvae, based on mean shrimp weight determined every three days, percent body weight and feed types utilized throughout a 22-day nursery period.

Day	Mean weight (mg)	Feed type	Feed (% body weight)
1 to 3	1.54	100 Artemia[a] / PL, PL redi[b] (1:2)	25
4 to 7	2.53	PL redi, Cru[c] #0 (1:2)	25
8 to 9	4.53	Cru # 0	25
10 to 12	5.91	Cru # 0	15
13 to 15	10.73	Cru # 0, Cru #1& #2 (1:1)	15
16 to 22	18.20	Cru # 1& #2	15

[a]INVE Americas, Inc. Salt Lake City, UT, USA.

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

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

Table 2. Water quality variables observed over a 22-day nursery period for *L. vannamei* post larvae, nursed in a recirculating system composed of six 6,000-L nursery tanks.

	Average	Minimum	Maximum	Standard deviation	CV[a]
Temperature (°C)	26.08	23.41	28.54	1.05	4.03
DO ($mg\ L^{-1}$)[b]	6.02	3.32	7.83	1.03	17.06
Salinity (ppt)	23.61	21.30	27.04	1.71	7.23
pH	7.60	7.20	7.90	0.15	1.91
TAN ($mg\ L^{-1}$)[c]	0.37	0.32	0.45	0.04	10.69

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

[b]Dissolved oxygen.

[c]Total ammonia-nitrogen.

Table 3. Mean production parameters of *L. vannamei* post larvae (initial weight of 1.5 mg), stocked at a density of 33 PL L^{-1} and nursed for 22 days in a recirculating system composed of six 6,000-L nursery tanks.

	Mean	Minimum	Maximum	Standard deviation	CV[a]
Final weight (mg)	38.09	33.91	44.46	3.68	9.66
Weight gain[b] (mg)	36.59	32.41	42.96	3.68	10.06
Survival (%)	57.94	47.00	66.24	7.57	13.07
FCR[c]	1.12	1.01	1.17	0.06	5.53
Yield (g $tank^{-1}$)	4,353	4,146	4,836	263.80	6.06
Standing crop[d] (kg m^{-3})	1.209	1.152	1.343	0.073	6.060

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

[b]Weight gain = Final weight - Initial weight.

[c]Food conversion ratio = Total feed offered / biomass increase.

[d]Standing crop (kg m^{-3}) = (Yield/1,000)/3.6 m^3.

Table 4. Ingredient composition (g 100 g^{-1} as is) of four experimental diets utilizing soybean-based diets in combination with 10% poultry by-product meal (PBM), fish meal (FM), distiller's dried grains with soluble (DDGS) or ground pea meal (PM) as a protein source.

Ingredient	10% PBM	10% FM	10% DDGS	10% PM
Soybean meal	55.12	53.71	58.01	58.00
Milo	24.81	26.19	16.34	15.33
PBM	9.99	-	-	-
FM	-	10.01	-	-
DDGS	-	-	10.00	-
PM	-	-	-	10.00
Corn gluten meal	-	-	4.83	4.83
Dicalcium phosphate	2.90	2.90	3.38	3.42
Fish oil	5.08	5.09	4.83	5.82
Bentonite	1.50	1.50	1.50	1.50
Squid meal	-	-	0.50	0.50
Vitamin premix[a]	0.33	0.33	0.33	0.33
Mold inhibitor	0.15	0.15	0.15	0.15
Mineral premix[a]	0.08	0.09	0.09	0.09
Stay-C, 35% vit. C activity	0.02	0.02	0.02	0.02
Copper sulfate	0.01	0.01	0.01	0.01
Total	100	100	100	100

Diets were formulated to contain 36% protein and 8% lipid.

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

[a]Vitamin premix and mineral premix are proprietary products, thus their composition is not listed.

Table 5. Nutrient composition (dry matter basis) of four experimental diets containing soybean meal in combination with 10% poultry by-product meal (PBM), fish meal (FM), distiller's dried grains with solubles (DDGS) or ground pea meal (PM).

Nutrient	10% PBM	10% FM	10% DDGS	10% PM
Crude protein	35.9	35.5	38.2	36.1
Moisture	8.85	8.11	8.43	8.43
Fat	8.16	8.26	7.66	7.41
Crude fiber	1.78	1.77	7.95	2.39
Ash	8.40	9.59	6.68	7.90

Diets were formulated to contain 36% protein and 8% lipid.

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

Diets were analyzed by New Jersey Feed Laboratory, Trenton, NJ, USA (Roy et al., 2009).

Table 6. Amino acid profile of four experimental diets containing soybean meal in combination with 10% poultry by-product meal (PBM), fish meal (FM), distiller's dried grains with solubles (DDGS) or ground pea meal (PM).

Amino acid	Percent as is			
	10% PBM	10% FM	10% DDGS	10% PM
Methionine	0.54	0.61	0.62	0.60
Cystine	0.53	0.54	0.62	0.61
Lysine	2.19	2.08	2.18	2.07
Phenylalanine	1.52	1.63	1.71	1.72
Leucine	2.65	2.78	2.91	2.99
Isoleucine	1.47	1.54	1.53	1.53
Threonine	1.31	1.33	1.35	1.34
Valine	1.27	1.33	1.30	1.29
Histidine	0.81	0.87	0.90	0.88
Arginine	2.42	2.41	2.54	2.51
Glycine	1.71	1.91	1.7	1.56
Aspartic Acid	3.61	3.8	3.9	3.83
Serine	1.72	1.72	1.82	1.86
Glutamic Acid	6.15	6.57	7.40	7.00
Proline	2.03	1.84	2.06	2.06
Hydroxyproline	0.24	0.15	0.15	0.07
Alanine	1.91	1.82	1.86	1.75
Tyrosine	1.08	1.10	1.20	1.21
Total	33.16	34.03	35.75	34.88

Diets were analyzed by New Jersey Feed Laboratory, Trenton, NJ,USA (Roy et al., 2009).

Table 7. Feeding regime for *L. vannamei* reared in the grow-out 0.1-ha ponds during an 18-week experimental period, based on estimated growth rate of 1.5 g wk^{-1} and assumed survival rate of 70%.

Week	Population	Feed input per day	
		kg $pond^{-1}$	kg ha^{-1}
1	35,000	0.24	2.36
2	34,382	0.89	8.93
3	33,765	3.00	30.00
4	33,147	2.79	27.86
5	32,529	8.36	83.65
6	31,912	7.62	76.20
7	31,294	8.05	80.47
8	30,676	7.32	73.25
9	30,059	7.18	71.77
10	29,441	7.03	70.30
11	28,824	7.57	75.71
12	28,206	7.57	75.71
13	27,588	6.50	65.00
14	26,971	6.50	65.00
15	26,353	6.50	65.00
16	25,735	4.46	44.64
17	25,118	5.71	57.14
18	24,500	4.29	42.86

Table 8. Summary of water quality variables monitored over an 18-week growth trial for *L. vannamei*, fed experimental diets containing high levels of soybean meal in various combinations and cultured in 0.1-ha ponds. Values represent the mean ± standard deviation and values in parenthesis represent minimum and maximum readings.

Parameter	10% PBM	10% FM	10% DDGS	10% PM
Temperature (ºC)				
am	29.36±1.72 (24.97, 33.08)	29.48±1.76 (25.21, 32.91)	29.37±1.71 (25.21, 32.87)	29.33±1.76 (25.01, 32.88)
noon	31.55±1.91 (26.76, 34.94)	31.63±1.98 (26.02, 35.65)	31.49±1.95 (26.48, 35.03)	31.65±1.99 (26.69, 35.28)
pm	31.22±1.95 (25.07, 34.96)	31.40±2.03 (25.47, 35.68)	31.25±1.95 (26.46, 35.40)	31.42±2.01 (25.37, 35.49)
DO[a] (mg L^{-1})				
am	4.21±1.12 (1.21, 10.69)	4.04±1.29 (0.23, 13.43)	4.30±1.24 (0.29, 13.84)	4.06±1.18 (0.96, 12.63)
noon	10.25±3.07 (2.22, 19.43)	10.92±3.35 (2.46, 20.99)	10.51±3.54 (2.14, 18.99)	11.98±3.38 (1.48, 21.36)
pm	8.31±2.99 (1.54, 17.80)	8.89±3.22 (0.52, 17.65)	8.52±3.12 (1.39, 19.41)	9.33±3.27 (0.16, 19.99)
Readings< 2.5(days)	5.0±3.8	9.0±6.1	4.5±3.1	6.0±3.2
pH				
am	7.75±0.55 (6.42, 9.20)	7.79±0.47 (6.95, 9.11)	7.70±0.46 (6.93, 9.00)	7.97±0.43 (7.09, 9.04)
noon	8.49±0.54 (7.15, 9.93)	8.55±0.48 (7.08, 9.46)	8.43±0.51 (6.70, 9.52)	8.67±0.43 (7.05, 9.91)
pm	8.45±0.58 (7.03, 9.82)	8.51±0.50 (7.19 ,9.47)	8.42±0.54 (7.10, 10.01)	8.65±0.43 (7.19, 9.81)
Salinity (ppt)				
am	28.10±1.44 (23.83, 31.25)	27.78±2.02 (22.35, 32.75)	28.30±1.68 (23.36, 32.72)	27.97±1.67 (21.93, 31.78)
noon	28.07±1.40 (23.92, 31.14)	27.75±1.97 (22.74, 32.58)	28.25±1.66 (23.42, 32.57)	27.92±1.64 (21.98, 31.52)
pm	27.96±1.49 (23.83, 31.15)	27.69±2.02 (22.70, 32.63)	28.17±1.71 (23.48, 32.58)	27.84±1.70 (21.90, 31.59)
Secchi (cm)	30.47±26.89 (5, 110)	27.37±23.23 (5, 110)	30.79±27.26 (5, 110)	25.53±22.94 (5,110)
TAN[b] (mg L^{-1})	0.72±1.59 (0.00, 7.39)	0.89±1.58 (0.00, 6.03)	0.93±1.95 (0.00, 10.00)	0.43±0.72 (0.00, 2.95)

[a]Dissolved oxygen.

[b]Total ammonia-nitrogen.

Table 9. Mean production parameters at 18 week experimental period of juvenile Pacific white shrimp, *L. vannamei* (initial weight of 40 mg), fed experimental diets containing high levels of soybean meal in combination with poultry by-product meals (PBM), fish meal (FM), distiller's dried grains with solubles (DDGS) or ground pea meal (PM), and cultured in 0.1-ha ponds.

Treatment	Yield (kg ha^{-1})	Final weight (g)	FCR[a]	Survival (%)
10% PBM	5187.4	15.98	1.33	93.74
10% FM	5054.8	16.86	1.35	86.60
10% DDGS	5265.5	16.28	1.33	92.20
10% PM	5194.8	16.25	1.37	88.58
P-value[b]	0.8215	0.7724	0.8622	0.6146
PSE[c]	73.9001	0.2825	0.0184	1.9943

[a]Feed conversion ratio = Total feed offered / biomass increase.

[b]Analysis of variance was used to determine significant differences ($P < 0.05$) among treatment means (n=4).

[c]Pooled standard error of treatment means.

Table 10. Water quality variables monitored over a 10-week growth period of juvenile *L. vannamei* (initial weight of 2.13 g), fed experimental diets containing high levels of soybean meal in combination with poultry by-product meals (PBM), fish meal (FM), distiller's dried grains with solubles (DDGS) or ground pea meal (PM), and reared under a semi-closed recirculating system. Values represent the mean ± standard deviation and values in parenthesis represent minimum and maximum readings.

	Temperature (°C)	DO (mg L^{-1})[a]	pH	Salinity (ppt)	TAN (mg L^{-1})[b]
am	27.83±1.73 (22.56, 30.91)	6.11±0.61 (3.77, 8.06)	7.84±0.15 (7.44, 8.44)	29.96±0.94 (23.86, 31.71)	0.13±0.27 (0.00, 1.19)
pm	30.39±1.93 (25.19, 33.98)	6.07±0.59 (3.00, 7.73)	8.09±0.18 (7.61, 8.78)	29.90±1.13 (22.18, 31.98)	

[a]Dissolved oxygen.

[b]Total ammonia-nitrogen.

Table 11. Mean production parameters at 10 week experimental period of *L. vannamei* (initial weight of 2.13±0.16 g), fed diets containing high levels of soybean meal in combination with poultry by-product meals (PBM), fish meal (FM), distiller's dried grains with solubles (DDGS) or ground pea meal (PM), and a commercial feed used as reference diet, and reared under a semi-closed recirculating system.

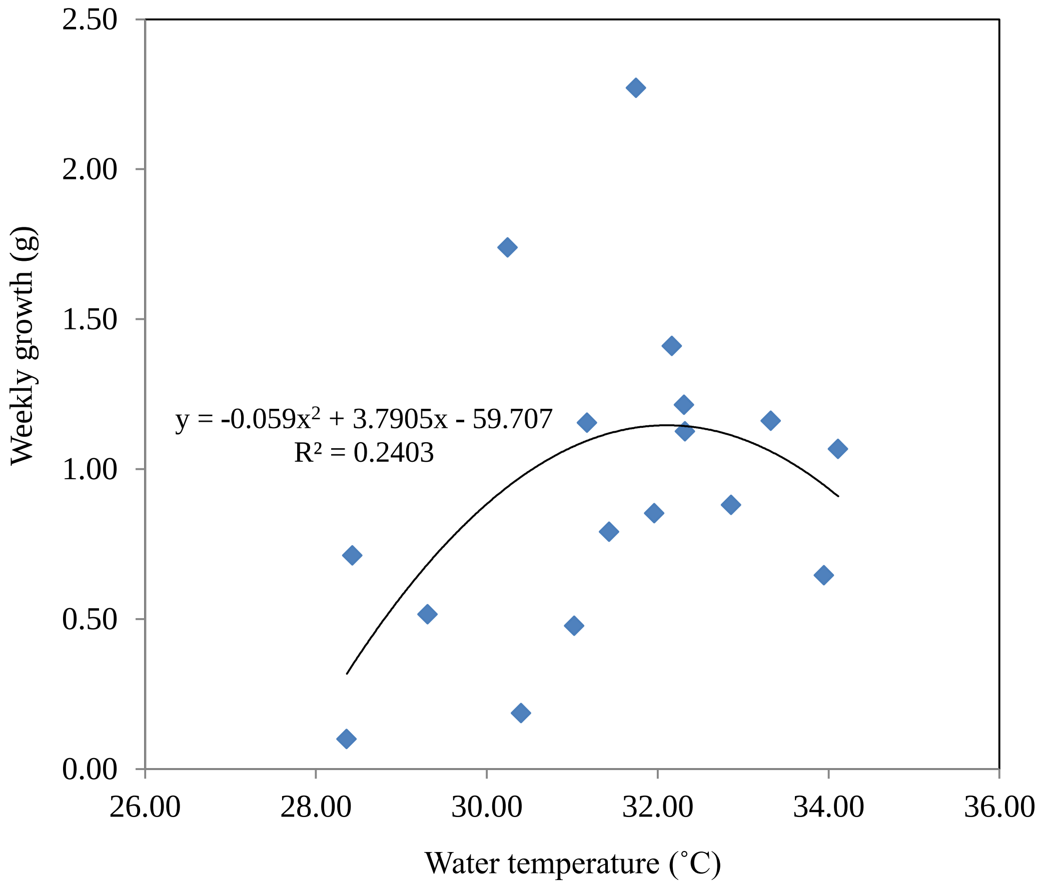
Treatment	Biomass (g $tank^{-1}$)	Final weight (g)	FCR	Survival (%)
10% PBM	553.0	18.7	1.32	98.7
10% FM	557.7	18.8	1.31	98.7
10% DDGS	548.3	18.5	1.33	98.7
10% PM	554.4	19.0	1.31	97.3
Reference diets[a]	579.8	19.7	1.25	98.3
P-value[b]	0.1032	0.0930	0.8722	0.1482
PSE[c]	3.8095	0.1354	0.4486	0.0109

[a]Reference diets = 35% Protein, Rangen 35,0 (Buhl, Idaho).

[b]Analysis of variance was used to determine significant differences ($p < 0.05$) among treatment means (n=4).

[c]Pooled standard error of treatment means.

Figure 1. Relationship between water temperature (°C) and mean weight gain (g) over an 18-week growth trial for *L. vannamei* (initial weight of 40mg), fed experimental diets containing high levels of soybean meal in combination with poultry by-product meals (PBM), fish meal (FM), distiller's dried grains with solubles (DDGS) or ground pea meal (PM), and cultured in 0.1-ha ponds.



CHAPTER III

REPLACEMENT OF SOLVENT EXTRACTED SOYBEAN MEAL BY SOY PROTEIN CONCENTRATE FOR PACIFIC WHITE SHRIMP (*Litopenaeus vannamei*) REARED UNDER CLEAR WATER CONDITION

Abstract

Solvent extracted soybean meal (SE-SBM) is one of the most efficient and widely used products at present; however, compare to fish meal which has a more optimal nutrient profile but higher price, soybean meal has lower nutrient contents such as protein, essential amino acids, essential fatty acids, and minerals which need be supplemented in feed formulation. As the nutrient density of the diet is increased the use of SE-SBM is inefficient as there is insufficient room in the formulation for other necessary components. Hence, higher protein ingredients are often required. Soy protein concentrate is a high protein ingredient that is produced from high quality, dehulled soybean seeds. By removing most of the oil, water soluble non-protein constituents, and many of the anti-nutritional factors, a variety of high protein products with varying characteristics can be produced. With increased availability and reduced prices of soy protein concentrate, which has higher protein content than SE-SBM, there is interest in using these meals in practical diet formulations. The objective of this study was to evaluate the growth response of Pacific white shrimp *Litopenaeus vannamei* fed practical diets containing different levels of soy protein concentrate (SPC) as a replacement of

soybean meal. In the first trials juvenile shrimp (0.12±0.01 g, initial weight) were stocked at a density of 15 shrimp per tank in 24, (340 L) square tank with a common biological filter. Six dietary treatments containing increasing percentages of SPC (0%, 5%, 10%, 20%, 40%, and 40% supplemented with DL-methionine) were offered to shrimp 2 times daily over a 55-day feeding trial using 4 replicates per treatment. In the second trial, juvenile shrimp (1.14±0.05 g, initial weight) were stocked under the same system and same conditions as the first trial. Diets were similar to those in the first experiment with the following modifications, crystalline DL-methionine was replaced with micro-encapsulated methionine (diet 6). Diet 7 that contained 40% SPC with additional fish soluble, and a commercial reference diet were also included. The test diets were fed to juvenile shrimp 2 times daily over a 53-day feeding trial using 3 replicates per treatment.

The results of these studies indicated that SPC in substitution of soybean meal at 20% and greater caused a reduction in mean final weights and an increase in FCR. Survival was not different among treatments. Thus, the results from this study suggest that up to 10%of SPC could be included in soybean meal based diet without causing adverse effect. Moreover, the addition of methionine or fish soluble to the diets with high level of SPC (40%) did not enhance growth of *L. vannamei*.

Introduction

Soybean has long been recognized as one of the most widely distributed oil seeds which produce a variety of products used in the animal feed industry. The use of soybean products, in the form of soybean meals or specialty soy products, have increased steadily over the past decade. This is a result of their consistent quality, especially with respect to protein and amino acid profile, and high digestibility. Soybean meal currently represents the predominant choice as an alternate protein source in commercial feed formulations for aquatic species.

However, the use of soybean meal in feeds for aquatic animals has limitations. Raw soybean seeds contain antinutritional factors that can cause negative impacts to shrimp digestive system and may influence its palatability (Forster et al., 2002). Additionally, soybean proteins are relatively low in sulfur amino acids (SAAs), e.g., methionine and cystine, as well as lack of n-3 highly unsaturated fatty acids (HUFAs), e.g., eicosapentaenoic acids (EPA) and docosahexaenic acids (DHA), which can have adverse effects on growth performance of shrimp (Lim and Dominy, 1990; Messina, 1999; Akiyama, 1989). However, exposing soybean to heat process can reduce the presence of antinutritional factors (ANF) and improve its palatability (Brown et al., 2008; Akiyama, 1989). Moreover, SAAs and EFAs in feed can be adjusted through well balanced formulations by supplementing synthetic methionine and various oil sources of marine origin to meet shrimp requirement.

The use of soy products as alternative protein sources in shrimp diets has been the focus of numerous research projects. Solvent extracted soybean meal (SE-SBM) is one of the most efficient and widely used protein sources in the feed industry. This is primarily because of its favorable protein content, suitable amino acid profile, economic opportunity, and good digestibility. The uses of SE-SBM as a protein source in diets of *L. vannamei* has received considerable attention with good results (Alvarez et al., 2007; Samocha et al., 2004; Lim and Dominy, 1990). However, in comparison to fish meal which has a more optimal nutrient profile but higher price, soybean meal has a lower nutrient content in terms of protein, essential amino acids (e.g. methionine), essential fatty acids, and minerals which need be adjusted with supplements or blending with other products. As the nutrient density (protein and energy) of the diet is increased, the use of SE-SBM is inefficient as there is insufficient room in the formulation for other necessary components.

Soy protein concentrate (SPC) is another high protein product that is produced through a series of different extraction and precipitation process from high quality dehulled soybeans. By removing most of the oil, water soluble non-protein constituents, and many of the anti-nutritional factors, a variety of high protein products with varying characteristics can be produced. There is approximately 65% crude protein in soy protein concentrate (SPC) which is similar to level found in fish meal, and considerably higher than those of SE-SBM (Paripatananont et al., 2002; Liu, 1997). Many feed manufacturers prefer a reasonable level of a high protein ingredient in their formulations to allow room to manipulate other ingredients. Hence, soy protein concentrate could be used as an alternative ingredient to soybean meal in shrimp diets. These products are increasingly being used by the animal feed industry. As the availability of these product increases, the price is likely to be reduced making them more cost effective solutions. As SPC has higher protein content than SE-SBM, there is interest in using these meals in practical diet formulations.

Although the use of soy protein concentrate in aquatic feed has been reported in many fish and shrimp species (Tibaldi et al., 2006; Refstie et al., 2001; Forster et al., 2002; Paripatananont et al., 2002; Mambrini et al., 1999; Storebakken et al., 1998; Kaushik et al., 1995), there is limited information available on the utilization of SPC in *L. vannamei* diets. Both soybean meal and SPC are deficient in methionine. Hence, methionine supplement may be required in shrimp diets with high levels of SPC. In addition, feed palatability is always a concern when using non-marine ingredients such as SPC. This may be improved by adding attractive materials such as fish soluble.

Therefore, the objective of this study is to evaluate the response of *L. vannamei* to increasing levels of SPC as replacement of SE-SBM in practical diets for *L. vannamei*, and to determine if methionine or fish soluble could improve their growth response.

Materials and Methods

A series of experiments were conducted at the Alabama Department of Conservation and Natural Resources, Marine Resource Division, Claude Peteet Mariculture Center in Gulf Shores, Alabama. Two growth trials were carried out to confirm the response of *L. vannamei* to increasing levels of SPC as replacement of SE-SBM in clear water tank system.

First trial

Experimental diets

Six diets formulated to be isonitrogenous (approximately 35% crude protein with 6% lipid) were tested using juvenile *L. vannamei.* Six experimental diets were formulated to contain vary levels of SPC (0, 5, 10, 20, 40 and 40% supplemented with crystalline DL-methionine) as replacement of SE-SBM (Table 1). The proximate composition including protein digestibility (using 0.0002% pepsin solutions) of experimental diets are listed in Table 2. Diets were prepared by mixing the ingredients in a mixer (Hobart, Troy, Ohio) for 30 minutes. Subsequently, hot water was added to the mixture until appropriate consistency for pelleting was obtained. Diets were then passed through a meat grinder and a 3-mm mesh sieve. Pellets were air dried (<50˚C) to a moisture content of less than 10%. After drying, pellets were crumbled, packed in sealed plastic bags and stored in a freezer until use.

Growth trial

Juvenile shrimp were obtained from nursery system and were selected by hand-sorting to a uniform size. Then juvenile shrimp (0.12±0.01 g, initial weight) were stocked into 24 square tanks (0.7x0.7x0.7 m, 340-L volume), under a recirculating system at a density of 15 shrimp per tank. Six experimental diets were randomly assigned with four replications (tanks) per treatment. Test diets were applied twice daily at 0800 and 1600 h for a 55-day experimental period. Feed input was pre-calculated on a weekly basis using an expected feed conversion ratio of 2:1 and a doubling in size until individual shrimp weighed one gram. Thereafter, a growth rate of one gram per week was assumed. Each tank was covered with nylon netting, and was continuously aerated with two air stones.

Shrimp were counted every two weeks to readjust the daily feed input. At the conclusion of the 10 week growth trial shrimp were counted and weighed individually. Percentage survival, mean final weight, biomass and feed conversion ratio were determined.

Second trial

Experimental diets

The second trial was a repeat of the first trial using the same increasing levels of SPC as replacements of SE-SBM except that micro-encapsulated methionine or fish soluble were used to replace crystalline DL-methionine (diet 6 and 7, respectively) (Table 3). Experimental diets were formulated to be isonitrogenous (approximately 35% crude protein) with constant lipid content (6%) were tested using juvenile *L. vannamei.* A commercial diet (Rangen Shrimp Grower, 35% protein, 8% lipid) was used as a reference diet. The proximate nutrient composition of experimental diets are listed in Table 4. Diets were prepared and kept by the same processes as the first trial.

Growth trial

Juvenile shrimp were obtained from nursery system and were selected by hand-sorting to a uniform size. Then juvenile shrimp (1.12±0.05 g, initial weight) were stocked into the same recirculating clear water system at a density of 15 shrimp per tank. Seven experimental diets and a commercial reference diet were randomly assigned to three replications tanks. Test diets were applied twice daily at 0800 and 1600 h for a 53-day experimental period. Based on the previous work, a growth of 0.8 g wk^{-1} and a FCR of 2 were used to calculate the amount feed needed each week, as feed were pre-weighed on a weekly basis. Shrimp were counted every two weeks to readjust the daily feed input. At the conclusion of the growth trial shrimp were counted and weighed individually. Percentage survival, mean final weight, biomass, and FCR were determined.

Water quality monitoring

For both experimentals, dissolved oxygen (DO), temperature, salinity and pH were measured twice daily in the biological filter and two of the rearing tanks at the 0800 and the 1600 h using a YSI 556MPS meter (Yellow Spring Instrument Co., Yellow Springs, OH, USA). Water samples were taken from the filter and two of the tanks to determine total ammonia-nitrogen (TAN) on a weekly basis using the Orion ammonia electrode probe (Thermo Fisher Scientific Inc., Waltham, MA, USA).

Statistical analysis

All data were statistically analyzed using one-way analysis of variance to determine significant differences ($P<0.05$), followed by the Student–Neuman–Keuls multiple comparison test to determine significant differences among treatment means (Steel and Torrie, 1980). All statistical analyses were carried out using SAS (V9.1. SAS Institute, Cary, NC, USA).

Results

First trial

Overall means, standard deviations, and ranges of morning and afternoon water quality variables observed over 55-day growth trial were within acceptable limits for the culture of this species (Table 5). Mean DO, temperature, salinity, pH, and TAN were 6.38 mg L^{-1}, 27.67 ºC, 22.1 ppt, 8.41 and 0.49 mg L^{-1}, respectively.

Although there were no differences (P > 0.05) in survival, significant differences in biomass, mean individual weight, and FCR among treatments were observed in the 55-day growth trial in *L. vannamei* fed the various diets (Table 6). Survival ranged from 98.3 to 100%. Mean individual weight ranged from 5.50 to 6.38 g with diets containing SPC at 20% or greater resulted in lower mean individual weight than diet containing lower level of SPC. Biomass ranged from 82.5 to 96.2 g $tank^{-1}$. FCR ranged from 2.19 to 2.57 with diets containing SPC at 20% or higher resulted in greater FCR than diet containing SPC at 10% and lower (Figure 1). Methionine supplementation in diet with 40% SPC did not improve growth and FCR compare to diet with same level of SPC containing no methionine.

Second trial

Overall means, standard deviations, and ranges of morning and afternoon water quality variables observed over 53-day growth trial were within acceptable limits for the culture of this species (Table 5). Mean DO, temperature, salinity, pH, and TAN were 7.6 mg L^{-1}, 26.5 ºC, 16.1 ppt, 7.7 and 0.10 mg L^{-1}, respectively.

Results from this trial yielded similar results to the first trial with lower mean individual weight and higher FCR at SPC level of 20% or greater. However, there were no differences in mean individual weight and FCR at the end of a 53-day growth trial in *L. vannamei* fed diet containing increasing level of SPC as replacement of SE-SBM and diets with fish soluble or micro-encapsulated methionine supplement (Figure 3-2).

Survival ranged from 77.8 to 100%. Mean individual weight ranged from 6.0 to 8.0 g with diets containing SPC at 20% or greater resulted in lower mean individual weight than diet containing lower level of SPC. Biomass ranged from 70.0 to 117.9 g $tank^{-1}$. FCR ranged from 1.82 to 2.54 with diets containing 40% SPC exhibiting higher FCR than diets containing lower level of SPC. The inclusion of micro-encapsulated methionine or fish solubles in diet containing 40% SPC resulted in no beneficial effects on either mean final weight or FCR. Shrimp fed on the commercial reference (control) diets were not significantly different from those maintained on the test diets.

Discussion

The results from the present study suggested that SPC can be included at 10% as replacement of SE-SBM in *L. vannamei* diet without causing adverse effect on growth performance of shrimp. In addition, lower biomass and higher feed conversion ratio were observed in diets containing SPC at 20% or greater in both growth trials. There were no differences in performance of shrimp fed experimental diets containing high level of SPC (40%) with and without methionine supplementation in both trials, or with the addition of fish solubles in the second trial. The results of this study are in agreement with Paripatananont et al. (2001) who evaluated diets containing SPC as a replacement of fish meal in diet for *Penaeus monodon*, reared under laboratory condition using diets containing 36% protein on dry weight basis. They found that 17.5% SPC can be incorporated in shrimp feed containing 12% SBM without affecting the growth of shrimp. However, significantly lower percent survival and weight gain, and higher FCR were observed in shrimp fed diets containing SPC at 26.5% or higher as replacements

75 and 100% of fish meal in diets. With the same species, Forster et al. (2002) suggested an applicable level of 27.2% SPC. It should be noted, however, their diets contained fish meal and supplemented with indispensible amino acid.

With rainbow trout, Mambrini et al. (1999) observed a decrease in growth when more than 50% fish meal in diets was replaced by SPC. In contrast, Kaushik et al. (1995) obtained no negative effect on the growth performance and nutrient utilization of rainbow trout fed diet containing SPC supplemented with L-methionine as a total replacement of fish meal. However, the same study report a reduction in growth rate of fish when up to 50% of fish meal was replaced by soy flour. Likewise, with shrimp, Lim and Dominy (1990) reported a decrease in weight gain when *L. vannamei* were fed with diets containing 42%SE-SBM or higher. For juvenile American lobster, Floreto et al. (2000) indicated that dietary inclusion of SBM at levels of 50% or higher resulted in significantly lower body weight gain than in treatment fed diets contain less than 50% SMB. The same study also showed that amino acid supplementation, e.g., arginine, leucine, methionine, and tryptophan, resulted in growth enhancement when SBM level was less than 50%. In addition, low palatability was observed when treatment were fed diets containing 50% SBM or higher. However, in the present study there was no sign of poor palatability of shrimp fed experimental diets containing various level of SPC.

The poor performance of shrimp fed higher levels (20% or higher) of SPC as a substitute for SE-SBM observed in this study may be caused by imbalanced of essential amino acid, or the presence of anti-nutritional factors (Guillaume, 1997). For example, lower levels of methionine are found when diets are formulated to contain increasing levels of soy protein as a direct replacement for fish meal (Storebakken et al., 1998; Mambrini et al., 1999).

Supplementation of methionine in diets containing high levels of soy protein found to have beneficial effect on growth performance of aquatic animals. Kaushik et al. (1995) reported that supplementation of L-methionine in the diet containing SPC as a primary protein ingredient for rainbow trout resulted in similar growth performance to fish fed fish meal diet (Kaushik et al., 1995). Similar results were stated by Floreto et al. (2000) that diets supplemented with crystalline L-methionine resulted in enhancing body weight gain and shortening molting cycle period of juvenile American lobster. Forster et al. (2002) suggested that SPC can be used to replace up to 75% fish meal when diets were supplemented with deficient indispensable amino acids.

Since the essential amino acid requirements for shrimp have not been determined (Fox et al., 1995), the amino acid profile of shrimp tissue has been used as a reference for diet formulation to balance amino acid level in order to sustain optimal growth (Cuzon et al., 2004). Methionine is considered the first limiting amino acids in shrimp diets containing high levels of soybean protein (Akiyama et al., 1991). Soy protein concentrate contains approximately 1.4 g methionine per 100 g crude protein (Perkins et al., 1995) as compare to 2.6 g per 100 g crude protein in *L. vannamei* tail muscle (Lim, 1993) or 2.4 and 3.5% of the dietary protein for methionine and methionine-cystine levels, respectively, for commercial shrimp production feeds (Akiyama et al., 1991). Millamena et al. (1996) reported the methionine requirement of *P. monodon* postlarvae at 0.89% of the diet or 2.4% of crude protein. In addition, the total sulfur amino acid requirement;

methionine plus cystine, would be 1.3% of the diet or 3.5% of protein in a diet containing 0.41% cystine.

The result from this study suggested that methionine supplementation in soy based diets containing 40% SPC in substitution to SE-SBM showed no improvement in growth and survival of *L. vannamei.* Likewise, Refstie et al. (2001) obtained similar growth in Atlantic salmon fed diets containing 30% SPC with or without DL-methionine supplementation as a replacement of fish meal. Diet containing only soy protein or soy protein supplemented with methionine have also been shown to be poorly utilized by red drum (Reigh and Simon,1992). Therefore, lower mean final weight and higher FCR of shrimp fed diets containing 40% SPC may not be related to methionine deficiency.

Chemoattractants or feed stimulants have been used to enhance feed palatability and feed intake when diets are formulated with soybean protein as alternative to fish meal. Nunes et al. (2006) obtained good feeding response of *L. vannamei* fed a commercial diet containing fish solubles. Fish solubles, a by-product of fish meal and fish oil production,contain high levels of water soluble vitamins. Fish solubles have been used in poultry and swine feeds as a source of unidentified growth factors (Soares et al., 1973). However, results obtained from our study suggested that addition of fish solubles in diet containing 40% SPC did not improve the performance of shrimp in terms of survival, growth and feed conversion ratio. Moreover, there was no indication of feed being rejected when shrimp were fed diets containing up to 40% SPC without the addition of fish solubles.

The pepsin digestibility of crude protein in all dietary treatments ranged from 81% to 89% with highest digestibility in diet containing 40% SPC. The digestibility values of our test diets are considered reasonable comparing to the apparent protein digestibility of soybean meal (90%) and of fishmeal (80.7%) reported for *L. vannamei* by Akiyama et al. (1989). Divakaran et al. (2000) also reported that the apparent digestibility of protein in soybean meal is at least 89% in *L. vannamei.* Therefore, protein digestibility of test diets is not a contributing factor to lower weight gain in treatment fed diets containing 20% SPC or higher.

Soy protein concentrate is produced by exposing soybean to heat process to inactivate antinutritional factors (ANF) and improve its palatability (Brown et al., 2008, Akiyama, 1989). Refstie et al. (1999) reported low levels of trypsin inhibitors in all commercial soy products. Nonetheless, Storebakken et al. (1998) reported the reduction of N apparent digestibility when Atlantic salmon were fed SPC diets with the present of phytate. Non-starch polysaccharides (NSP) fractions I soybean have been reported to negatively affect the digestive system of Atlantic salmon (Refstie et al., 1999). Dabrowski et al. (1989) reported that a decrease of protein digestibility in rainbow trout fed soybean meal was due to trypsin inhibitors. However, Sessa and Lim (1991) indicated that *L. vannamei* can tolerate relatively high levels of trypsin inhibitors (TI) and levels of 0.64-6.14 mg TI per g diet) had no effect on weight gain, FCR, and survival. It has also been suggested that ANF present in SBM diets may interfere with protein and lipid digestion and affect the palatability and nutrient absorption (Amaya, 2006; Dersjant-Li et al., 2002).

Results from the present study suggested that 10% SPC in combination with SBM is acceptable and can be utilized as the primary protein source in shrimp diet without causing negative effect on biomass, growth, survival, and feed conversion ratio of

L. vannamei reared under laboratory conditions. Furthermore, the results from this study confirmed that supplementation of DL-methionine, micro-encapsulated methionine or fish solubles to the diet containing 40% SPC are not required. However, it was not sure that the reduction in growth performance in treatments fed diets containing SPC at 20% or greater may cause by imbalanced nutrient content in feed or other unknown factors. There for, further research must be done to evaluate the nutrient composition of SPC use in shrimp feed formulation as well as utilization of SPC in outdoor production conditions.

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Table 1. Ingredient composition (g 100 g^{-1} dry weight) of six experimental diets containing increasing percentages of SPC (0, 5, 10, 20, 40 and 40% supplemented with methionine) as a substitute for SE-SBM.

Ingredient	0%	5%	10%	20%	40%	40%+ Met
Soybean meal solvent-extracted	58.8	52.2	45.5	32.5	6.2	6.2
Soy concentrate	0.0	5.0	10.0	20.0	40.0	40.0
Menhaden fish oil	4.3	4.3	4.3	4.3	4.3	4.3
Soy oil	0.0	0.0	0.1	0.1	0.1	0.1
Corn starch	2.2	3.8	5.5	8.5	14.7	14.6
Whole wheat	20.0	20.0	20.0	20.0	20.0	20.0
Trace mineral premix[a]	0.5	0.5	0.5	0.5	0.5	0.5
Vitamin premix[a]	2.0	2.0	2.0	2.0	2.0	2.0
Stay C, 35% vit. C activity	0.1	0.1	0.1	0.1	0.1	0.1
CaP-diebasic	3.0	3.0	3.0	3.0	3.0	3.0
Soy lecithin	1.0	1.0	1.0	1.0	1.0	1.0
Cholesterol	0.1	0.1	0.1	0.1	0.1	0.1
Corn gluten meal	8.0	8.0	8.0	8.0	8.0	8.0
DL-methionine	0.0	0.0	0.0	0.0	0.0	0.1
Total	100	100	100	100	100	100

[a]Vitamin premix and mineral premix are proprietary products, thus their composition is not listed.

Table 2. Nutrient composition (dry matter basis) of six experimental diets containing increasing percentages of SPC (0, 5, 10, 20, 40 and 40% supplemented with methionine) as a substitute for SE-SBM.

Nutrient	0%	5%	10%	20%	40%	40%+ Met
Crude protein	40.00	39.70	39.60	40.08	40.20	40.40
Moisture	5.71	6.69	7.58	7.13	7.00	6.16
Fat	6.69	6.45	6.43	6.15	5.88	5.95
Crude fiber	2.69	2.81	2.87	2.86	2.79	2.96
Ash	6.21	6.16	5.98	5.79	5.57	5.68
Protein difestibility (0.0002% pepsin)	87.77	81.23	83.29	83.52	89.66	

Diets were formulated to contain 35% protein and 6% lipid.

Diets were analyzed by New Jersey Feed Laboratory, Trenton, NJ, USA.

Table 3. Ingredient composition (g 100 g^{-1} dry weight) of six experimental diets containing increasing percentages of SPC (0, 5, 10, 20, 40, 40% supplemented with methionine and 40% with fish solubles) as a substitute for SE-SBM.

Ingredient	0%	5%	10%	20%	40%	40%+ Met	40%+ fish sol.
Soybean meal solvent extracted	54.50	48.10	41.70	29.00	3.50	2.30	3.50
Soy protein concentrate	0.00	5.00	10.00	20.00	40.00	40.00	40.00
Menhaden fish oil	4.60	4.60	4.60	4.60	4.65	4.60	4.60
Corn starch	0.19	1.59	2.99	5.69	11.14	11.39	10.79
Whole wheat	27.81	27.81	27.81	27.81	27.81	27.81	27.81
Trace mineral premix[a]	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Vitamin premix[a]	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Stay C, 35% vit. C activity	0.10	0.10	0.10	0.10	0.10	0.10	0.10
CaP-diebasic	3.20	3.20	3.20	3.20	3.20	3.20	3.40
Soy lecithin	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Cholesterol	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Corn gluten meal	6.00	6.00	6.00	6.00	6.00	6.00	6.00
Micro-encapsulated methionine	0.00	0.00	0.00	0.00	0.00	0.02	0.00
Fish solubles	0.00	0.00	0.00	0.00	0.00	0.00	1.00
Total	100	100	100	100	100	100	100

[a] Vitamin premix and mineral premix are proprietary products, thus their composition is not listed.

Table 4. Nutrient composition (dry matter basis) of seven experimental diets containing increasing percentages of SPC (0, 5, 10, 20, 40, 40% supplemented with methionine and 40% with fish solubles) as a substitute for SE-SBM.

Nutrient	0%	5%	10%	20%	40%	40%+ Met	40%+ fish sol.
Crude protein	36.80	37.30	35.90	35.80	35.80	40.40	37.10
Moisture	7.35	6.90	7.39	8.96	11.04	6.16	7.54
Fat	7.15	6.93	6.80	6.62	6.02	5.95	6.35
Crude fiber	3.15	3.31	3.18	3.26	2.91	2.96	3.15
Ash	6.68	6.49	6.47	6.26	5.82	5.68	6.04

Diets were formulated to contain 35% protein and 6% lipid.

Diets were analyzed by New Jersey Feed Laboratory, Trenton, NJ, USA.

Table 5. Water quality variables for growth trials with juvenile *L. vannamei* reared under clear water system for both trials. Values represent the mean ± standard deviation and values in parenthesis represent minimum and maximum readings.

Variable	First Trial	Second Trial
DO (mg L^{-1})[a]	6.38 ± 0.50	7.6 ± 0.95
Temperature (˚C)	22.67 ± 1.46	26.5 ± 1.68
Salinity (ppt)	22.06 ± 1.28	16.1 ± 0.54
pH	8.41 ± 0.32	7.7 ± 0.20
TAN (mg L^{-1})[b]	0.49 ± 0.19	0.10 ± 0.20

[a]Dissolved oxygen.

[b]Total ammonia-nitrogen.

Table 6. Growth performance of *L. vannamei* (initial weight of 0.12±0.01 g) reared under clear water system in polyethylene tanks during 55-day experimental period (first trial), fed diets containing increasing percentages of SPC (0, 5, 10, 20, 40 and 40% supplemented with methionine) as a substitute for SE-SBM.

Treatment	Biomass (g tank^{-1})	Final weight (g)	FCR[a]	Survival (%)
0% SPC	92.98ab	6.31ab	2.19a	98.33
5% SPC	95.63a	6.38ab	2.17a	100.00
10% SPC	96.23a	6.30a	2.14a	98.33
20% SPC	86.70bc	5.78bc	2.42b	100.00
40% SPC	86.60bc	5.77bc	2.42b	100.00
40% SPC+Met	82.50c	5.50c	2.57b	100.00
P-value[b]	0.001	0.005	0.001	0.564
PSE[c]	1.322	0.096	0.038	0.384

[a]Feed conversion ratio = Total feed offered / biomass increase.

[b]Means (n = 4) not sharing a common superscript within a row are significantly different based on analysis of variance (P > 0.05) followed by Student Newman-Keuls multiple range test.

[c]Pooled standard error of treatment means.

Table 7. Growth performance of *L. vannamei* (initial weight of 0.14±0.05 g) reared under clear water system in polyethylene tanks during 53-day experimental period (second trial), fed diets containing increasing percentages of (0, 5, 10, 20, 40, 40% supplemented with methionine and 40% with fish solubles) as a substitute for SE-SBM, compare to a commercial reference diet.

Treatment	Biomass (g $tank^{-1}$)	Final weight (g)	FCR[a]	Survival (%)
0% SPC	109.1	7.6	1.89	95.6a
5% SPC	111.9	8.0	1.82	93.3a
10% SPC	110.2	7.5	1.92	97.8a
20% SPC	96.0	7.0	2.08	91.1ab
40% SPC	90.1	7.1	2.10	84.4ab
40% SPC+Met	70.0	6.0	2.54	77.8b
40% SPC+fish sol.	85.7	6.7	2.21	84.4ab
Reference diet[b]	117.9	7.9	1.84	100.0a
P-value[c]	0.9362	0.2081	0.3906	0.0028
PSE[d]	10.4565	0.6148	0.3130	2.8868

[a]Feed conversion ratio = Total feed offered / biomass increase.

[b]Diets were commercially manufactured by Rangen® (Angleton, TX, USA) using extrusion processing to contain 35% Protein and 8% lipid.

[c]Means (n = 4) not sharing a common superscript within a row are significantly different based on analysis of variance (P > 0.05) followed by Student Newman-Keuls multiple range test.

[d]Pooled standard error of treatment means.

Figure 1. Mean final weight (g) and FCR of *L. vannamei* post larval (initial weight of 0.12±0.01 g) reared under clear water system in polyethylene tanks during 55-day experimental period (first trial), fed diets containing increasing percentages of SPC (0, 5, 10, 20, 40 and 40% supplemented with methionine) as a substitute for SE-SBM..

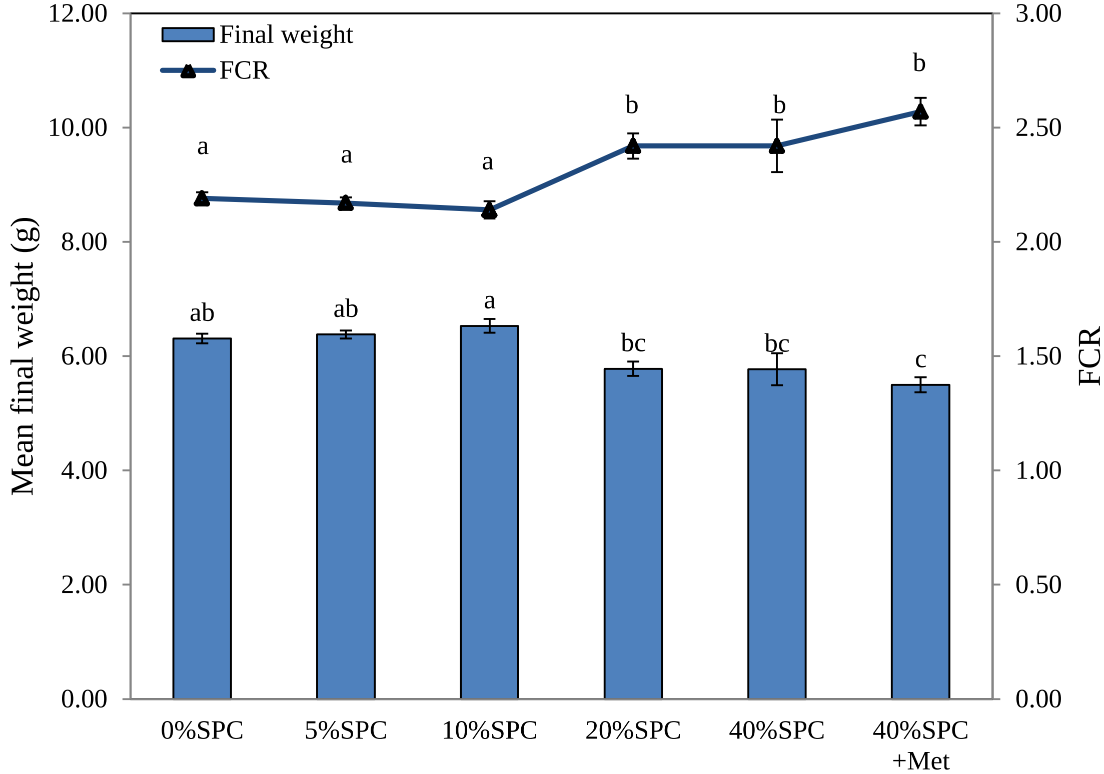
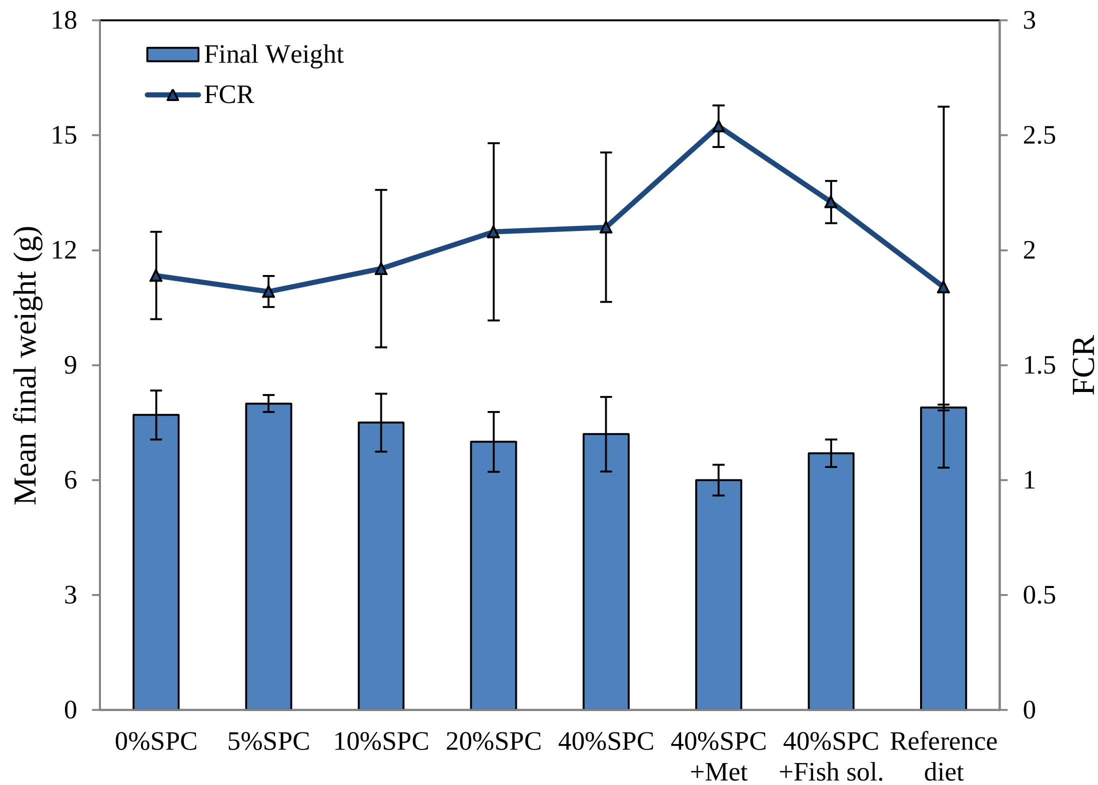


Figure 2. Mean final weight (g) and FCR of *L. vannamei* post larval (initial weight of 0.14±0.05 g) reared under clear water system in polyethylene tanks during 53-day experimental period (second trial), fed diets containing increasing percentages of (0, 5, 10, 20, 40, 40% supplemented with methionine and 40% with fish solubles) as a substitute for SE-SBM, compare to a commercial reference diet.



CHAPTER IV

USE OF SOY PROTEIN CONCENTRATE IN PRACTICAL DIETS FOR PACIFIC WHITE SHRIMP (*Litopenaeus vannamei*) REARED UNDER VARIOUS CULTURE CONDITIONS

Abstract

The objective of this project was to demonstrate the feasibility of diets formulated to contain increasing percentages of soy protein concentrate (SPC) in production diets for *Litopenaeus vannamei* reared under different culture conditions. A series of growth trials using different culture systems which included clear water recirculating system, a green water, semi-closed, recirculating system, and production ponds, were conducted at Claude Peteet Mariculture Center in Gulf Shores, Alabama. Four practical diets containing increasing percentages (0, 4, 8, and 12%) of SPC were utilized to evaluate the response of the shrimp over the culture period. The clear water trial was conducted in 600-L polyethylene tanks using three replicates per diet. Juvenile *L. vannamei* (mean weight ± S.D., 0.94 ± 0.04 g) were stocked at a density of 15 shrimp m^{-2} and cultured for 10 weeks. The same four dietary treatments and a commercial reference diet were offered to juvenile shrimp (initial weight of 1.0 ± 0.03 g) maintained in outdoor green water system over a 10 week period at a stocking density of 30 shrimp per tank. In addition, test diets were tested in 0.1-ha ponds using four replicates per diet. Post larvae were obtained from commercial sources and nursed for three weeks. Juvenile shrimps (mean weight ±

S.D., 0.013 ± 0.001 g) were stocked at 35 shrimp m^{-2} and were cultured under standardized pond production conditions for 18 weeks. At the conclusion of these experiments; net yield, mean individual weight, survival, and FCR were evaluated. The results in green water tank trial and pond trial demonstrated the feasibility of soy protein concentrate inclusion up to 12% SPC in soybean based diet without causing negative effect on growth, feed conversion, survival and net yield. The results in clear water tank trial show no consistent trend in mean final weight among treatments, but no differences were observed in net yield, survival and FCR among treatments. Based on the results from the previous study as well as of this study results, SPC at a level up to 12% can be used in commercial feed formulations for *L. vannamei*.

Introduction

Protein is the most expensive component of the diet and is a major nutrient required for maintenance and growth of shrimp (Shiau, 1998). An insufficient daily intake of protein will result in reduction in growth or abnormality. The minimum protein requirements for shrimp to maintain optimal performance varied depending on age or size of shrimp, quality of dietary protein (essential amino acid profiles and digestibility), availability of alternative food sources, environmental parameters, and culture management (D'Abramo and Sheen, 1994; Lim and Akiyama, 1995; Venero, 2006). Generally, commercial shrimp feeds contain 30% to 50% crude protein, composed mostly of marine animal protein such as fish meal, shrimp meal and squid meal (Mente et al., 2002; Lim and Dominy, 1990). These ingredients have excellent nutritional value with high palatability, but are expensive and not always readily available (Lim and Persyn, 1989). Considerable research has been conducted to find alternative protein sources which are inexpensive, have a stable supply and are sustainable to replace marine animal protein in shrimp feed formulations.

Among alternative protein ingredients, soybean meal is considered one of the most promising ingredients and widely used as a protein source in fish and shrimp feeds because of its acceptable amino acid profile, consistent composition, availability and reasonable price (Dersjant-Li, 2002; Forster et al., 2002; Hardy, 1999). Several studies have been conducted to evaluate the nutritional value of soybean products in shrimp diets (Lim and Dominy, 1990, 1992; Amaya et al., 2007a,b; Cruz-Suarez et al., 2001; Ray et al., 2009; Sudaryono et al., 1995; Samocha et al., 2004; Piedad-Pascaul et al., 1990; Smith et al., 2001). However, the use of soybean meal as a sole protein ingredient in shrimp feed is limited because of its shortage in sulfur amino acids, highly saturated fatty acids, and mineral such as phosphorus and the presence of anti-nutritional factors such as trypsin inhibitors that may affect digestibility and palatability of feed (Akiyama, 1989; Dersjant-Li, 2002). Therefore, nutrient content of the feed should be well formulated to meet nutritional requirements of the shrimp when soy products are utilized. As the nutrient density (protein and energy) of the diet is increased the use of solvent-extracted soybean meal (44-48% protein) is inefficient as there is insufficient room in the formulation for all necessary components. Hence, higher protein ingredients are often required.

Compared to solvent-extracted soybean meals, soy protein concentrate (SPC) contains higher protein and generally lower levels of antinutrients. Soy protein concentrate contains approximately 65% crude protein, a level similar to that found in fish meal, and considerably higher than SE-SBM (Paripatananont et al., 2002; Liu, 1997). Likewise, several anti-nutritional factors such as trypsin inhibitors and soluble oligosaccharide can be inactivated through the alcohol extraction process of SBM (Kaushik et al., 1995). Soy protein concentrate (SPC) is produced through a series of different extraction and precipitation process from high quality de-hulled soybeans. By removing most of the oil and water soluble non-protein constituents, a variety of high protein products with varying characteristics can be produced.

Numerous published studies have indicated the feasibility of SPC inclusion in feeds for many fish and shrimp species such as European sea bass (Tibaldi et al., 2006), rainbow trout (Kaushik et al., 1995; Mambrini et al., 1999), Atlantic halibut (Berge et al., 1999), Atlantic salmon (Refstie et al., 2001; Storebakken et al., 1998, 2000), tiger shrimp

(Liu et al., 2000; Paripatananont et al., 2002), and Pacific white shrimp (Forster et al., 2002). However, there is limited information on the utilization of SPC in combination with soybean meal in commercial feeds for *L. vannamei*. According to our previous studies which have evaluated increasing levels of SPC in soybean based diet for *L. vannamei* under laboratory conditions, inclusion of 20% SPC or higher in soybean based diet resulted in adverse effect on shrimp growth. Inclusion of lower levels of SPC in soybean based diet, however, resulted in good performance of shrimp. Thus, inclusion of SPC at levels from 0 to 12% in combination with soybean meal were used to evaluate feasibility of SPC use in commercial feed for *L. vannamei*.

As the presence of natural foods can have a synergistic effect on the performance of the shrimp, studies were carried out through a series of growth trials under both laboratory and field conditions. Nutrition studies are often carried out under laboratory conditions where water quality management is easier and there is no natural productivity (Amaya et al., 2007a). On the other hand, the presence of natural food sources in green water tank system and in ponds may enhance shrimp growth and maintain acceptable water quality (Moss, 2000). Shrimp have the ability to harness food and nutrient particles suspended in water column and through benthic foraging, (Tacon et al., 2004). Hence, the evaluation of shrimp performance in the presence of natural feeds should be evaluated. Both pond and green water tank systems allow shrimp to have access to natural foods. However, green water tanks systems are less variable and less costly than ponds. Given there is still limited information regarding to the utilization of SPC under commercial conditions, this study was carried out to evaluate the feasibility of diets formulated to contain increasing percentages of SPC (0, 4, 8 and 12%) in production diets for *L.*

vannamei reared under different culture environments, e.g., clear water tank system, green water tank system, and production ponds.

Methods

A series of experiments were conducted at the Alabama Department of Conservation and Natural Resources Marine Resource Division, Claude Peteet Mariculture Center in Gulf Shores, Alabama. The growth trials of postlarvae *L. vannamei* were conducted under three rearing systems; a clear water recirculating tank system, a green water tank system and an outdoor pond system.

Experimental animals

Shrimp post larvae (PL) were obtained from a commercial hatchery, Shrimp Improvement System, Key West, FL. Dissolved oxygen (DO), temperature, salinity, and pH were randomly measured among the shipping bags using a YSI 556 MPS meter (Yellow Spring Instruments Co., Yellow springs, OH, USA). Two water samples were collected in order to determine the total ammonia-nitrogen (TAN) using the Orion ammonia electrode probe (Thermo Fisher Scientific Inc., Waltham, MA, USA). An oxygen tank with one air stone was operated to provide oxygen during the acclimation period. Newly hatched brine shrimp, *Artemia salina* (INVE Americas, Inc., Salt Lake City, UT, USA), were prepared one night before PL reception, and were fed to PL in the acclimation tank. PL were slowly acclimated to temperature and salinity prior to releasing into the acclimation tank. Water quality variables were measured to control temperature and salinity change in the range of 4 ˚C per hour and 2 ppm per hour, respectively. After acclimation PL were concentrated by drain harvesting into a 57-L concentration tank and quantified volumetrically. Six sub-samples were taking using a 60-ml beaker while the water in the concentration tank was mixing vigorously. Average numbers of PL per ml were then determined. Sixty shrimps were randomly collected and individually weighed to determine the average weight and predict the biomass for determination of feed input.

The shrimp were divided and spread equally into four nursery tanks (6,000 L). The nursery system was stocked at a density of 54 postlarvae per liter. Each nursery tank was equipped with three air lifts and two air stones to help with water circulation and aeration which was provided by a common regenerative blower. The recirculating nursery system was composed of six nursery tank, a biological filter, a pressurized sand filter and a 1.5-hp circulation pump (Aquatic Eco-systems, Apopka, Florida, USA). Feeds were applied on daily basis four times per day. Uneaten feed was siphoned as needed to maintain water quality in the nursery tanks (Table 1). PL were reared in the nursery system for 3 weeks. Water Quality was monitored two times per day at 800 and 1600 h, and Total ammonia-nitrogen (TAN) was measured bi weekly using a spectrophotometer (Spectronic 20 Genesys, Spectronic Instrument Inc. Rochester, NY, USA) following the Nesslerization method (APHA, 1989) (Table 2). Every three days, 60 PL per tank were randomly collected and sub-samples of 10 shrimps each were pooled weighed in order to evaluate growth and adjust daily feed inputs.

At the end of two weeks, each tank was partially drained to concentrate the shrimp. The shrimp were then collected by dip nets. Six sub-samples were taken from each tank to determine average wet weight of the shrimp (g). Once the number of shrimp per unit weight was determined, shrimp were harvested and small groups weighed (around 100g per group) and then distributed into one of 16 buckets (1 bucket per pond) each representing one production pond. Shrimp from each nursery tank were distributed across all ponds to minimize any bias from variation in nursery tanks. The shrimp were then transferred to the corresponding pond and stocked. Final population, weight gain (g), percentage survival, final weight (g), and feed conversion ratio for each nursery tank was then determined (Table 3).

Experimental diets

Four experimental diets (Table 4) were formulated to contain graded levels of soy protein concentrate (SPC). The experimental diets were manufactured as a sinking extruded pellet by Rangen Inc. (Angleton, TX, USA) under commercial feed manufacturing condition. All experimental diets contain approximately 35% protein and 8% lipid with lysine and methionine plus cystine contents of 5% and 3% of the total protein and squid meal at 0.5% of the diet was also included. The proximate composition including protein digestibility (using 0.0002% pepsin solutions) of the test diets are presented in Table 5. The basal diet was designed to contain 58% solvent extracted de-hulled soybean meal which was replaced with graded levels of soy protein concentrate (0, 4, 8 and 12%) for test evaluations.

Clear water tank trials

Juvenile shrimp obtained from the nursery system were selected by hand-sorting to a uniform size. Then juvenile shrimp (0.94±0.04 g, initial weight) were stocked at a density of 15 shrimp per tank. The clear water tank system was a semi-closed recirculating system consisted of 12 culture tanks (0.53 m height x 1.19 m diameter, 600-L volume), a central reservoir, a biological filter and a circulation pump. Four experimental diets were randomly assigned with three replications (tanks) per treatment. Test diets were offered to shrimp twice daily at 0800 and 1600 h for a 10-week experimental period. Feed input was pre-calculated on a weekly basis based on an expected feed conversion ratio of 1.5 and a growth rate of one gram per week. Shrimp were counted every two weeks to readjust the daily feed input. Nettings were used to cover all tanks to prevent shrimp from jumping out.

During the 10-week growth trial, dissolved oxygen concentration, temperature, salinity, and pH were monitored twice daily in the central filter and two of the rearing tanks at 600 h and 1600 h using a YSI meter, (Yellow Spring Instrument Co., Yellow Springs, OH, USA). Water samples were taken bi-weekly from central reservoir and two tanks to measure Total ammonia-nitrogen (TAN) using the Orion ammonia electrode probe (Thermo Fisher Scientific Inc., Waltham, MA, USA). Water quality results are summarized in Table 6.

At the conclusion of the 10-week growth trial shrimp were counted and weighed individually. Percentage survival, mean final weight, biomass and feed conversion ratio were determined.

Green water tank trials

Juvenile *L. vannamei* (1.0±0.03g initial weight) were collected from the research ponds and were size-sorted by hands. Juvenile shrimp were randomly selected and stocked at a rate of 30 shrimp per tank (38 shrimp per m^3) in an outdoor recirculating system. The outdoor tank system consisted of a central reservoir (800 L) with a trickle filter, a 0.33-hp submersible pump, and 24, circular polyethylene tanks (0.85 m height x 1.22 m upper diameter, 1.04 m lower diameter) designed to contain 800 L. A 0.33-hp submersible pump was used to move water from one of the production ponds to the central reservoir at a rate of 8 liters per minute between 0800 h and 1400 h to mimic a production pond setting. This exchange rate allowed a 100% water exchange in the recirculating system every six days. Each tank and central reservoir were provided with two air stones connected to a 0.5-hp regenerative blower (Sweetwater Aquaculture Inc. Lapwai, ID, USA) to supply aeration. Netting was used to cover all tanks to protect the shrimps from jumping out.

During the 10-week growth trial, dissolved oxygen concentration, temperature, salinity, and pH were monitored twice a day in the central filter and two of the system tanks at 0600 h and 1600 h using a YSI meter, (Yellow Spring Instrument Co., Yellow Springs, OH, USA). Water samples were taken bi-weekly from central reservoir and two tanks to measure Total ammonia-nitrogen (TAN) using the Orion ammonia electrode probe (Thermo Fisher Scientific Inc., Waltham, MA, USA). Water quality results are summarized in Table 7.

Five test diets were randomly assigned amongst the 24 tanks (750 L). Four experimental diets used in production ponds were tested using five replicates per diet, and the fifth diet, a reference commercial diet (Rangen Shrimp Grower, 35% protein, 8% lipid), was tested using four replicates. Juvenile shrimp were offered test diets twice per day at 0800 h and 1600 h. Daily ration of feed was calculated based upon an expected weekly growth rate of 1.3 g wk^{-1} and expected FCR of 1.2.

At the conclusion of the 10 week growth trial shrimp were counted and weighed individually. Mean final weight, final net yield, percent survival, and feed conversion ratio were determined.

Pond Production Trial

Juvenile *L. vannamei* (initial weight of 0.01±0 mg) were collected from nursery system and stocked in the production ponds at the rate of 35 shrimp m^{-2}. Ponds used for the grow-out phase were approximately 0.1 ha in surface area, (rectangular 46 x 20 m) with a 1.0 m average depth. Each pond was equipped with a 20-cm diameter standpipe, a concrete catch basin (3.0 x 2.0 x 0.5 m), and lined with 1.52 mm thick high-density polyethylene lining (HDPE). The bottom of each pond was covered with a 25-cm deep layer of sandy-loam soil. After each culture cycle, ponds were dried and tilled in order to allow oxidation and mineralization of organic matter. Approximately three weeks before stocking, ponds were filled with brackish water from the intra-costal canal between Mobile and Perdido Bay, AL. Incoming water was filtered through a three foot 250 μm-mesh nylon filter sock (Micron Domestic Lace Mfg., Inc) to prevent the introduction of predators, as fish larvae, crab, or other planktonic organisms, but allow the introduction of phytoplankton in ponds. Two weeks before stocking juvenile shrimp, the combination of inorganic liquid fertilizers of 32-0-0 mixed with 10-34-0 were applied in a rate of 1,697 ml and 303 ml per pond, which provided 5.73 kg of N and 1.03 kg of P_20_5 ha^{-1}, respectively, in order to promote natural productivity in all ponds. The second fertilization was reapplied at half of the initial rate when the Secchi disk reading was greater than 40 cm. All ponds were provided aeration about 10 hp ha^{-1} using 1-hp paddlewheels aerators (Little John Aerator, Southern Machine Welding Inc. Quinton, AL) with either 1-hp or 2-hp Aire-O_2 aerators (Aire-O_2, Aeration Industries International, Inc. Minneapolis, Minnesota) to maintain dissolved oxygen above 3 mg L^{-1}, additional aerators were provided as needed when dissolved oxygen fell below 3 mg L^{-1}. This study was a sustainable semi-intensive system which was well managed feeding, and minimized water exchanged.

Four test diets were randomly assigned to 16 production ponds using four replicates per diet. Test diets were divided to two feedings per day, early morning 0800 h and late afternoon 1600 h. Feed inputs were based on historical results as well as current observations. A small amount of commercial feed was applied to promote natural productivity in pond water during the 1st to 4th weeks. Thereafter, feed input was calculated based upon an expected weight gain of 1.3 g wk^{-1}, a feed conversion of 1.2, and a survival of 75% during the pond culture period (Table 8).

During the experimental period, 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 two 5 foot cast nets (monofilament net, 1.52 m radius and 0.95 cm opening) for the remaining of the culture period.

Water quality variables, e.g., dissolved oxygen (DO), temperature, salinity, and pH were monitored three times a day, at sunrise (0500 h - 0530 h), during the day (1500 h - 1530 h), and at night (2300 - 2400 hr), using a YSI 556 MPS meter, (Yellow Spring

Instrument Co., Yellow Springs, OH, USA). Secchi disk reading and TAN were monitored on a weekly basis. Water samples were taken from all ponds at the depth of 80 cm in the water column and TAN was determined using the Orion ammonia electrode probe (Thermo Fisher Scientific Inc., Waltham, MA, USA). Water quality results for the pond trial are summarized in Table 9.

The shrimp were harvested at the end of the 18-week study period, three feedings were stopped before harvest in order to clear the shrimp gut. The night before harvest, the water was drained down about two thirds of the ponds and aeration provided using paddlewheel aerators to keep shrimp alive and minimize erosion of the pond bottom. On harvest day, the remaining water was drained and the shrimp were pump harvested 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 pumped, de-watered and collected in a hauling truck. The shrimp were rinsed and weighed. Approximately 150 shrimps for each pond were randomly selected to measure individual weight and size distribution. At the conclusion, mean final weight, net yields, percent survival, and FCR were determined.

Statistical analysis

All data were statistically analyzed using one-way analysis of variance to determine significant differences ($P<0.05$) among treatments, followed by the Student–Neuman–Keuls multiple comparison test to determine significant differences between treatment means (Steel, Torrie, 1980). All statistical analyses were carried out using SAS (V9.1. SAS Institute, Cary, NC, USA).

Results

Nursery system

Average water quality variables throughout the 15-day nursery period were as follows: dissolved oxygen 6.44 ± 1.45 mg L^{-1}; temperature 26.22 ± 1.44 ^{o}C; pH 7.55 ± 0.33; salinity 28.63 ± 5.03 ppt; and TAN 0.15 ± 0.14 mg L^{-1} (Table 2). Water quality conditions were maintained within acceptable limits for the culture of this species. At the conclusion of the shrimp nursery period, mean individual weight 0.013± 0.006 g, weight gain 0.012 ± 0.006 g, survival 58.07 ± 5.27%, and FCR 1.42 ± 0.04 were observed (Table 3).

Clear water tank trial

Overall means, standard deviations, and ranges of morning and afternoon water quality variables observed over 10-week growth trial were within acceptable limits for the culture of this species (Table 6). The results of one replicate from treatment fed diet 0% SPC was excluded from the statistical analysis because of the occurrence of algae in the tank which caused better performance of shrimp. Growth performance of *L. vannamei* reared under clear water recirculating system is summarized in Table 10. There were no differences ($P > 0.05$) in survival, FCR, and net yield. However, significant differences in mean individual weight among treatments were observed in the 10-week growth trial in *L. vannamei* fed diet contain increasing percentage of 0, 4, 8, and 12% SPC in substitution to soybean meal as a protein source. Survival ranged from 88.9 to 97.8%. Net yield ranged from 77.1 to 100.6 g $tank^{-1}$. FCR ranged from 2.65 to 3.61. Mean individual weight ranged from 5.3 to 7.2 g with the highest mean individual weight in shrimp fed diet containing % SPC (Figure 1a).

Green water tank trial

The overall means, standard deviations, and ranges of morning and afternoon water quality variables observed over 10-week growth trial were within a reasonable range for the culture of this species(Table 7). Growth performance of *L. vannamei* reared under green water tank system is summarized in Table 11. Results indicated that there were no differences (P > 0.05) in survival, mean final weight, and FCR of *L. vannamei* fed diet containing increasing percentage of SPC in substitution to soybean meal as a protein source. However, significant differences in net yield among treatments were observed l. Survival ranged from 95.8 to 100.0%. Mean individual weight ranged from 13.5 to 15.0 g. FCR ranged from 1.17 to 1.28. Net yields ranged from 397.2 to 431.6 g $tank^{-1}$ with the highest yield in treatment fed 8% SPC. Shrimp maintained on the commercial reference diets showed significantly higher in net yield and lower FCR than shrimp fed on the test diets albeit; treatment fed diet containing 8% SPC had similar yield to treatment fed reference diet. Additionally, similar FCR to treatment fed reference diet was observed in treatment fed 8 and 12% SPC (Figure 1b).

Production pond trial

The overall means, standard deviations, and ranges of morning, afternoon, and night water quality variables observed throughout the 18-week growth trial are displayed in Table 9. Water quality conditions were within a reasonable range for the culture of this species under pond production conditions. High dissolved oxygen readings (above 10 mg L^{-1}) were observed in the afternoon due to photosynthesis activity. On the other hand, low dissolved oxygen readings (below 2.5 mg L^{-1}) were observed due to the phytoplankton died off (Boyd, 2007, 2009).

General trends in production results from the pond trial were similar to those observed in the outdoor tanks. The results of one replicate from treatment fed diet containg 0% SPC was excluded from the statistical analysis because of low survival of shrimp. Growth performance of *L. vannamei* reared under production pond condition is summarized in Table 12. There were no differences ($P > 0.05$) in any of the measured production parameters. Treatment fed diet containing 4% SPC had slightly larger yield of 5051 kg ha^{-1} compared with 8% SPC (4508 kg ha^{-1}), 12% SPC (4479 kg ha^{-1}), and 0% SPC (4190 kg ha^{-1}), respectively. Likewise, treatment fed diet containing 4% SPC had numerally lower FCR at 1.30 compared with 12% SPC (1.48), 8% SPC (1.50), and 0% SPC (1.59), respectively. However, there were no differences in survival and mean final weight among treatments. Shrimp survival ranged from 86.7% (0% SPC) to 93.3% (12% SPC), and mean final weight ranged from 13.5 g (0, 8 and 12% SPC) to 15.7 g (4% SPC), but these differences were not significant.

Discussion

The results from this study indicate that SPC at a level up to 12% may be include in diets containing high level of soybean meal without adversely affecting growth and survival of *L. vannamei*. Although, there is no consistent trend in mean final weight of shrimp results from the clear water tank trial, significant difference in mean final weight among treatments were observed. However, there were no differences in final biomass among treatments. This finding is similar to growth response of shrimp in green water tank trial or pond trial. Furthermore, the results from the previous study in clear water system with shrimp fed diets containing from 0 to 10% SPC also showed no difference in growth performance.

In general, high level of soybean proteins inclusions in shrimp feed, with the absence of expensive fish meal, are viable and result in acceptable performances of shrimp, when essential nutrients in diet are properly balanced to meet shrimp requirement. Similar results was reported by Kaushik et al. (1995) who found that diet containing SPC with L-methionine supplement could completely replace fish meal in rainbow trout diet without causing negative effect on growth performance and nutrient utilization of fish.

On the other hand, Lim and Dominy (1990) found the decrease in weight gain of *L. vannamei* fed diets containing 42% SE-SBM or higher. Likewise, Floreto et al. (2000) indicated that diets containing 50% soybean meal and higher resulted in significantly lower body weight gain in juvenile American lobster than treatment fed diets with lower level of soybean meal inclusion.

In the clear water tank system, shrimp growth is the result of the availability of compound feed entering the system to satisfy the nutritional requirements as there are little or no other food sources presented (Moss ,1995). There were no differences (P > 0.05) in survival, FCR, and net yield. However, significant differences in mean individual weight among treatments were observed in the 10-week growth trial Mean final weight of shrimp fed 0% SPC was significantly higher than shrimp offered the diets with 4% SPC, but there were no differences in the growth performances among dietary treatments containing 0, 8,and 12% SPC or 4, 8 and 12% SPC. This does not follow the expected dose response and does not match previous experiments or results for final biomass. In addition, there was an evidence of the present of filamentous algae in 0% SPC treatment which may have serves as a potential supplemental food that could certainly improve growth performance of shrimp in this dietary treatment. Based on the results in clear water systems from the previous chapter which shrimp fed diets containing increasing level of SPC from 0 to 10% resulted in no differences in growth response, the differences observed in this trial is more likely caused by the extrinsic factors or random variation.

In the green water tank trial, the results at the conclusion of the 10-week growth trial indicated that net yield in treatment fed diets containing higher level of SPC (4, 8 or 12%) resulted in significantly better net yield than treatment fed diet without SPC (0% SPC). However, there were no differences ($P > 0.05$) in survival, mean final weight, and FCR for shrimp offered diets containing various levels of SPC. Shrimp maintained on the commercial reference diets had significantly higher mean final weight than shrimp fed the test diets. This could be the results of slight inadequacies of the plant based diet or simply variation across treatments. These results confirm that SPC at a level up to 12% could be used in shrimp feed formulations and that higher levels provided numerically better results. As has been previously reported growth performance of the shrimp was considerably better in the green water trial comparing to that of the clear water system, (1.44 g wk^{-1} versus 0.61 g wk^{-1}, respectively). This is caused by the availability of additional nutrient from natural food organisms present in the water column and sediment in the green water tanks that serve as growth-enhancing factor in shrimp (Moss, 1995; Tacon et al., 2002).

Similar to the results to the green water tank trial, production parameters results from the semi-intensive pond trial over an 18-week culture period were not different ($P > 0.05$) among treatments. Net yields (3,900 – 5,051 kg ha^{-1}) were in acceptable production range for semi-intensive shrimp production. Overall mean final weight (14.3 g) from this trail was found to be slightly lower than previous historical data from the same site. This could be the results of overstocking of juvenile shrimp as percent survivals in several ponds were over 100%. Since, feed inputs were calculated based on the expected survival of 75% the higher than expected survival may have resulted in feed limitations to shrimp. Shrimp reared under the green water tank system which were obtained from the production ponds showed better performance in term of growth per week than those in production ponds, 1.44 g wk^{-1} comparing with 0.86 g wk^{-1}, respectively. These observations may indicate that underfeeding may be one of the reasons for the reduction in shrimp growth. In addition, shrimp growth can be affected by unknown water quality conditions or shrimp genetics.

Based on the observed response of the shrimp from this experiment, there were no differences across the SPC treatments in all culture environments, except in the clear water system. Thus, we can conclude that the use of SPC at a level up to 12% is acceptable and promote growth in Pacific white shrimp. The pepsin digestibility of crude protein in all dietary treatments ranged from 76.9 to 80.5%. The digestibility values of our test diets are considered reasonable comparing to the apparent protein digestibility of soybean meal (90%) and of fishmeal (80.7%) reported for *L. vannamei* by Akiyama et al.

(1989). Consequently, the results of this study confirm that the use of SPC in commercial feed formulations is possible regarding to promote SPC use as a high protein ingredient for shrimp feeds.

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Table 1. Feeding regime for *Litopenaeus vannamei* post larvae, based on mean shrimp weight determined every three days, percent body weight and feed types utilized throughout a 15-day nursery period.

Day	Mean weight (mg)	Feed type	Feed (% body weight)
1 to 3	1.01	100 Artemia[a] / PL, PL redi[b] (1:1)	25
4 to 6	2.24	PL redi , Cru[c] #0 (1:1)	25
7 to 9	3.53	Cru # 0	25
10 to 12	5.03	Cru # 0	15
13 to 15	8.10	Cru # 0, Cru #1& #2 (1:1)	15

[a]INVE Americas, Inc. Salt Lake City, UT, USA.

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

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

Table 2. Water quality variables observed over a 15-day nursery period for *L. vannamei* post larvae, nursed in a recirculating system composed of six, 6,000-L nursery tanks.

	Average	Minimum	Maximum	Standard deviation	CV[a]
Temperature (°C)	26.22	23.30	29.50	1.44	5.50
DO (mg L^{-1})[b]	6.44	2.75	9.71	1.45	23.06
Salinity (ppt)	28.63	17.10	34.00	5.03	17.58
pH	7.55	7.11	8.11	0.33	4.42
TAN (mg L^{-1})[c]	0.15	0.01	0.49	0.14	95.57

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

[b]Dissolved oxygen.

[c]Total ammonia-nitrogen.

Table 3. Mean production parameters of *L. vannamei* post larvae (initial weight of 1.0 mg), stocked at a density of 52 PL L^{-1} and nursed for 15 day in a recirculating system composed of four, 6,000-L nursery tanks.

	Mean	Minimum	Maximum	Standard deviation	CV[a]
Final weight (mg)	12.88	12.10	13.40	0.57	4.41
Weight gain[b] (mg)	11.87	11.09	12.39	0.57	4.78
Survival (%)	58.05	55.13	61.02	3.27	5.63
FCR[c]	1.42	1.35	1.45	0.04	3.08
Yield (g $tank^{-1}$)	2,440	2,381	2,554	77.19	3.16
Standing crop (kg m^{-3})[d]	0.678	0.661	0.709	0.021	3.164

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

[b]Weight gain = Final weight - Initial weight.

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

[d]Standing crop (kg m^{-3})= (Yield/1,000)/3.6 m^3.

Table 4. Ingredient composition (g 100 g^{-1} as is) of four experimental diets designed to contain increasing percentage of 0, 4, 8 and 12% soy protein concentrate (SPC) as a substitute for soybean meal and used for *Litopenaeus vannamei* growth trials.

Ingredient	0% SPC	4% SPC	8% SPC	12% SPC
Soybean meal (47%)	58.01	52.01	46.01	39.67
Milo	16.34	18.34	20.34	22.67
Corn distillers grain	10.00	10.00	10.00	10.00
Corn gluten meal	4.83	4.83	4.83	4.83
SPC	-	4.00	8.00	12.00
Dicalcium phosphate	3.38	3.38	3.38	3.38
Fish oil	4.33	4.33	4.33	4.33
Bentonite	1.50	1.50	1.50	1.50
Squid meal	0.50	0.50	0.50	0.50
Soy lecithin	0.50	0.50	0.50	0.50
Vitamin premix[a]	0.33	0.33	0.33	0.33
Mold inhibitor	0.15	0.15	0.15	0.15
Mineral premix[a]	0.09	0.09	0.09	0.09
Stay-C 35% (C)	0.02	0.02	0.02	0.02
Copper sulfate	0.01	0.01	0.01	0.01
Total	100	100	100	100

Diets were formulated to contain 35% protein and 8% lipid and commercially manufactured by Rangen® (Angleton, TX, USA) using extrusion processing.

[a]Vitamin premix and mineral premix are proprietary products, thus their composition is not listed.

Table 5. Nutrient composition of the experimental diets and a commercial reference diet used for *L. vannamei* reared under different culture conditions.

Nutrient	Proximate analyses (%)				
	0% SPC	4% SPC	8% SPC	12% SPC	Reference diet
Crude protein	35.8	37.0	36.7	35.9	35.8
Moisture	8.34	8.61	7.70	7.71	11.39
Fat (AH)	9.34	8.51	8.69	8.49	9.47
Crude fiber	2.87	3.06	2.91	2.96	2.57
Ash	9.08	9.26	9.48	9.27	7.92
Protein digestibility (0.0002% pepsin)	79.89	80.54	77.66	76.88	

Diets were formulated to contain 35% protein and 8% lipid.

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

Diets were analyzed by New Jersey Feed Laboratory, Trenton, NJ,USA.

Table 6. Water quality variables monitored during 10 week growth period of juvenile *L. vannamei* (initial weight of 0.94 g), fed experimental diets containing increasing level of SPC at 0, 4, 8 and 12% as a substitute for soybean meal, and reared under a clear water recirculating system. Values represent the mean ± standard deviation of daily and weekly determinations. Values in parenthesis represent minimum and maximum readings.

	Temperature (°C)	DO (mg L^{-1})[a]	pH	Salinity (ppt)	TAN (mg L^{-1})[b]
am	26.29±1.16 (21.73, 28.34)	6.10±0.63 (4.58, 8.61)	7.82±0.29 (6.72, 8.36)	16.25±0.63 (12.67, 17.47)	0.04±0.02 (0.02, 0.10)
pm	28.80±0.92 (25.37, 31.16)	5.98±0.56 (4.60, 7.15)	8.20±0.30 (7.33, 8.67)	16.25±0.67 (14.89, 17.49)	

[a]Dissolved oxygen.

[b]Total ammonia-nitrogen.

Table 7. Water quality variables monitored during 10-week growth period of juvenile *L. vannamei* (initial weight of 1.0 g), fed experimental diets containing increasing level of SPC at 0, 4, 8 and 12% as a substitute for soybean meal, and reared under a semi-closed recirculating system. Values represent the mean ± standard deviation of daily and weekly determinations. Values in parenthesis represent minimum and maximum readings.

	Temperature (°C)	DO (mg L^{-1})[a]	pH	Salinity (ppt)	TAN (mg L^{-1})[b]
am	27.16±1.27 (21.97, 29.61)	5.66±0.77 (3.71, 7.26)	7.93±0.36 (6.66, 8.51)	15.60±0.46 (14.49, 16.22)	0.23±0.20 (0.04, 0.70)
pm	30.08±1.27 (27.24, 33.15)	5.55±0.68 (3.66, 6.72)	8.26±0.32 (7.19, 8.70)	15.51±0.42 (14.19, 16.20)	

[a]Dissolved oxygen.

[b]Total ammonia-nitrogen.

Table 8. Feeding regime for *L. vannamei* reared in the grow-out pond during an 18-week experimental period, at estimated survival rate of 75%.

Day	Week	Population	Feed input per day	
			kg pond^{-1}	kg ha^{-1}
1	0	35,000	1.00	10.00
8	1	34,485	1.50	15.00
15	2	33,971	3.00	30.00
22	3	33,456	5.00	50.00
29	4	32,941	5.86	58.57
36	5	32,426	4.04	40.42
43	6	31,912	6.60	66.04
50	7	31,397	7.00	69.97
57	8	30,882	6.88	68.82
64	9	30,368	6.88	68.82
71	10	29,853	6.18	61.78
78	11	29,338	6.54	65.38
85	12	28,824	4.84	48.35
92	13	28,309	5.40	54.01
99	14	27,794	6.19	61.94
106	15	27,279	6.34	63.42
113	16	26,765	6.50	65.00
120	17	26,250	2.50	25.00

Table 9. Summary of water quality variables monitored over an 18-week growth trial for *L. vannamei*, fed experimental diets with varying level of SPC and cultured in 0.1-ha ponds. Values represent the mean ± standard deviation of daily and weekly determinations. Values in parenthesis represent minimum and maximum readings.

Parameter	0% SPC	4% SPC	8% SPC	12% SPC
Temperature (ºC)				
am	28.25±1.93 (19.26, 31.70)	28.52±1.95 (21.37, 33.59)	28.26±2.08 (20.51, 31.67)	28.24±2.09 (21.14, 33.30)
noon	30.52±2.57 (23.98, 34.92)	30.74±2.65 (24.00, 35.38)	30.69±2.66 (23.99, 34.74)	30.83±2.70 (24.14, 36.03)
pm	30.88±2.64 (22.48, 34.09)	30.30±2.76 (22.76, 34.80)	30.23±2.71 (20.54, 34.17)	30.37±2.84 (21.82, 35.16)
DO[a] (mg L^{-1})				
am	5.36±1.63 (0.78, 9.74)	5.20±2.06 (0.35, 14.85)	5.48±1.79 (1.69, 11.48)	5.36±1.75 (1.57, 10.01)
noon	10.39±3.43 (2.62, 23.12)	10.40±3.39 (2.32, 21.61)	10.58±3.40 (3.03, 27.09)	10.56±3.43 (2.52, 21.88)
pm	7.94±2.27 (1.40, 14.26)	8.16±2.47 (1.77, 14.80)	8.14±2.44 (1.66, 14.71)	8.13±2.51 (1.63, 17.70)
Readings < 2.5 (days)	5±3.83	9±6.06	4.5±3.11	6±3.16
pH				
am	8.03±0.46 (6.47, 9.42)	8.08±0.45 (6.56, 9.12)	8.12±0.51 (6.41, 9.37)	7.96±0.48 (6.61, 9.20)
noon	8.85±0.55 (7.02, 10.14)	8.82±0.49 (7.55, 9.87)	8.93±0.56 (6.77, 10.14)	8.88±0.54 (7.33, 10.16)
pm	8.65±0.50 (6.91, 9.79)	8.69±0.49 (7.37, 9.74)	8.75±0.56 (6.77, 10.16)	8.64±0.50 (7.21, 10.08)
Salinity (ppt)				
am	15.36±1.37 (11.34, 18.41)	15.42±1.24 (12.70, 18.88)	14.92±1.44 (11.64, 18.75)	15.51±1.38 (12.73, 19.49)
noon	15.56±1.27 (11.14, 19.17)	15.48±1.19 (12.87, 19.49)	14.84±1.33 (11.65, 18.55)	15.60±1.33 (12.74, 19.85)
pm	15.49±1.13 (11.14, 19.17)	15.52±1.25 (12.89, 19.44)	15.03±1.33 (11.66, 18.88)	15.59±1.37 (12.75, 19.70)
Secchi (cm)	37.35±38.00 (10, 110)	38.16±36.12 (10, 110)	41.41±40.70 (5, 110)	36.90±36.73 (10,110)
TAN[b] (mg L^{-1})	0.69±1.18 (0.01, 5.00)	0.64±1.13 (0.01, 4.00)	0.73±1.39 (0.01, 6.00)	0.36±0.84 (0.01, 4.00)

[a]Dissolved oxygen.

[b]Total ammonia-nitrogen.

Table 10. Mean (n = 3) production parameters at 10-week experimental period of *L. vannamei* (initial weight of 0.94±0.04 g), fed diets containing increasing level of SPC at 0, 4, 8 and 12% as a substitute for soybean meal, and reared under a clear water recirculating system.

Treatment	Biomass (g $tank^{-1}$)	Final weight (g)	FCR[a]	Survival (%)
0% SPC[b]	100.6	7.2a	2.65	93.3
4% SPC	78.2	5.3b	3.54	97.8
8% SPC	77.1	5.8ab	3.61	88.9
12% SPC	93.9	6.4ab	2.85	97.8
P-value[c]	0.159	0.044	0.130	0.713
PSE[d]	7.302	0.350	0.289	6.443

[a]Feed conversion ratio = Total feed offered / biomass increase.

[b]Results with only 2 replicates.

[c]Means (n = 4) not sharing a common superscript within a row are significantly different based on analysis of variance (P > 0.05) followed by Student Newman-Keuls multiple range test.

[d]Pooled standard error of treatment means.

Table 11. Mean production parameters at 10-week experimental period of *L. vannamei* (initial weight of 0.99±0.03 g), fed diets containing increasing level of SPC at 0, 4, 8 and 12% as a substitute for soybean meal, and a reference commercial diet, and reared under a green water recirculating system.

Treatment	Biomass (g $tank^{-1}$)	Final weight (g)	FCR	Survival (%)
0% SPC	398.9c	13.5b	1.28a	98.0
4% SPC	397.2bc	13.7b	1.28a	96.7
8% SPC	416.8ab	13.9b	1.22ab	100.0
12% SPC	411.5bc	13.9b	1.23ab	98.7
Reference diet[a]	431.6a	15.0a	1.17b	95.8
P-value[b]	0.001	0.008	0.002	0.152
PSE[c]	5.448	0.263	0.018	1.170

[a]Reference diets = 35% Protein, Rangen 35,0 (Buhl, Idaho).

[b]Means (n = 5) not sharing a common superscript within a row are significantly different based on analysis of variance (P > 0.05) followed by Student Newman-Keuls multiple range test.

[c]Pooled standard error of treatment means.

Table 12. Mean (n = 4) production parameters at 18 week experimental period of juvenile Pacific white shrimp, *L. vannamei* (initial weight of 10 mg), fed experimental diets containing increasing level of SPC at 0, 4, 8 and 12% as a substitute for soybean meal, and cultured in 0.1-ha production ponds.

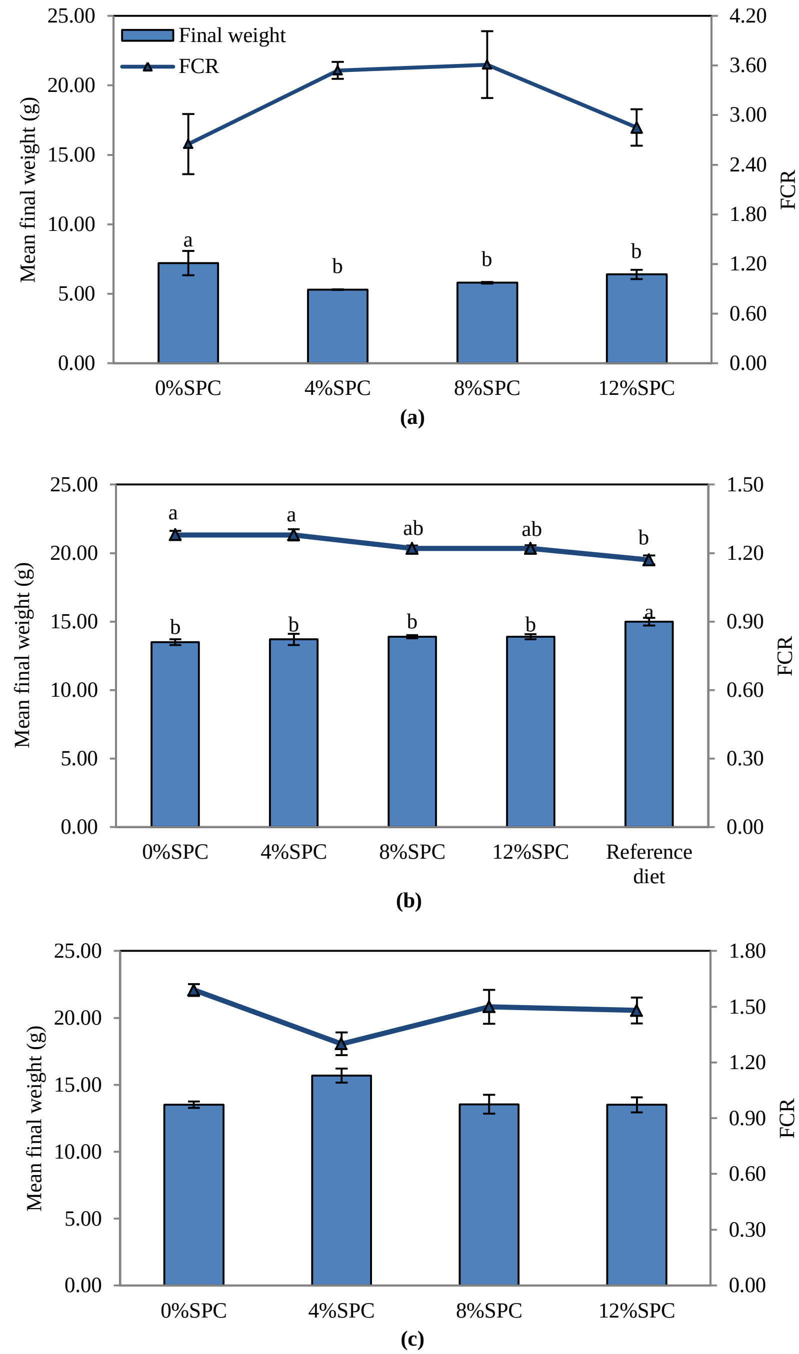
Treatment	Yield ($kg\ ha^{-1}$)	Final weight (g)	FCR	Survival (%)
0% SPC[a]	4190.2	13.5	1.59	86.7
4% SPC	5051.4	15.7	1.30	89.5
8% SPC	4508.6	13.5	1.50	92.9
12% SPC	4479.4	13.5	1.48	93.3
P-value[b]	0.137	0.363	0.136	0.861
PSE[c]	234.378	1.020	0.071	6.019

[a]Results with only 3 replicates.

[b]Analysis of variance was used to determine significant differences ($p < 0.05$) among treatment means (n=4).

[c]Pooled standard error of treatment means.

Figure1. Mean final weight (g) and FCR of *L. vannamei* juveniles fed diets containing increasing level of SPC at 0, 4, 8 and 12% as a substitute for soybean meal, and reared under a clear water recirculating system (a), a green water semi-closed recirculating system (b), and production pond conditions (c).



CHAPTER V

SUMMARY AND CONCLUSIONS

Increasing the use of plant-based ingredients and reducing the use of marine ingredients in shrimp feed formulations has been a primary goal of many researchers. Towards this goal, there is considerable interest in using soybean products in shrimp feeds because of their acceptable nutrient contents as well as low cost compared to marine animal protein sources. Soybean meal contains a moderate protein content, high digestibility, relatively well-balanced amino acid profile, and steady supply with a reasonable price. However, the use of soybean meal as a primary protein source in shrimp feed is limited because of its shortage in sulfur amino acids, highly unsaturated fatty acids, and minerals such as phosphorus as well as the presence of antinutritional factors such as trypsin inhibitors that may affect digestibility and palatability of feed. In addition, as the nutrient density (protein and energy) of the diet is increased, the use of solvent-extracted soybean meal (44-48% crude protein) is inefficient as there is insufficient room in the formulation for all necessary components. Hence, higher protein ingredients are often required. Various mixtures of soybean meal and other protein sources or utilization of soybean products containing higher protein can provide solutions to more balanced feed formulations.

Results from this study confirm that utilization of soybean meal is an acceptable ingredient in feed for Pacific white shrimp, as long as dietary nutrients are properly balanced to meet their requirements. For example, soybean meal-based diets with the inclusion of either 10% of poultry by-product meal, fish meal, distiller's dried grains with solubles or ground pea meal resulted in excellent performance of shrimp reared under outdoor conditions. Shrimp fed soybean-based diets in combination with poultry meal or other plant based proteins produced similar performances to shrimp fed the diet containing fish meal or a commercial reference diet. Consequently, we can conclude that fish meal can be totally replaced by a combination of plant and animal protein sources (soybean meal and poultry by-product meal), or all plant protein sources (DDGS, or pea meal with the inclusion of corn gluten meal and squid meal) when diets are formulated to contain acceptable nutrient levels and proper balance of essential amino acids.

The inclusion of soy protein concentrate as a high protein ingredient in combination with soybean meal and corn gluten meal in practical diets resulted in good performance of shrimp at an inclusion level of 10%. Increasing the inclusion levels of SPC to 20% or higher caused a reduction in shrimp growth. The supplementation of DL-methionine, micro-encapsulated methionine or fish solubles to the diets containing high level of SPC (40%) as a replacement for soybean meal had no effect on the growth performance of shrimp.

Based on the observed response of the shrimp fed practical diets containing increasing level of SPC under various culture conditions, SPC inclusion at a level up to 12% SPC in soybean-based diet caused no negative effect on growth, feed conversion, survival and yield of shrimp under both laboratory and field conditions. Consequently, the inclusion of SPC in shrimp feed formulation at a level of up to 12% is acceptable and promotes good growth in Pacific white shrimp.

In conclusion, based on laboratory, outdoor tank and pond based research soybean meal can be used as the primary protein source in *L. vannamei* feed formulations provided that proper combination with other low cost protein sources such as poultry by-product, distiller's dried grains with solubles, pea meal or soy protein concentrates is made. To develop such feed formulations, it is critical that the ingredients are of high quality and the essential nutrients in diets are properly balanced to meet shrimp requirements. Such diets should facilitate reduced feed costs and promote the use of sustainable plant protein sources in shrimp production.

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