Use of Corn Protein Products in Practical Diets for the Pacific White Shrimp

Denghang Yu[a,b], Xiaoyun Fang[a], Yangen Zhou[a], Melanie Rhodes[a], D. Allen Davis[a,*]

[a] School of Fisheries Aquaculture and Aquatic Sciences,
Auburn University, AL 36849-5419, USA
[b] College of Fisheries, Huazhong Agricultural University,
Wuhan 430070, China Tel.: +1 334 844 9312; fax: +1 334 844 9208
E- Mail address: davisda@auburn.edu

Abstract

Corn protein products are commonly used to balance the amino acid profile of practical diets especially those with low levels of methionine. Yet, there is little information regarding the efficacy of their use. Consequently, the objective of this study was to evaluate the production response of *Litopenaeus vannamei* fed diets with increasing percentages of corn protein products. Three standard indoor tank trials and a pond production trial were conducted. In trial 1, three diets were formulated to contain corn gluten meal (CGM) (8%) and increasing levels (6.5 and 13.0%) of corn protein concentrate (CPC). Five diets were evaluated in Trial 2, with diets 1 to 4 using increasing levels (0, 4, 8 and 16%) of CPC replacing soybean meal, while diet 5 contained a CPC with a better AA profile specifically higher levels of lysine, designated CPCL. The production diets contained increasing percentages (0, 4, 8, and 12%) of CPC as a replacement for fish meal. The diets were commercially produced and evaluated in 0.1 ha production ponds using four replicates per diet. Nursed juvenile shrimps (0.023 g initial weight) were stocked at 38 shrimp m^{-2} and were cultured under standardized pond production conditions for 16 weeks. At the conclusions; net yield (5007-5440 kg/ha), final mean weight (17.2-20.5 g), survival (64.9-83.6 %), and FCR (1.27-1.38) were evaluated with no significant differences between dietary treatments. The results from this study demonstrated that corn protein concentrate inclusion up to 12% CPC in diet can be used in commercial feed formulations for *L. vannamei* without causing negative effect on growth, feed conversion, survival and net yield.

Keywords: Pacific white shrimp, Corn protein concentrate, Production pond

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Introduction

Pacific white shrimp (*Litopenaeus vannamei*) is one of the most popularly cultured shrimp in the world. It is a good species for intensive production because of its rapid growth, low mortality in high-density culture and disease resistance (Williams *et al*. 1996; Ponce-Palafox *et al*. 1997). To reduce culture costs, considerable amount of work has been put towards reducing the cost of shrimp feed formulations by replacing relatively expensive ingredients with more economical alternatives. The study to reduce feed costs has concentrated on development of alternative protein sources, which is the most expensive component of the diet and is a major nutrient required for maintenance and growth of shrimp (Shiau 1998). The development of commercial aquatic feeds has traditionally been dependent on fish meal (FM) as the main protein source because of its balanced essential amino acid (EAA) profile and high protein content (Hardy 1996). Fish meal is also an excellent source of essential fatty acids (EFAs), digestible energy, vitamins and minerals. However, because of the high cost and limited availability feed formulations have shifted to increasing levels of alternative protein sources using limited amounts of fish meal or animal proteins.

Among alternative protein ingredients, soybean meal is a widely available, economical protein source with relatively high digestible protein and energy contents and good amino acid profile (Hertrampf & Piedad-Pascual 2000). Several studies have been conducted to evaluate the nutritional value of soybean products in shrimp diets (Lim & Dominy 1990, 1992; Piedad-Pascaul *et al*. 1990; Sudaryono *et al*. 1995; Cruz-Suarez *et al*. 2001; Smith *et al*. 2001; Samocha *et al*. 2004; Amaya *et al*. 2007a,b; Ray *et al*. 2009). The use of soybean meal in shrimp feed formulations has been reviewed by Sookying *et al.* (2013). However, the utilization of soybean meal has limitations because of its moderate protein content and its relatively low level of essential amino acids such as methionine. The low level of methionine often becomes limiting requiring the blending of other ingredients or the supplementation of synthetic forms. High protein plant ingredients are being studied as their nutrient density allows for added room in the formulations and if appropriately chosen, they can complement the amino acid profiles of the other protein sources.

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Various processes are being used to concentrate the protein in cereal grains and are being studied to compliment and/or replace other protein sources. Corn Protein Concentrate is the dried protein fraction of the corn primarily originating from the endosperm after removal of the majority of the non-protein components by enzymatic solubilization of the protein stream obtained from the wet-mill process. Corn protein concentrate, used in the present study, is high in protein (79.7%) and a rich source of methionine (1.77%). Corn gluten meal is another high protein corn product produced by wet milling. In the wet milling process, corn is soaked in a solution to soften the kernel in order to facilitate the separation of the various component parts to produce a range of co-product including corn gluten meal. For the product used in these studies the protein content was analyzed at 64.8% with 1.45% methionine. These corn by-products could be used as nutrient dense protein sources that have the advantage of having a high methionine level and thus help balance the amino acid profile of plant-based feed formulations. Consequently, the objective of this line of research was to evaluate the use of corn byproducts as components of practical diets for marine shrimp.

Materials and Methods

Three corn products, Corn protein concentrate (CPC: Empyreal 75, Cargill Corn Milling, Cargill, Inc., Blair, NE, USA), corn protein concentrate produced using a modified process which enhances the AA profile and increases the lysine content (CPCL: Lysto, Cargill Corn Milling, Cargill, Inc., Blair, NE, USA) and corn gluten meal (CGM, Grain Processing Corporation, Muscatine, IA) were obtained for the evaluation of their potential as ingredients in aquaculture feeds for *L. vannamei.* Primary ingredients used in the diet formulations were characterized for proximate analysis and amino acid analysis (Table1) and incorporated into the test diets for independent growth trials. Pacific white shrimp, *L. vannamei*, post larvae were obtained from Shrimp Improvement Systems (Islamorada, FL) and nursed before stocking experimental systems.

Research test diets (36% protein, 8% lipid) were prepared in the Aquatic Animal Nutrition Laboratory at the Department of Fisheries and Allied Aquaculture, Auburn University (Auburn, AL, USA) using standard procedures for the laboratory production of shrimp feeds. Pre-ground dry ingredients and oil were mixed in a food mixer (Hobart Corporation, Troy, OH, USA) for 30 min. Hot water was then blended into the mixture to obtain a consistency appropriate for pelleting. Diets were pressure-pelleted using a meat grinder with a 3-mm die, air dried (<50 C) to a moisture content of 8-10 %. After drying, pellets were crumbled, packed in sealed plastic bags and stored in a freezer until needed.

Growth Trial I

Three experimental diets (Table 2), using soybean meal as the primary protein source, were utilized to evaluate the biological response of shrimp. Diets 1 to 3 were formulated to contain CGM (8%) and increasing levels (6.5 and 13.0%) of CPC and 36% protein and 8% lipid. Juvenile shrimp (0.52 ± 0.04 g) were hand sorted for uniform size and stocked into 12 tanks (60 L) at 10 shrimp $tank^{-1}$. Group weights were recorded by tank and a one-way ANOVA was performed to ensure there were no statistically significant differences between mean weights of the shrimp. Dissolved oxygen (DO), temperature, and salinity were monitored twice daily using an YSI 650 multi - parameter instrument (YSI, Yellow Springs, OH).

Shrimps were fed four times daily with the initial ration of feed determined based on an estimated weight gain (0.8 g/week), FCR of 2 and 100% survival. This data is based on the results of previous studies conducted in this system. Shrimp were counted weekly and the feed was adjusted based on survival and observations of feeding. At the conclusion of the 6 weeks growth trial, shrimp were counted and group weighed. Mean final weight, final biomass, percent survival, and feed conversion ratio were determined.

Growth Trial II

Five experimental diets (Table 2) were evaluated. Diets 1 to 4 were formulated to contain increasing levels (0, 4, 8 and 16% diet) of CPC replacing soybean meal on an iso-nitrogenous basis. This represents 0, 8.8, 17.7, 35.4 and 36.6% of the total protein for diets 1 to 5, respectively. Diets 2, 3 and 4 were supplemented with crystalline lysine to ensure lysine was replete. Diet 5 utilized 9.7% corn protein concentrate with a modified AA profile having a higher lysine content (CPCL) as a partial replacement for CPC and crystalline lysine. All the five experimental diets contained approximately 36% protein and 8% lipid and were prepared as previously described.

This growth trial utilized five treatments with four replicates. Fifteen Pacific white shrimp were stocked in each of the twenty 220L- polyethylene tanks (bottom area of 0.36 m^2) in an indoor recirculating system, with mean initial weight of 0.36g. Tanks in the experimental system were connected to an Aquadyne bead filter (1.2 Ft^2 media, 24" x 42") and a 0.25 hp centrifugal pump (RK2) to maintain water quality. All the water quality parameters were measured as previously described and maintained in acceptable ranges for pacific white shrimp. A diel light: dark cycle was set at 14:10 h.

Shrimp were offered test diets four times daily. Daily ration of feed was calculated based upon an estimated weight gain of 0.8g/week and expected FCR of 1.8. At the conclusion of the 10-week growth trial, shrimp were counted and group weighed. Mean final weight, final biomass, percent survival, and feed conversion ratio were determined. Five shrimp from each aquaria were frozen at -20 C until analysis of protein and dry matter were conducted. Upon analysis, each subsample was defrosted and blended in a food processor to produce a homogenous sample. Dry matters of shrimp were determined by placing representative portions of each sample in an oven at 95 ºC until constant weight was obtained. Protein content of whole shrimp samples were determined by the micro-Kjeldahl method (Ma & Zuazaga 1942).

Commercial diets

To evaluate the potential of corn protein concentrate under commercial conditions, four diets were manufactured by Rangen Inc. (Angleton, TX, USA) as a sinking 3 mm extruded pellet. All experimental diets contained approximately 36% protein and 10% lipid (Table 3). The basal diet was designed to contain 15% fish meal which was replaced with graded levels of CPC (0, 4, 8 and 12%).

Growth Trial III

The 44 day growth trial was conducted using similar methods as previously described, in terms of Pl source, water quality measurements and feed management. This growth trial utilized the four commercial diets with 15 replicates. Ten juvenile shrimp (0.128g $\pm$ 0.005g) were stocked in each of 60 aquaria (80 l). Each aquaria was connected to a common sump tank, aquadyne bead filter (1.2 Ft^2 media, 24" x 42"), 150 gal fluidized bed biological filter and a 0.25 hp centrifugal pump (RK2) and submerged thermostat-controlled heater.

Pond Trial

Pond trials 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 post larvae *L. vannamei* were conducted under an outdoor pond system.

Experimental animals

Shrimp post larvae (PL) were obtained from a commercial hatchery, Shrimp Improvement System, Islamorada, FL. The shrimp were nursed using standard practices as described by Garza de Yta *et al*. (2004). At the end of two weeks nursery period, 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.

Pond Management

Juvenile *L. vannamei* (initial weight of 0.023 g) were collected from nursery system and stocked in the production ponds at the rate of 38 shrimp m^{-2}. Standard production practices were conducted as described by Amaya *et al*. (2007a). In summary, 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 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. All ponds were provided base 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) as backup and/or supplemental aeration to maintain dissolved oxygen above 3 mg L^{-1}, the additional aerator was used when dissolved oxygen fell below 3 mg L^{-1}. This study was developed on a sustainable, semi-intensive system which was well managed. Feeding schemes were appropriate and water exchanged was minimized.

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 1^{st} to 4^{th} 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. The feed input initially started with 7.9 kg/ha and increased to maximum, input of 79.7 kg/ha after which it was slowly decreased to account for mortality up to week 12; thereafter, input remained at 76.4 kg/ha. During the

experimental period, shrimp growth was monitored on a weekly basis by sampling 70 to 100 animals per pond.

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 (1400 h - 1430 h), and at night (2000 - 2100 hr), using a YSI ProPlus meter (Yellow Spring Instrument Co., Yellow Springs, OH, USA). Secchi disk readings 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).

The shrimp were harvested at the end of the 16-week culture period. 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 from 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. The results of one replicate from treatment fed diet containing 0% CPC and two replicates from 8% CPC were excluded from the statistical analysis because of low survival of shrimp caused by aerator failure.

Statistical analysis

Differences in yield, final mean weights, FCR, and survival were statistically analyzed using one-way analysis of variance to determine if significant differences ($P<0.05$) existed among treatment means, 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

The growth performance of *L. vannamei* in the 3 tank trials is presented in Table 4. At the conclusion of the 6 week culture period for Trial 1, there were no significant differences in final mean weight, weight gain, FCR or survival. There were also no significant differences at the conclusion of trial 2, the dry matter content of shrimp after 10-week culture ranged from 23.1% to 25.4%, with no significant differences among treatments (P = 0.3738). The crude protein of shrimp (dry weight) ranged from 62.1% to 66.9 %, with no significant differences among treatments (P = 0.1133). No significant difference was found among treatments for protein retention efficiency (P = 0.1114).The results from trial 3 showed significant differences in final biomass, final weight and FCR. Water quality parameters remained in acceptable levels for each of the growth trials (Table 5).

The overall means, standard deviations, and ranges of morning, afternoon, and night water quality variables observed throughout the 16-week pond production trial are displayed in Table 6. 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 die off.

Growth performance of *L. vannamei* reared under production pond condition is summarized in Table 7. There were no differences (P > 0.05) in any of the measured production parameters. Treatment fed diet containing 12% CPC had slightly larger yield of 5440.1 kg ha^{-1} compared with 8% CPC (5420.5kg ha^{-1}), 4% CPC (5190.0 kg ha^{-1}), and 0% CPC (5007.8 kg ha^{-1}), respectively. Likewise, treatment fed diet containing 8% CPC had numerally lower FCR at 1.27 compared with 12% CPC (1.29), 4% CPC (1.34), and 0% CPC (1.38), respectively. However, there were no differences in survival and mean final

weight among treatments. Shrimp survival ranged from 64.9% (0% CPC) to 75.9% (12% CPC), and mean final weight ranged from 20.5 g to 18.7 g, but these differences were not significant (Table 7). The estimated value of the produced shrimp was determined based on the size distribution and the local price for each size class. The cost of each feed was also provided by the feed mill to allow for the calculation of feed costs per unit of production. As feed costs were different, costs per unit of shrimp were also significantly different (Table 7).

Discussion

The quality and cost effectiveness of commercial feeds are a primary concern for the farmer and feed manufacturer. In order to produce a quality feed one has to understand variations in ingredient cost, quality, and availability. Additionally, the effects of the ingredient on processing, nutrient content of the ingredient and the response of the animal must be understood to consistently make a high quality diet that meets the nutrient requirements of the culture species. Such diets are typically a mixture of a few primary ingredients that produce the desired results. Despite the common practice of using corn protein products in commercial feed formulations for shrimp, there are few studies evaluating the efficacy of these products. In the reported research, a series of diets were formulated with corn gluten and corn protein concentrate as replacements for SBM and/or fishmeal in practical feed formulations to systematically evaluate the response of the shrimp.

The basal diet used in this research is a variation to diets used in a range of culture systems and in a number of research trials with positive results (Ray *et al*. 2009; Sookying & Davis 2011; Sookying *et al*. 2011, Zhu *et al*. 2013). In the first two trials, the replacement of CGM with CPC as well as increasing levels of CPC replacing soybean meal. In the later experiment, increased levels of CPC represented 0, 8.8, 17.7, 35.4 and 36.6% of the total protein for diets CP0, CP4. CP8, CP16 and CP+LCP, respectively. The similar performance seen in trials I and II, indicates that it is applicable to replace soybean meal with up to 16% CPC representing around 36% of the protein, thus indicating that digestibility and nutrient availability of the meals should be similar.

Lysine, arginine and methionine are often thought of as the most limiting essential amino acids for shrimp in commercial feed formulations (Akiyama *et al*. 1991). Soybean meal is considered to be high in lysine and low in methionine, while corn protein is low in lysine and high in methionine. Consequently, replacement of soybean meal by CPC will improve the methionine levels but if used at high levels it will reduce lysine levels, which could be solved by the blending of other intact proteins or supplements of crystalline lysine. Fox *et al*. (1995) reported that the requirement for lysine by *L. vannamei* fed 35% protein diet was 1.6% of the diet, and 2.1% of the diet if fed 45% protein diet. The total lysine content of the diets from the second growth trial were designed to be in slight excess of the reported requirement which was confirmed by the similar growth performance of shrimp. Additionally, the present study demonstrated that there were no significant growth performance differences between shrimp fed diet 4 (16% CPC+L) and diet 5 (6.81% CPC and 9.7% CPCL). This result provides initial evidence that this co-product provided adequate dietary lysine levels for *L. vannamei* in diet 5, and can be used to partially substitute soybean meal without addition of crystalline lysine.

The primary reason for the inclusion of CPC is to enhance the methionine level of the diet without using crystalline sources. In the present study, calculated methionine contents of the experimental diets were 1.68%, 1.73%, 1.78%, 1.88% and 1.93%, while the sulfur amino acid (methionine + cystine) contents were 2.99%, 3.05%, 3.11%, 3.21% and 3.35% of crude protein, respectively for the five diets in the second growth trial. A methionine requirement for *L. vannamei* has not been firmly established, but the requirement for black tiger shrimp *Penaeus monodon*, Atlantic ditch shrimp *Palaemonetes varians* and kuruma shrimp *Marsupenaeus japonicus* are 2.4-2.9%, 2.0-2.4%, 1.4% of dietary protein, respectively (Millamena *et al*. 1996; Teshima *et al*. 2002; Palma *et al*. 2009; Richard *et al*. 2010). The similar growth performance of shrimp fed five experimental diets indicated that all the five diets should meet the total sulfur amino acid requirement of *L. vannamei*.

The second component of this research was the commercial production of feeds using increasing levels of CPC as a replacement for fishmeal. The feeds were formulated to contain 35% protein and 8% lipid. Based on proximate analysis of the diets, the lipid

content was significantly higher than the formulated value, which may confound the results as lipid levels generally increased with reductions in fish meal. The increased lipids and/or reduced fishmeal and increase CPC did result in significant difference under clear water conditions with the higher levels of CPC and lower levels of fishmeal resulting in reduced performance of the shrimp. As results from experiment 1 and 2 were favorable, the reduced performance is most likely linked to the elevated lipids. As these differences were not observed under pond production conditions the use of high levels of CPC in production diets is still warranted.

Under pond production conditions, the four test diets were fed to shrimp under semi-intensive conditions, leading to a final average production of 5,264 kg/ha and FCR of 1.32. Which are typical production values for this facility when shrimp are farmed at around 35 individuals per meter over a 16 week culture period. Using ingredient and manufacturing costs determined by the feed mill, a production cost ($/kg shrimp) can be calculated. This cost ranged from $1.11 to $1.60 per kg of shrimp produced (Table 7). These results demonstrate that the use of alternative feed formulations such as CPC results in reduced feed cost but also reduced production costs. Hence, the use of CPC as an alternative protein source should be encouraged.

In general, high levels of CPC inclusions in shrimp feed, with the concomitant reduction of expensive fish meal, are viable when essential nutrients in the diet are properly balanced to meet nutrient requirement of shrimp. Corn protein concentrate has been studied as an alternative protein source in many aquatic species such as, turbot *Psetta maxima* (Kaushik *et al*. 1999), Japanese flounder *Paralichthys olivaceus* (Kikuchi, 1999), rainbow trout *Oncorhynchus mykiss* (Morales *et al*. 1994), gilthead sea bream *Sparus aurata L*. (Olivia-Teles & Pereira, 2003), yellowtail *Seriola_quinqueradiata* (Shimeno *et al*. 1993) and Nile tilapia *Oreochromis niloticus* (Wu *et al*. 1995). Oliva-Teles and Pereira (2003) indicated that corn gluten meal can replace up to 60% fish meal protein in diets for gilthead sea bream juveniles without negative effects on fish growth.

In summary, CGM and more recently CPC have been used by commercial feed manufacturers as a protein-rich ingredient having high levels of methionine. Results of the reported trials indicate that both CGM and CPC are viable protein sources for use in commercial shrimp feed formulations.

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Table 1. Proximate compositions and amino acid profile (As-is basis, units %) for the major ingredients.

	Menhaden fish meal[a]	Solvent extracted soybean meal[b]	Corn gluten meal[c]	CPC[d]	CPCL[e]
Moisture	9.39	11.64	8.41	9.84	11.61
Protein	64.3	49.9	64.8	79.7	79.8
Fat	10.7	1.19	0.46	2.36	2.58
ADF[f]	<0.2	2.83	2.82	9.8	7.5
Ash	15.1	5.34	6.63	0.91	0.91
Alanine	6.45	1.72	5.32	8.26	6.17
Arginine	4.99	3.26	2.03	2.11	2.16
Aspartic acid	6.84	6.55	3.72	3.89	4.10
Cystine	0.43	0.88	1.10	1.28	1.27
Glutamic acid	9.70	9.64	12.89	14.2	14.06
Glycine	6.05	1.89	1.79	1.84	1.83
Histidine	2.21	1.16	1.30	2.05	1.40
Isoleucine	3.10	1.86	2.51	2.36	2.99
Leucine	5.50	3.36	10.04	10.4	11.95
Lysine	6.04	2.81	1.03	1.37	5.66
Methionine	1.47	0.82	1.45	1.77	1.67
Phenylalanine	2.97	2.16	3.88	5.00	4.57
Proline	4.48	2.36	5.82	7.42	6.78
Serine	3.37	1.95	2.97	3.53	2.78
Threonine	3.46	1.56	2.02	2.42	2.19
Tyrosine	2.50	1.49	3.08	3.74	3.75
Tryptophan	1.10		0.34	0.55	0.37
Valine	4.09	1.78	3.03	2.85	3.29

[a]Omega Protein Inc., Reedville, Virginia, USA.

[b]De-hulled solvent extracted soybean meal, Faithway Feed Co. Inc., Guntersville, Alabama, USA.

[c]Corn gluten meal, Grain Processing Corp. Muscatine, Iowa

[d]Corn protein concentrate, Empyreal® 75, Cargill Corn Milling, Cargill, Inc., Blair, NE, USA

[e]Corn protein concentrate (CPCL) Lysto™, Cargill Corn Milling, Cargill, Inc., Blair, NE, USA.

[f]ADF: Acid Detergent Fiber

Table 2. Ingredient composition (g $100g^{-1}$ of feed) of experimental diets use in a six weeks growth trial. All diets were developed to contain 36% protein and 8% lipids. Diets were designed to use various soybean meals produced from different varieties of soybeans at an equal protein inclusion level.

	Trial 1			Trial 2				
	Diet 1	Diet 2	Diet 3	CP0	CP4	CP8	CP16	CP+L
Corn Gluten meal[a]	8.00	-	-	-	-	-	-	-
CPC [b]	-	6.50	13.00	0.00	4.00	8.00	16.00	6.81
CPCL[b]	-	-	-	-	-	-	-	9.7
Soybean meal[c]	52.70	52.7	42	58.83	52.40	45.74	32.43	32.43
Menhaden fish meal [d]	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00
Menhaden Fish Oil [d]	0.27	0.27	0.27	5.76	5.76	5.76	5.76	5.76
Soy oil [e]	5.90	5.90	6.00	-	-	-	-	-
Corn oil [e]	-	-	-	0.06	0.04	0.03	-	-
Corn Starch [f]	7.98	9.48	13.38	5.35	7.61	9.97	14.69	14.60
Whole wheat [f]	13.00	13.00	13.00	18.00	18.00	18.00	18.00	18.00
Trace Mineral premix [g]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Vitamin premix [h]	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Stay C 250 mg/kg using 25% [i]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
CaP-diebasic [j]	2.50	2.50	2.50	2.35	2.52	2.70	3.05	3.05
Lecithin, soy [k]	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Cholesterol [f]	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Lysine [f]				-	0.02	0.16	0.42	-

[a] Grain Processing Corporation, Muscatine, IA, USA.

[b] CPC (Empyreal® 75); CPCL (Lysto®), Cargill Corn Milling, Cargill, Inc., Blair, NE, USA

[c] Faithway Feed Co., Guntersville, AL, USA.

[d] Omega Protein Inc., Reedville, VA, USA.

[e] Archer Daniels Midland,

[f] MP Biomedicals Inc., Solon, Ohio, USA

[g] Trace mineral premix without Mg (g 100g-1): cobalt chloride 0.004, cupric sulphate pentahydrate 0.250, ferrous sulfate 4.0, manganous sulphate monohydrate 0.650, potassium iodide 0.067, sodium selenite 0.010, zinc sulfate heptahydrate 13.193, filler 81.826.

[h] Vitamin premix (g kg-1): thiamin HCl 0.5, riboflavin 3.0, pyridoxine HCl 1.0, DL Ca-pantothenate 5.0, nicotinic acid 5.0. [i] Stay C®, (L-ascorbyl-2-polyphosphate), Roche Vitamins Inc., Parsippany, NJ, USA.

[j] T. Baker®, Mallinckrodt Baker, Inc., Phillipsburg, NJ, USA.

[k] Enhance D-97, The Solae Company, St. Louis, MO, USA

Table 3. Ingredients composition (g $100g^{-1}$ of feed) of commercially produced feed formulations and proximate analysis. Diets were used in the third growth trial and the pond production trial.

	CPC0	**CPC4**	**CPC8**	**CPC12**
Soybean meal	46.69	46.63	46.48	46.32
Milo	26.20	26.31	26.48	26.67
Menhaden Fish Meal	15.00	9.99	5.00	
Corn protein concentrate		4.00	8.00	12.00
Squid meal	2.00	2.00	2.00	2.00
Menhaden fish oil (sprayed)	4.58	4.86	5.15	5.45
Soy lecithin	1.00	1.00	1.00	1.00
Vitamin premix	0.33	0.33	0.33	0.33
Trace mineral premix	0.52	0.52	0.52	0.52
Stay C 350 mg/kg (35%)	0.02	0.02	0.02	0.02
Calcium phosphate dibasic	2.00	2.68	3.36	4.03
Copper sulfate	0.01	0.01	0.01	0.01
Bentonite	1.50	1.50	1.50	1.50
Mold inhibitor	0.15	0.15	0.15	0.15
Crude Protein	36.38	36.61	36.48	37.68
Crude Fat	10.28	10.47	13.52	12.91
Moisture	7.62	7.89	5.15	3.79
Crude Fiber	2.22	2.18	2.03	2.17
Ash	10.76	9.60	8.95	8.83

Table 4. Growth performance of Pacific white shrimp for each tank trial. Trial 1, mean initial weight 0.52 g cultured for 6 weeks. Trial 2 mean initial weight 0.35 g cultured for 10 weeks. Trial 3, mean initial weight 0.148 g cultured for 6 weeks. PSE=Pooled Standard Error

Trial	Final Weight (g)	Weight gain (%)	Feed conversion ratio	Survival (%)
1 (n=4)				
Diet 1	4.3	698.1	2.2	87.5
Diet 2	4.6	763.0	2.1	80.0
Diet 3	4.2	743.8	2.2	75.0
PSE	0.1639	44.35	0.109	7.02
P-value	0.4377	0.5877	0.2767	0.4777
2 (n=4)				
CP0	10.17	2747.6	1.72	83.3
CP4	9.83	2700.6	1.78	98.3
CP8	10.37	2885.7	1.59	95.0
CP16	10.03	2727.3	1.75	93.4
CP+L	10.25	2781.7	1.72	90.0
PSE	0.14	39.5	0.03	1.9
P-value	0.76	0.63	0.25	0.17
3 (n=15)				
CPC12*	4.61^{c}	3058.2^{b}	1.56^{a}	80.71
CPC8	4.95^{bc}	3196.3^{b}	1.45^{ab}	82.00
CPC4	5.38^{ab}	3515.7^{a}	1.32^{bc}	81.33
CP0	5.71^{a}	3752.9^{a}	1.25^{c}	86.00
PSE	0.0796	54.9910	0.0247	1.5570
P-value	<0.0001	0.0002	0.0003	0.6252

*n=14

Table 5. Summary of water quality variables for each of the growth trials for *L. vannamei* cultured in recirculating systems. Values represent the mean±standard deviation.

	Temperature (C)	Salinity (ppt)	Dissolved Oxygen (mg/L)
Trial 1	26.0 ± 1.49	4.55 ± 0.83	5.56 ± 0.76
Trial 2	28.5 ± 0.86	24.4 ± 0.73	5.57 ± 0.28
Trial 3	28.5 ± 1.06	6.44 ± 0.59	6.78 ± 0.26

Table 6. Summary of water quality variables monitored over a 16-week growth trial for *L. vannamei* cultured in 0.1-ha ponds. Values represent the mean±standard deviation and values in parenthesis represent minimum and maximum readings.

	Temperature (^{0} C)	Salinity (ppt)	Dissolved Oxygen (mg/L)	pH	Secchi Depth (cm)	TAN (mg/L)
Morning	28.34 ± 2.89 (18.00, 32.40)	11.51 ± 3.36 (4.01, 17.10)	3.91 ± 1.21 (0.08, 10.41)	7.01 ± 0.54 (4.91, 9.12)	30.56 ± 16.25 (10, 105)	0.57 ± 1.22 (0, 10)
Afternoon	30.50 ± 2.84 (21.30, 35.30)	11.54 ± 3.33 (3.99, 17.03)	9.68 ± 2.59 (1.12, 19.41)	8.40 ± 0.78 (4.90, 9.96)		
Night	30.40 ± 2.73 (21.00, 34.60)	11.50 ± 3.36 (4.01, 17.07)	8.04 ± 2.42 (0.03, 7.56)	8.17 ± 0.80 (5.13, 10.06)		

Table 7. Growth performance of Pacific white shrimp after 16 weeks of culture. Average initial weight of 0.023 ± 0.002g.

	Final weight (g)	Yield (kg/ha)	Survival (%)	FCR	Production value ($)	Feed Cost ($)	Feed $/Kg Shrimp
CPC0	20.51[a]	5007.8[a]	64.92[a]	1.38[a]	2106.72[a]	791.41[a]	1.60[a]
CPC4	17.48[a]	5190.0[a]	77.58[a]	1.34[a]	1808.40[a]	715.69[b]	1.39[ab]
CPC8	17.17[a]	5420.5[a]	83.60[a]	1.27[a]	1844.05[a]	651.31[c]	1.20[b]
CPC12	18.71[a]	5440.1[a]	75.91[a]	1.29[a]	2018.08[a]	598.16[d]	1.11[b]
PSE	0.5289	117.3565	2.3024	0.03487	65.2261	4.0777	0.0369
P-value	0.2112	0.5601	0.1423	0.6898	0.3727	<0.0001	0.0049

FCR: Feed Conversion Ratio