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Project Summary

As shrimp prices have fallen and production costs increased, shrimp farmers are more concerned with economic efficiencies of the feed. This means they are quite receptive to moving away from traditional high fish meal diet to less expensive protein sources. Additionally, there are social pressures to minimize the use of fish meal and other marine ingredients in shrimp feed formulations. The most logical replacement for protein from fish meal is to increase the level of protein originating from soybeans which means that inclusion levels in shrimp feeds will need to be increased. To date we have identified most of the limiting nutrients in soy based diets and we have increased the inclusion levels as high as 58% of the ration. Although some nutrient restriction still need to be defined, nutrient density of the diet is a problem as the level of soybean meal is increased. This simply means, that a high protein ingredient is required to provide room in the formulations. Soy protein concentrate (SPC) can meet this need as it is suitably priced to not only replace fish meal but also provide the required room in diet formulation. Hence, the objective of this project was 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 production conditions. Consequently, two parallel growth trials were conducted in outdoor tanks and ponds to evaluate the production potential of the various diets. In both trials, growth, feed conversion and survival were good and there were no significant differences between the four open formulation feeds containing up to 12% SPC. Based on present results, growth, feed conversion, survival and production yields were not effected by the use of SPC up to 12% of the diet. Hence, once can recommend that levels up to 12% are reasonable to use in commercial feed formulations for shrimp.

Background

Currently, opportunities exist for further research and marketing efforts to increase the use of soy products in aquaculture feeds. The aquaculture feed industry is one of the fastest growing components of agriculture as well as the compounded feed industry supplying feed to an industry that has an average annual rate of growth of 8.8% since 1950. The commercial culture of shrimp has been one of the most successful components of the aquaculture industry, representing around 20% of the world value of aquaculture products with an expected 2008 growth rate of 8-10% . Although this industry represents 4% of the world aquaculture production by volume, shrimp feed manufacturers are using around 23% of the fish meal used in aquaculture feeds. This use is not only unwarranted but it is no longer economically or politically sustainable. Given the political and economic situation, feed mill manufacturers and producers are eager to find alternatives to both improve their cost effectiveness and image. Increasing the use of soybean meal and other soy products in shrimp make economic sense, it enhances the long term viability of the industry and provides a better public image.

Due to both economic pressures from high fish meal prices as well as political pressures from major buyers such as Walmart requiring sustainable practices, the use of high (20-30 %) levels of fish meal is no longer desirable. The idea of replacing fish meal with plant proteins in diets is not new and reduces the dependence on the fish meal industry and provides alternative choices when formulating feeds. Soybean meal currently represents the predominant choice as an alternate protein source in commercial feed formulations for aquatic species. Hence, the shrimp research program is designed to identify restrictions to the use of high levels of soy products as replacements for fish meal in commercial shrimp feeds and demonstrate their use in feeds under practical pond conditions. A systematic stepwise approach is being utilized to develop good scientific data that in turn ensures that pond based trials have the highest potential for success. To date, we have been quite successful and have identified the primary nutrient limitations such as total sulfur amino acids, highly unsaturated fatty acids, cholesterol and phosphorus which has allowed us to increase maximum inclusion levels of soybean meal.

We have systematically identifying limiting nutrients and compared a series of commercially produced diets, containing animal protein, as well as soy based diets that were developed for this research program. These diets have been successfully tested using soybean meal levels as high as 58% of the diet under both laboratory and field conditions. Although this demonstrates that we can utilize high levels of soybean meal, most feed mill manufactures would prefer not to use such high levels of soybean meal as there is limited "room" in the formulation. Most feed manufacturers prefer a reasonable level of a high protein ingredient to allow room in formulations. This is particularly important when dealing with feeds with higher levels of protein. Soy protein concentrate is produced through a series of different extraction and precipitation process which remove most of the oil and water soluble non-protein constituents of soybeans to produces a high quality high protein product. Consequently, developing protocols of the use of SPC in shrimp feeds will provide a soy based product that can serve as a nutrient dense ingredient for shrimp feeds. Hence, the primary objective of this research was to evaluate the use of varying levels of soy protein concentrate under production conditions.

Methods

The primary goal of the project is to promote the use of soy products in shrimp feeds. Hence, the project has built off of a stepwise program of work designed to optimize inclusion levels of soy products in shrimp feeds. A series of test diets (Table 1) containing graded levels of soy protein concentrate (SPC) were formulated to contain 35% protein and 8% lipid and manufactured by Rangen Inc specifically for this project. The proximate composition of the test diets are presented in (Table 2). The basal diet was designed to contain 58% solvent extracted de-hulled soybean meal which were replaced with graded levels of soy protein concentrate (0, 4, 8, 12 %) for test evaluations.

Research was conducted at the Claude Peteet Mariculture Facility in Gulf Shores Al, under the direction of Auburn University faculty. Two growth trials were conducted with commercially obtained post larvae under practical grow out conditions. One was run in outdoor production ponds and the other using a green water tank system.

Pond Production Trials

Pacific white shrimp *Litopenaeus vannamei* post-larvae were obtained from Shrimp Improvement Systems, (Plantation Key, FL) and nursed for two weeks using standard protocols and feed inputs (Table 3). Results from the nursery phase are summarized in Table 4. After the nursery phases, juvenile shrimp (0.0129 g mean weight) were stocked into the ponds located in the same facilities, at a density of 35 shrimp per squared meter. Ponds used for the grow-out phase are 0.1 ha in surface area (46 x 20 m), 1.0 m average depth and lined with a 1.52 mm thick, high-density polyethylene. Each pond bottom is covered with a 25-cm deep layer of sandy-loam soil and equipped with a 20-cm diameter screened standpipe and a concrete catch basin. Prior to use, pond soils was dried and tilled to allow oxidation and mineralization of organic matter. Two weeks before stocking, the ponds were filled with brackish water (~15 ppt) from the Intracostal Canal between Mobile and Perdido Bay, Alabama. Inlet water was filtered through a 250-μm nylon filter sock (Micron Domestic Lace Mfg., Inc) in order to prevent the introduction of predators and minimize the introduction of larval species. Inorganic liquid fertilizers were mixed and applied one week before stocking at a rate of 1,697 ml of 32-0-0 and 303 ml of 10-34-0 per pond, therefore, providing 5.73 kg of N and 1.03 kg of P_20_5/ha.

Dietary treatments were randomly assigned to one of four replicate ponds. Feeds were produced by Rangen, Inc. (Angleton TX, USA) under commercial manufacturing conditions and offered twice a day to the shrimp, as a sinkable, extruded 3 mm pellet. Feed inputs were based on historical results as well as current observations and are presented in Table 5. Shrimp growth was monitored on a weekly basis by determining the average weight in a sample of 70 to 100 animals per pond. Sampling was carried out by capturing shrimp by seine during first two weeks and cast net (monofilament net, 1.52 m radius and 0.95 cm opening) for the remaining of the culture period.

During the experimental period, dissolved oxygen, temperature, salinity and pH concentrations were measured at sunrise (05:00-05:30 h), during the day (15:00-15:30 h), and at night (20:00-22:00 h), using a YSI meter (Yellow Spring Instrument Co., Yellow Springs, OH, USA). Total ammonium-nitrogen (TAN) and Sechii disk readings will be determined on a weekly basis. Water samples for TAN analysis will be taken 40 cm from the pond surface and measured with a spectrophotometer (Spectronic Instrument Inc. Rochester, NY, USA) by the Nesslerization method (APHA 1989). In order to maintain minimum dissolved oxygen levels of 3 mg/L, each pond is provided with a base aeration capacity of 10 hp/ha (7.5kW/ha). Paddle wheel aerators of 1-hp (Little John Aerator, Southern Machine Welding Inc. Quinton, AL) or propeller aspirators aerators of 1-hp (11.2 Ampers) or 2-hp (20 Ampers) (Aire-$O_{2,}$ Aeration Industries International, Inc. Minneapolis, Minnesota) are used for this purpose. When required, additional aeration (up to 30 hp/ha) was used to maintain adequate DO levels. Water quality results are summarized in Table 6.

Harvesting took place over a three day period, after around17 weeks of pond culture. Feed inputs were stopped two days prior to harvesting a given pond. The night before harvest, two thirds of the water from each pond was drained and aeration provided using paddlewheel aerators to keep shrimp alive and minimize erosion on pond bottoms. On harvest day, shrimp were pumped from the catch basin using a hydraulic fish pump equipped with a 25-cm diameter suction pipe (Aqualife-Life pump, Magic Valley Heli-arc and Mfg, Twin Falls, Idaho, USA). The pump was placed in the catch basin and shrimp pumped, de-watered and collected in a hauling truck. Shrimp were then transferred for washing and weighing. During weighing, a random sample of 150 shrimp was collected for individual weight determinations. Individual weights will be used to calculate mean final yield, mean final weight, survival and size distributions. After quantifying the biomass from each pond, mean final yield (final biomass), feed conversion ration (total feed offered / biomass increase), size distribution and survival were determined.

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

Tank trials

Juvenile (~1 g) Pacific white shrimp (*Litopenaeus vannamei)* were obtained from 16 shrimp production ponds located at the Claude Peteet Mariculture Center, in Gulf Shores, Alabama. Shrimp were stocked at a density of 30 shrimp per tank (38 shrimp/m^3) in an outdoor recirculating system. The system is composed of 24, 800-L polyethylene circular tanks and central reservoir with biological filter. Before stocking, the system was filled with brackish water from a shrimp production pond. Daily water exchanges were performed during the experimental period between 8:00 am and 2:30 pm, when pond water was pumped into the central reservoir at a rate of 8 liters per minute. This exchange rate allowed a 100% water exchange in the recirculating system every six days. Hence, maintaining a green water culture environment. In each system, aeration is provided to each tank by two air stones connected to a common air supply.

During the 10 week growth trial, dissolved oxygen (DO), temperature, salinity and pH concentrations were measured in the central filter and two of the system tanks at 06:00 in the morning and at 16:00 in the afternoon. Water quality parameters were measured using a YSI meter, (Yellow Spring Instrument Co., Yellow Springs, OH, USA). Total ammonia-nitrogen (TAN) was determined on a weekly basis, with water samples taken from the filter and two of the tanks and measured with a spectrophotometer (Spectronic Instrument Inc. Rochester, NY, USA) using the Nesslerization method (APHA 1989).

A total of five dietary treatments were randomly assigned to shrimp in replicate tanks. Five replicates were used for the four test diets (Table 1) and four for a commercial reference diet. Feeds were produced by Rangen Inc. (Angleton, TX. USA) under commercial manufacturing conditions and offered to the shrimp as a sinking extruded pellet.

Daily feed inputs were back-calculated, based upon an expected shrimp weight gain of 1.3 g per week and an expected feed conversion ratio of 1.2:1. Feed was provided twice per day. Feed inputs were stopped one day before harvesting to allow the shrimp to purge themselves. Shrimp production was determined at the end of the growth trial by determining the following parameters: mean final weight, weight gain (%), weekly weight gain, final net yield, feed conversion ratio (FCR) and survival. Data was then analyzed using the previously mentioned procedures.

Results and Discussion

Feed is generally the largest variable cost associated with commercial shrimp operations and protein is the most expensive component of the shrimp feed. Hence, it is no surprise that there is a considerable effort geared towards the optimization of protein sources. In traditional feed formulations, fish meal serves as the primary protein source as it is highly nutritious. In many markets, the high cost of shrimp feed is partially due to the use of expensive fish meal products that can be replaced with plant based protein sources, e.g. soybean meal, as long as the diets are properly balanced for essential nutrients. In addition to economic pressures to remove fish meal, consumers have started to put pressure on buyers to provide products based on renewable resources further supporting the reduction of fish meal levels. Hence, this is an opportune time for producers to reduce their reliance on fish meal as it is both economical and provides a more favorable image.

The present study builds off of previous research which has developed and demonstrated the applicability of high levels of inclusion of soybean meal in shrimp feeds. Although, we have developed techniques to use very high inclusion levels, there are disadvantages to maximize the level of soybean meal. The primary disadvantage is that you limit the room left in the feed formulations which limits ingredient replacement strategies. In the case of high protein diets, there are also physical limitations to the use of soybean meal due to nutrient density or protein content of the meal. Consequently, there is considerable interest in further processing the meal to produce a nutrient dense ingredients such as soy protein concentrate (SPC).

Soy protein concentrate (SPC) is a high protein ingredient that is produced through a series of different extraction and precipitation process from high quality dehulled soybeans. By removing most of the oil and water soluble non-protein constituents, the protein content can be increased and a number of anti-nutrients removed or reduced. Soy protein concentrates are increasingly being used by the animal feed industry as they are a renewable source of protein. As SPC has a higher protein content than SE-SBM, there is interest in using SPC in practical diet formulations. Hence, the present research was based on previous work and designed to evaluate the use of increasing levels of SPC in soy based diets containing 58% soybean meal. The soybean meal in the basal diet was then replaced on a protein to protein basis with graded levels (0, 4, 8, 12%) of soy protein concentrate. Diets were produced under commercial conditions and then tested in outdoor green water tanks as well as pond systems.

With regards to the growth response of shrimp maintained in the green water tank system, water quality and performance of the shrimp offered the various test diets are presented in Tables 6 and 7, respectively. Water quality was maintained within reasonable levels for this species and the growth response of juvenile shrimp in the out door tank system was typical for this system. Based on the observed response of the shrimp, increasing the level of SPC did not influence the final weight (13.5-13.9g), FCR (1.22-1.28), and survival (96.7-100%). There were no significant difference among the test diets. Results of shrimp maintained on the commercial reference diet was significantly better in terms of final weights (15 g). However, there were no significant differences in total biomass as well as FCR when comparing the reference diet to several of the plant based diets. These results confirm that SPC could be used up to 12% inclusion level in shrimp feed formulations and that higher levels provided numerically better results. Comparison to that of the commercial reference diet was marginal. This could be due to slight inadequacies of the plant based diet or simply variation across treatments.

The four test diets were also evaluated under semi-intensive pond production conditions. The ponds were stocked at 35 shrimp/m^2 and reared over an 18 week period. Harvest results are presented in Table 8. Production At the conclusion of approximately 18 weeks of pond culture the shrimp were harvested and quantified. Production was typical for this site with total harvests ranging from 4.2-5 metric tons per hectare. These are good production values for this site and would be considered quite acceptable by industry standards. Final weights (13.5-15.7 g) of the shrimp was within historical production parameters for this site but considerably lower than more recent harvest weights of the shrimp. The small size of the shrimp is most likely due to feed limitations due to higher than expected survival. Typical survival at this site is 70-80% whereas the observed survival for this production run ranged from 86-93%. The high survival would appear to be due to overstocking of juvenile shrimp as there were several ponds with over 100% survival. The FCR for shrimp maintained on the various diets ranged from 1.53 to 1.28 which are typical for this site and would be considered very good values. Shrimp used in the green water tank system were from the production ponds and they had better growth (1.44 g/wk vs 0.86 g/wk) than those in the pond. Hence, we can conclude that under feeding is the most likely reason for reduced growth in the ponds.

The present research was carried out in parallel systems which include green water tanks as well as research ponds. The out door tank system serves as a less variable and a more reproducible system which mimics pond passed production. As there were no difference across the SPC treatments in both the tanks system as well as the pond system we can conclude that the use of SPC up to 12 % inclusion is acceptable. Hence, the results of this work confirm that the use of SPC in commercial feed formulations should be promoted as a high protein ingredient for shrimp feeds.

Table 1: Composition (g 100 g^{-1} dry weight) of experimental diets designed to contain 35% protein and 8% lipid. Diets were produced by Rangen Inc at their Texas Plant.

Ingredients	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
CFS corn distillers grain	10.00	10.00	10.00	10.00
Corn gluten yellow	4.83	4.83	4.83	4.83
Soycomil SPC	-	4.00	8.00	12.00
Dicalcium phosphate 21 P	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, crude	0.50	0.50	0.50	0.50
Vitamin premix	0.33	0.33	0.33	0.33
Mold inhibitor	0.15	0.15	0.15	0.15
Trace mineral premix	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

Table 2: Nutrient composition of test diets for *L. vannamei* reared in polyethylene tank trail of 10 week period.

Nutrient	Proximate Analyses (%)				
	0% SPC	4% SPC	8% SPC	12% SPC	Reference
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
Fiber	2.87	3.06	2.91	2.96	2.57
Ash	9.08	9.26	9.48	9.27	7.92
Pepsin (0.0002%)	79.89	80.54	77.66	76.88	

Table 3: Feeding rates as percentage biomass and feed type utilized through a 15-day nursery period for *Litopenaeus. vannamei,* post-larvae. Feed inputs were based on mean shrimp weight determined every three days and an assumed 84% survival.

Day	Mean weight (mg)	Feed type	Feed ratio	Feed rate (%body weight)
1	1.01	Artemia (1/2), PL redi (1/2)[a]	1:1	25
2	1.01	Artemia (1/2), PL redi (1/2)	1:1	25
3	1.01	Artemia (1/2), PL redi (1/2)	1:1	25
4	2.24	PL redi (1/2), Cru #0 (1/2)[b]	1:1	25
5	2.24	PL redi (1/2), Cru #0 (1/2)	1:1	25
6	2.24	PL redi (1/2), Cru #0 (1/2)	1:1	25
7	3.53	PL redi (1/4) Cru # 0 (3/4)	1:3	25
8	3.53	Cru # 0	1	25
9	3.53	Cru # 0	1	25
10	5.03	Cru # 0	1	15
11	5.03	Cru # 0	1	15
12	5.03	Cru # 0	1	15
13	8.10	Cru # 0, Cru #1& #2 (1:1)	1:1	15
14	8.10	Cru # 0, Cru #1& #2 (1:1)	1:1	15
15	8.10	Cru # 0, Cru #1& #2 (1:1)	1:1	15

[a] PL Redi-reserve 400-600 microns. 50% Protein, produced by Zeigler Bros, Inc., Gardners, PA, USA
[b] Crumble feed, 45% protein, produced by Rangen Inc., Buhl, Idaho, USA

Table 4: Final population, weight gain, percentage survival, final weight, and FCR of PL *Litopenaeus vannmei* after 15 day nursery period.

	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)	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] Coefficient of variation = Standard deviation / mean x 100

[b] Weight Gain = Final Weight - Initial Weight

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

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

Table 5: Feeding regime for *L. vannamei* reared in the grow-out pond during 18 week experimental period, at estimated survival rate of 75 percent.

Day	Week	Population	Feed input/day	
			Kg/Pond	Kg/ha
1	0	35,000	1.00	10.00
8	1	34,486	1.50	15.00
15	2	33,971	3.00	30.00
22	3	33,457	5.00	50.00
29	4	32,942	5.86	58.57
36	5	32,428	4.04	40.42
43	6	31,913	6.60	66.04
50	7	31,399	7.00	69.97
57	8	30,884	6.88	68.82
64	9	30,370	6.77	67.68
71	10	29,855	6.18	61.78
78	11	29,341	6.54	65.38
85	12	28,826	4.84	48.35
92	13	28,312	5.40	54.01
99	14	27,797	6.19	61.94
106	15	27,283	6.34	63.42
113	16	26,768	6.50	65.00
120	17	26,254	5.83	58.33

Table 6: Water quality parameters for growth trials with juvenile *L. vannamei* reared in pond trial and green water systems. Values represent the mean ± standard deviation.

Parameter	Pond Trial	Green water system
Dissolved O_2 (mg L^{-1})	6.59 ± 2.50	5.65 ± 0.84
Temperature (C)	29.13 ± 2.60	28.44 ± 1.93
Salinity (g L^{-1})	14.85 ± 3.00	15.45 ± 1.39
pH	8.33 ± 0.59	8.08 ± 0.38
TAN (mg L^{-1})	0.53 ± 1.09	0.26 ± 0.21

Table 7: Growth performance of *L. vannamei* reared under green water system in polyethylene tanks during 10 week experimental period fed diets containing different levels of soy protein concentrate. Shrimp has a mean initial weight of 1.0 g and were stocked at 30/tank in five replicate tanks per dietary treatment and four replicate tanks for reference diet.

Treatment	Yield (g/tank)	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]	11.937	0.576	0.040	2.563

[a] Reference diet = 35% Protein, Rangen 35,0 (Buhl, Idaho)
[b] P value $\alpha = 0.05$
[c] Pooled Standard Error

Table 8: Growth performance of *L. vannamei* reared under pond condition during 18 week experimental period fed diets containing different levels of soy protein concentrate. Four replicate ponds were utilized, however, one pond in the 0% SPC suffered high mortality and was excluded from the study.

Treatment	Yield (kg/ha)	Final Weight (g)	FCR	Survival %
0%SPC [a]	4190	13.5	1.53	86.6
4%SPC	5051	15.7	1.28	89.5
8%SPC	4509	13.5	1.44	92.9
12%SPC	4479	13.5	1.44	93.3
P-value [b]	0.1368	0.3626	0.1358	0.8605
PSE [c]	236.3	1.028	0.0718	6.068

[a] Means of three replicates, as one pond had poor survival (56.6%) due to aeration failure.
[b] P value $\alpha = 0.05$
[c] Pooled Standard Error