Final Report

Shrimp Summary:

Project Title: Soy-in-Aquaculture Research Program -Shrimp

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Introduction

World shrimp production has been increasing for the past four decades. In 2004, production reached over 6 million tons. This unprecedented expansion has been primarily attributed to increased production from the shrimp farming activities. In 2004, more than 41% (2.5 million tons) of the world's total shrimp production was from farming. The primary cost of production in shrimp farming is feed, with protein being the most expensive macronutrient in these diet formulations. Although marine fish meals and fish oils provide excellent sources of high quality essential amino acids, lipids, vitamins, minerals and attractants in aquaculture diets, we must consider the finite availability these resources and the impact their procurement has on the environment. In addition, demand for fishery products from other high profit sectors, such as the pet food industry, will force fish meal prices up until its usage in aquatic feeds will no longer be economically feasible. If aquaculture is to continue to be an increasing contributor to the human food supply, it is critical that aquaculture feeds become less reliant on marine fisheries products. Due to both economic pressures from high fish meal and fish oil prices and pressures from buyers and consumers requiring sustainable practices, the use of high levels of fish meal and fish oils is no longer acceptable. Currently, feed mill manufacturers and producers are taking a pragmatic approach by looking into practices that will not only reduce feed production costs, but also improve their public image. Increasing the use of soybean meal and other soy products in shrimp diet formulations should reduce feed costs and improve both the image and sustainability of this growing industry.

This study was part of a continuing project focusing on developing plant-based feeds for aquaculture; the current work was geared toward enhancing the lipid component of plant-based feeds. A systematic, stepwise approach has been developed to produce good scientific data and to ensure that larger scale pond based trials can follow with the highest potential for success. The ultimate goal of the research was to provide adequate information to the feed formulators to allow them to use the maximum level of soy products (soybean meal, soybean oil, and soy lecithin) in their feed formulations.

Previous studies have shown that fish meal can be replaced either singularly with animal by-product meal or in combination with plant protein sources without affecting the physical and nutritional quality of the feeds. However, the use of alternative protein sources is often done in combination with the use of marine oils to supply essential fatty acids and enhance the palatability of the diet. Soybean meal has been identified as a good alternative protein source due to its high protein content and relatively balanced amino acid profile. In previous studies we have maximized the use of soybean meal in shrimp feeds, however, there are numerous other products derived from soybeans that can be utilized in aquaculture diets including soy oil and soy lecithin which have yet to be optimized. Although it is unlikely that soy oil will be suitable as a complete fish oil replacement due to a lack of essential fatty acids, it may be possible to reduce fish oil use in plant-based shrimp feeds by diluting fish oil with soy oil. Another approach to enhancing the lipid component of plant-based feeds is the inclusion of soy lecithin. Phospholipids play an important role in maintaining cellular structure. They also enhance cholesterol transport, increase lipid retention, and may facilitate the pelleting process by acting as a lubricant. Soy lecithin, a primary source of phospholipids, has the potential to improve the lipid component of plant-based feeds.

The objectives of this study were: 1. To evaluate the response of Pacific White Shrimp to diets with increasing levels of dietary lecithin (0-2%) in conjunction with decreasing levels of fish oil to maintain the same dietary lipid level, 2. To evaluate the response of the shrimp to diets with decreasing levels of fish oil supplemented with increasing levels of soybean oil to maintain the targeted lipid content.

Materials & Methods

Shrimp postlarvae were obtained from Shrimp Improvement Systems, Islamorada, FL. Juvenile shrimp were conditioned for one month on commercial shrimp diets containing 35% crude protein (Rangen #2, 3, 4, Rangen, Buhl, ID). Shrimp were graded for similar weight (1.37 ± 0.05 g) and stocked at a density of 26/tank (31 m^2 or $40/m^3$). Dead shrimp were replaced the following day after stocking with animals of similar size.

Feed trials utilized a recirculating system composed of 84 high-density polyethylene cylindrical tanks. Each tank had a working volume of 650 L with 0.85 m^2 bottom surface area. Netting was used to cover the tanks to prevent the shrimp from escaping. Tanks were filled with previously chlorinated seawater from the nearby Laguna Madre. Tanks were positioned within an open-air structure with a roof that enabled partial penetration of direct sunlight for development of primary productivity within tanks. No external biofiltration was provided and each tank received aeration from two airstones (7-10 L/min/stone). Flow restrictors maintained a recirculation rate of 1.9 L/min/tank.

Dissolved oxygen (DO), temperature, salinity, and pH were monitored twice daily (0830 and 1630) from eight randomly selected tanks using a YSI 650 multi-parameter instrument (YSI, Yellow Springs, OH). Total ammonia-nitrogen (TAN) was determined weekly in samples taken from eight randomly-selected tanks using an ion specific probe (EA 940, Orion Research Inc, Cambridge, MA).

Experimental diets for the lecithin study (Table 1) were formulated without fish meal and contained crude protein levels of 36% with five levels of lecithin (0.00, 0.25, 0.5, 1, and 2%). Diets for the lipid study (Table 2) were formulated to contain fish:soy oil ratios of 100:0, 50:50, 60:40, 70:30, 80:20, 90:10. Two additional diets were included in the study to compare two sources of soybean meal (Archer Daniels Midland Company, Decatur, IL and Owens Grain Inc, Canton, IL).

Test diets were prepared in the laboratory at the Department of Fisheries and Allied Aquacultures, Auburn University (Auburn, AL) using standard procedures for manufacture of shrimp feeds: Dry ingredients and oil were mixed in a food mixer (Hobart Corporation, Troy, OH) for 30 min. Hot water was then blended into the mixture to attain 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%, and stored at 4 C. Dietary treatments were randomly assigned and each experiment was run using a double blind experimental design. The trials were conducted at the Texas AgriLife Research Mariculture Laboratory in Flour Bluff, Corpus Christi, TX, USA.

The fish:soy oil study evaluated six diets and the lecithin study evaluated five diets, each with five replicates per treatment. Two additional diets containing different sources of soybean meal were also included for comparison purposes. A commercial shrimp grow-out feed (35% CP diet, Rangen Inc., Buhl, ID) served as a reference diet, and was tested in four replicate tanks. Initial rations were determined assuming weight gain of 1.2 g/wk, FCR of 1.5 and 100% survival. These assumptions were based on the results of previous studies conducted in this system. One tank from each treatment was equipped with a feed tray for qualitative monitoring of feed consumption, and feed rates were reduced when excess feed was observed on the trays. The same tanks were also used to monitor mean weekly shrimp weight gain (as the average of five shrimp). Feed was offered three times daily according to the following schedule: 25% of the ration at 0830 and 1130, and 50% of the ration at 1630. The study was terminated on Day 74, once mean shrimp weight had exceeded 20 g. Upon harvest, tanks were drained, shrimp collected and, counted and weighed by tank.

Table 1. Ingredient composition of lecithin test diets as g/100g, analyzed to contain 36% CP and 7.2% lipid.

Ingredient	**Lecithin%**				
	0.0	**0.25**	**0.5**	**1.0**	**2.0**
Soybean meal	54.50	54.50	54.50	54.50	54.50
Corn gluten meal	6.00	6.00	6.00	6.00	6.00
Corn starch	0.97	0.82	0.71	0.47	0.00
Whole wheat	27.81	27.81	27.81	27.81	27.81
Trace Mineral premix	0.50	0.50	0.50	0.50	0.50
Vitamin premix	1.80	1.80	1.80	1.80	1.80
Choline chloride	0.20	0.20	0.20	0.20	0.20
Stay C 250 mg/kg	0.10	0.10	0.10	0.10	0.10
CaP-diebasic	3.00	3.00	3.00	3.00	3.00
Cholesterol	0.05	0.05	0.05	0.05	0.05
Menhaden fish oil	**5.10**	**4.97**	**4.84**	**4.57**	**4.04**
Lecithin	**0**	**0.25**	**0.50**	**1.00**	**2.00**

The juveniles for the study were hand sorted for uniform size and group weight was recorded tank-wise. One way ANOVA showed no statistically significant differences between treatments in mean shrimp weight at stocking. Differences between treatments with respect to mean final weights, total yield, FCR, and survival were determined using one way ANOVA ($\alpha = 0.05$). All differences were determined to be significant at $P < 0.05$ significance level.

Table 2. Ingredient composition of lipid ratio test diets as g/100g, analyzed to contain 36% CP and 7.2% lipid.

Ingredient	**Fish oil : Soy oil**					
	100:0	**50:50**	**40:60**	**30:70**	**20:80**	**10:90**
Soybean meal	54.50	54.50	54.50	54.50	54.50	54.50
Corn gluten meal	6.00	6.00	6.00	6.00	6.00	6.00
Corn starch	0.47	0.47	0.47	0.47	0.47	0.47
Whole wheat	27.81	27.81	27.81	27.81	27.81	27.81
Trace Mineral premix	0.50	0.50	0.50	0.50	0.50	0.50
Vitamin premix	1.80	1.80	1.80	1.80	1.80	1.80
Choline chloride	0.20	0.20	0.20	0.20	0.20	0.20
Stay C 250 mg/kg	0.10	0.10	0.10	0.10	0.10	0.10
CaP-diebasic	3.00	3.00	3.00	3.00	3.00	3.00
Lecithin	1.00	1.00	1.00	1.00	1.00	1.00
Cholesterol	0.05	0.05	0.05	0.05	0.05	0.05
Menhaden fish oil	**4.57**	**2.29**	**1.83**	**1.37**	**0.91**	**0.46**
Soy oil	**0**	**2.29**	**2.74**	**3.20**	**3.66**	**4.11**

Results

The studies were terminated 74 days from initiation. Table 3 summarizes the mean, and maximum and minimum values of the daily and weekly water indicators monitored in this study. The values recorded are within the range reported for suitable growth of this species.

After 10 weeks on the fish:soy oil ratio test diets, shrimp final weights, yields, growth per week, and FCR were significantly different between treatments (Table 4). There was a general depression in performance at higher levels of soy oil inclusion. Survival was not significantly different between treatments. The commercial diet performed similarly to the 100% fish oil and the 50:50 soy to fish oil treatments.

After 10 weeks on the lecithin diets, shrimp final weights, yields, growth per week, and FCR were significantly different between treatments (Table 5). Survival was not significantly different between treatments. The commercial diet performed similarly to the 0.5, 1, and 2% lecithin treatments. There were no significant differences in shrimp performance between the two sources of soybean meal.

Table 3. Summary of daily and weekly water quality parameters.

Indicator	Average	Max	Min
AM Dissolved oxygen (mg/L)	6.3	7.5	4.4
PM Dissolved oxygen (mg/L)	6.5	8.0	4.5
AM Temperature (C)	26.9	28.4	22.1
PM Temperature (C)	29.2	29.3	25.8
AM pH	7.9	8.3	7.5
PM pH	8.0	8.4	7.6
Salinity (ppt)	29.3	30.7	27.4
TAN (mg/L)	0.32	1.8	ND

Table 4. Response of shrimp after 10-weeks on fish:soy oil ratio study diets.

Treatment Fish oil:Soy oil	Av. Wt. (g)	Survival (%)	Growth (g/wk)	FCR	Yield (kg/m^3)
100:0	21.68	97.7	1.99	1.30	0.886
50:50	21.85	99.2	1.91	1.33	0.887
40:60	20.97	98.5	1.83	1.40	0.826
30:70	20.45	98.5	1.78	1.44	0.806
20:80	20.78	98.5	1.81	1.42	0.819
10:90	19.93	100	1.73	1.46	0.797
P value (ANOVA)	0.001	0.733	0.001	0.004	0.002

Table 5. Response of shrimp after 10-weeks on lecithin test diets.

Treatment Lecithin (%)	Av. Wt. (g)	Survival (%)	Growth (g/wk)	FCR	Yield (kg/m^3)
0.0	21.77^{ab}	96.9	1.90^{ab}	1.36^{a}	0.843^{a}
0.25	21.54^{a}	99.2	1.88^{a}	1.35^{ab}	0.855^{ab}
0.5	22.27^{ab}	97.7	1.95^{ab}	1.32^{ab}	0.870^{abc}
1.0	22.68^{b}	97.7	1.99^{b}	1.30^{ab}	0.886^{bc}
2.0	22.42^{ab}	100	1.96^{ab}	1.28^{b}	0.897^{c}
P value (ANOVA)	0.026	0.351	0.023	0.020	0.011

Discussion

The results of the lecithin study indicate a general increase in shrimp performance up to 1% inclusion of lecithin. Although higher levels of inclusion did not improve performance, they did not decrease it, and could be used if positive effects are seen at the mill in terms of through put and pellet quality.

In the fish:soy oil study, shrimp performance was depressed as soy oil levels were increased. However, one would expect this response only at the lowest levels of fish oil inclusion. We suspect the most likely reason for these unexpected results is due to oxidation of the soy oil used in this study. Another explanation may be an essential fatty acid deficiency, however, again this should be observed only at very low levels of fish oil inclusion. An issue that has recently come to the forefront is an imbalance of the omega-6 to omega-3 ratio. Although this is not likely to have been a contributing factor in the poor shrimp performance observed in the current study, both omega-6 (n-6) and omega-3 (n-3) fatty acids play significant roles in human health. Recently, the contribution of vegetable oils high in omega-6 fatty acids to total dietary fat intake has increased for Western populations, and some published data suggests that this excessive amount of omega-6 PUFAs results in a very high n-6/n-3 ratio (~15:1) and promotes the pathogenesis of many diseases including cardiovascular disease, cancer, and inflammatory autoimmune diseases. Plant-based feed ingredients contain a high n-6/n-3 ratio, thus one challenge in producing a shrimp for health conscious consumers is to ensure that the farmed shrimp maintain the very low n-6/n-3 fatty acid ratio typical of wild caught shrimp. For these reasons, this study will be repeated to confirm our results. In addition, the planned study will compare the effects of traditional soy oil with high n-6/n-3 fatty acid ratio to a newly formulated soybean oil product with lower n-6/n-3 fatty acid ratio (manufactured by the American Soybean Association) on shrimp performance and fatty acid content of shrimp tissue.

Acknowledgement

Special thanks to American Soybean Association and to Texas AgriLife Research for the funding. Special thanks to Shrimp Improvement Systems, Islamorada, FL for providing the postlarvae at reduced cost and to the AgriLife Research Mariculture Laboratory staff for their help.