United Soybean Board
Final Report Form

Project # and Title	Project: 1340-512-5262. Use of Soy-based Products in Practical Diets for Florida Pompano, *Trachinotus carolinus.* Phase II
Organization & Project Leader	University of Miami Dr. Daniel Benetti & Dr. Jorge Suarez
Reporting Period	March 01, 2013- Feb 28, 2014

Introduction: Statement on the rationale and background for the studies

Research is needed to advance the fish feeds sector in the United States and the world by increasing the cost-effectiveness and environmental sustainability of the aquafeeds used during the growout stages. This project addresses the need to develop practical diets for Florida Pompano (*Trachinotus carolinus*), using combinations of various soy protein products at different ages in order to formulate cost-effective and environmentally-friendly diets.

The proposed goal is to maximize fish meal replacement for Florida Pompano, *Trachinotus carolinus*, at different ages in order to optimize the soy-based products in aquaculture feeds. The use of protein sources such as standard soybean meal, soy protein concentrate and soy by selective breeding program could greatly improve the profitability and appeal of the industry. In essence, the main objective was to conduct scientific experiments with practical application aimed at improving the ecological and economic efficiency of feeds for the Florida pompano and eventually for other commercially important species as well. The present proposal represented the second research phase of a study financed by United Soybean Board (USB) in 2012.

Florida pompano is a truly euryhaline species of high market value and demand. With the recent interest in developing aquaculture of high-value carnivorous species, production costs are a primary concern since carnivorous fish require greater amounts of protein and fishmeal represents the primary protein source in feeds formulations. Lack of adequate feeds for the growout of pompano remains a serious bottleneck preventing pompano aquaculture expansion to commercial scale. No basic or applied research has been conducted to develop ecologically and economically efficient diets for larger size pompano, at the later stages of the growout period, when the vast majority of the feeds are used. We proposed to conduct innovative scientific research to address and resolve this problem.

Studies completed - brief summary of the number and type of studies conducted, including general study design and approach on how and where the studies were conducted, but without details of the materials and methods

Experimental Diets

Five isonitrogenous and isoenergetic experimental diets were formulated to produce diets in which 50 (D500) g/kg $^{-1}$ of protein from fishmeal was replaced by that from: [1]Combination of standard dehulled SBM+Brazilian SPC (Selecta 60% CP), [2] Combination of standard dehulled SBM+ HP 300 Hydrolyzed Soy Protein (Hamlet Protein), [3] Combination of standard dehulled SBM+Schillinger Navita, [4]Combination of standard dehulled SBM+Soy Protein Concentrate (Solae Profine). The formulation and proximate composition of the experimental diets are shown in Table 1.

All diets were formulated to provide ~40% crude protein and ~13% total lipid (as-feed) and supply approximately 20 kJ gross energy/g dry diet. All diets were supplemented with an amino acid mix. The reference diet was formulated to be similar to commercial formulations for pompano with 30% fish meal and the experimental diets were formulated with 15% fish meal.

Experimental Conditions

The Florida pompano used in this experiment were spawned and reared at the University of Miami Experimental Hatchery (UMEH). The fish (avg. wt. 27.8 ± 8.8 g at stocking) were randomly distributed into a flow-through system consisting of eighteen 1,000-L conical polyethylene plastic tanks. Each tank was fitted with a central standpipe and an oxygen stone. Stocking density was 35 fish per tank at average biomass of 975±50.0 (0.9 kg/ m^3). Fish were hand-fed the experimental diets (three tanks per diet) twice daily, at 0900 and 1600 h for 34 days. Fish were hand-fed as much as they could consume in 5 min up to apparent satiation. Sand filtered, well water and seawater was pumped into each tank at an exchange rate of 600-900% per day. The tanks were siphoned as needed and the system was backwashed every other day. Throughout the 5-week experimental period, water temperature was 25.2± 0.2 °C, salinity 33-35 ppt, and dissolved oxygen maintained at 8.1± 1.2 mg/L.

Harvest, sample collection and production performance

The first harvest was performed 34 days after the start of the experiment, with a final density of 1.4 kg/m^3 and an average weight at harvest of 44.8 ± 14.8 g.

Results - sequential summary of results, ending with recommendations on soy diet formulations, feeding protocols, economics and other related recommendations

The most important activities conducted in this phase of the project were:

- Carried out the spawning and larval rearing of the Florida pompano used for the different experiments at the University of Miami Experimental Hatchery,UMEH.
- Five isonitrogenous and isoenergetic experimental diets were formulated to produce diets in which 50 g/kg $^{-1}$ of protein from fishmeal was replaced by soy-based products.
- This investigation yielded the optimum combinations of various soy protein products for the optimal growth of Florida pompano for one weight range (28-44 g) (Figure 1). The biological performances of the experimental diets are shown in Table 2.
- Cultured pompano for the growout study through the fattening phase until they reached an average weight of 150 g. This was the target for the second weight range (150-450g) (Figure 2).

Meeting expectation/performance measures:

The most relevant results obtained in this project included the following:

- SPC Solae diet produced significantly higher average daily growth (ADG), feed efficiency (FE) and protein efficiency ratio (PER), while yielding a significantly lower feed conversion ratio (FCR) and fish in:fish out (FIFO) ratio (Table 2).
- When compared to the Reference and SPC Solae diets, the young pompano fed the SPC Brazilia (Selecta) diet showed significantly lower average daily growth (ADG), feed efficiency (FE) and protein efficiency ratio (PER), while yielding a significantly higher feed conversion ratio (FCR) (Table 2).
- Fish in:fish out (FIFO ratio) values from this study ranged between 1.8 (Solae diet) to 2.9 (Reference diet). Among the soy-based products, Brazilian SPC yielded the highest FIFO ratio at 2.24.
- The University of Miami's graduate student Stephen Sutton has actively participated in the activities of this project and is completing his Master's thesis based on this study. Besides his thesis, a peer-reviewed publication is anticipated when the study is complete.

Changes: Problems, obstacles, new developments or market/industry/research changes that impacted or may impact the completion date, cost or scope of the project.

As the experimental diets were being produced for this experiment and the pompano juveniles reached their target start weight of about 150 g, we experienced a rare mortality event with this species in our facilities. A severe outbreak of the ecto-parasite known as *Brooklynella hostilis* caused a massive mortality of all 458 fish we had raised to complete the first objective of this project. Appendix 1 provides a detailed explanation of the events and our attempts to prevent the proliferation and recurrence of *Brooklynella* in our facilities. Since the mortality event, there have been no further incidences of occurrences of external parasites in any of the marine fish species maintained at our hatchery facilities. To complete the objectives outlined in this study, we have recently acquired juvenile pompano (around 20 g average weight) that will be grown to 100 g over the next two months and then utilized to replace the fish that were lost. As previously outlined, we plan to perform 4 months of a growth-performance study, analysis of the data and produce a complete final report. Data collection is scheduled to commence around mid-June and last until mid- October. Therefore, due to the circumstances, we kindly request to delay completion of this project until December 2014, when we commit to submit a updated version of the final report presenting the final data with detailed information.

Conclusions - summarize overall value of research results and application opportunities by industry

This project will provide valuable data for the formulation of more efficient diets with high levels of soy-based products, enabling nutritionists to formulate feeds specifically for fish at the respective size ranges, therefore reducing the high operation costs associated with the feeds.

The results of this project can and will likely be readily used by the aquaculture industry and the scientific community. There is a widespread interest in developing Florida pompano aquaculture by the industry (e.g. both Open Blue Sea Farms in Panama and Marine Farms Belize, our two most important industry partners, have requested fingerlings to stock their cage production systems). Global Sea Farms from the Dominican Republic is also raising Florida pompano in cages and expressed interest in novel diets that are more economically viable.

The collective efforts of the US soybean industry and university graduate programs created funded opportunities for research in soybean utilization in aquaculture nutrition that are already having an impact in the field. The most conspicuous indirect benefit of these initiatives is that they ignited widespread interest, and is now forming a new generation of bright, talented and qualified graduate students who are and will continue to contribute with new ideas to the development of novel technologies. This will have a great positive impact in the years to come and most likely generate great benefits to the soybean industry in the near future.

Table 1. Formulation and proximate composition of the experimental diets (*g/100 g* as feed) fed to adults, Florida pompano *Trachinotus carolinus.*

Experimental diet	Ref. diet	Brazilian SPC diet	Hamlet protein diet	Navita diet	Solae SPC diet
Menhaden meal	30.0	15.0	15.0	15.0	15.0
Soybean meal	35.0	35.0	35.0	35.0	35.0
SPC (Brazilian Selecta)		24.0			
Hamlet protein			24.0		
SG-Navita				24.0	
SPC (Solae)					24.0
Corn gluten	10.0	1.0	4.5	4.0	1.5
Menhaden oil	6.6	8.0	8.0	8.0	8.0
Lecithin (Soy refined)	1.0	1.0	1.0	1.0	1.0
Mineral-vitamin premix	6.0	6.0	6.0	6.0	6.0
Choline chloride	0.5	0.5	0.5	0.5	0.5
Taurine	0.47	0.58	0.58	0.58	0.58
Lysine HCl				0.05	
DL-Methionine		0.34	0.29	0.30	0.33
Ca P-dibasic	0.5	1.0	1.0	1.0	1.0
Dextrin	9.0	6.50	4.13	4.44	6.91
Cellufil	0.93	1.08		0.13	0.18
Proximate analysis g/100 g (as feed).					
Crude protein (g/100 g)	40.5	41	41	40.5	41
Crude lipid (g/100 g)	13	13	13	13	12.5
Energy (kJ/g)	20	20	20	20	20

[1]Mineral Premix composition (g/kg): Ca(H2PO4)2 ·H2O, 136.00; Ca(C6H10O6) ·5H2O, 348.553; FeSO4 ·7H2O, 5.00; MgSO4 ·7H2O, 132.00; K2HPO4, 240.00; NaH2PO4 ·H2O, 88.00; NaCl, 45.00; AlCl3 ·6H2O, 0.084; KI, 0.15; CuSO4 ·5H2O, 0.50; MnSO4 ·H2O, 0.70; CoCl2 ·6H2O, 1.00; ZnSO4 ·7H2O, 3.00; NaSeO3, 0.0127. Vitamin Premix composition (g/kg): Ascorbic acid, 50; dl-calcium pantothenate, 5.0; Choline chloride, 36.2; Inositol, 5.0; Menadione sodium bisulfite, 2.0; Niacin, 5.0; Pyridoxine HCl, 1.0; Riboflavin, 3.0; Thiamine mononitrate, 0.5; dl-alpha-tocopherol acetate (250 IU/g), 8.0; Vitamin A palmitate (500,000 IU/g), 0.2; Vitamin micro-mix[c], 10.0; Cellulose, 874.1 Vitamin Micro-mix composition (g/100g): Biotin, 0.50; Folic acid, 1.8; Vitamin B12, 0.02; Cholecalciferol (40 IU/ug), 0.02; Cellulose, 97.66

Table 2. Experiment 1. Mean selected efficiency parameters, and fish in:fish out (FIFO) in juvenile Florida pompano, *Trachinotus carolinus* (means of three replicates).

Sample Period 34d First Harvest	**Final Weight** g	**WG** %	**ADG** g/fish/day	**SGR** %/d	**MDI** g/fish/day	**GEI** kJ/fish/d
Dietary Treatment						
Reference	46.4[a]	64.6	0.54[a]	1.47	1.17	23.3
Brazilian SPC	42.1[b]	53.6	0.43[b]	1.26	1.15	23.0
Hamlet protein	41.6[b]	57.6	0.45[b]	1.34	1.09	21.8
Navita	43.7[ab]	57.9	0.47[b]	1.34	1.15	22.9
Solae SPC	46.0[a]	65.3	0.53[a]	1.48	1.15	23.0
Pr>F	0.016	0.071	0.012	0.07	0.113	0.109
P.S.E	2.48	6.27	0.054	0.115	0.039	0.782

Sample Period 34d First Harvest	**GPI** g/fish/day	**FE** g gain/ g fed	**PER** %	**FCR** g fed/ g gain	**FIFO**
Dietary Treatment					
Reference	0.53[ab]	0.46[a]	1.02[a]	2.18[a]	2.90[c]
Brazilian SPC	0.52[b]	0.38[b]	0.83[b]	2.69[b]	2.24[b]
Hamlet protein	0.48[c]	0.41[ab]	0.93[ab]	2.45[ab]	2.05[ab]
Navita	0.51[b]	0.41[ab]	0.92[ab]	2.44[ab]	2.04[ab]
Solae SPC	0.52[b]	0.47[a]	1.04[a]	2.16[a]	1.80[a]
Pr>F	0.037	0.047	0.044	0.039	0.0002
P.S.E	0.019	0.046	0.102	0.039	0.414

Values that do not share superscripts within the same column were found to be significantly different ($P<0.05$).

ADG = average daily gain; SRG = specific growth rate; MDI = mean daily intake; GEI = gross energy intake; GPI = gross protein intake; FE = feed efficiency; PER = protein efficiency ratio; FCR = feed conversion ratio; FIFO = fish in:fish out

Figure 1. Juvenile Pompano at first harvest size (44±2.5 g).

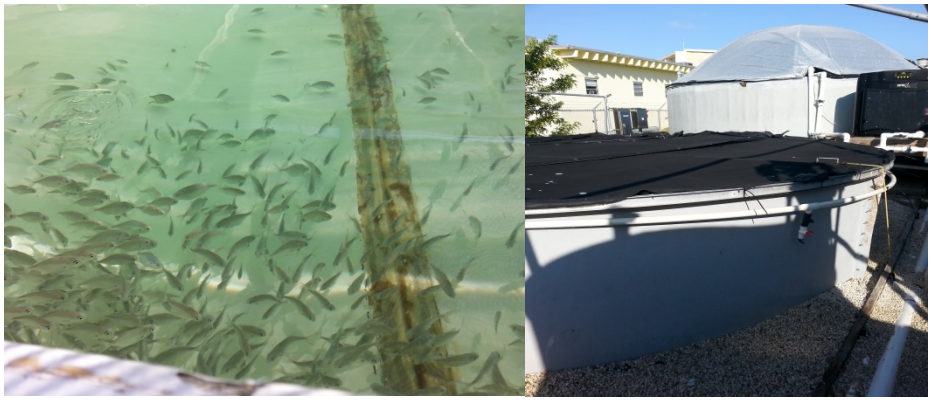

Figure 2. Cultured Pompano for the growth study through the fattening phase until they reached an average weight of 150 g.

Appendix 1

Brief report of a disease outbreak at UMEH facilities

An outbreak of *Brooklynella hostilis* infected our juvenile Florida Pompano, *Trachinotus carolinus* held at the University of Miami Experimental Hatchery (UMEH). *Brooklynella hostilis* is an aggressive marine ectoparasite commonly infecting tropical marine fish. This pathogenic cyrtophorine ciliate attacks the host gills and skin of the host fish causing severe hemorrhages and fusion of the gill lamellae, which in turn causes the fish to suffocate and die (Lom and Nigrelli, 1970). At the University of Miami, we follow diligent protocols and procedures to prevent parasites from infecting our fish (Benetti and Alarcon 2000). Water quality parameters are taken daily and extensive observations to ensure fish health are made and recorded routinely. In accordance with said protocols, on Tuesday, February 11, 2014, 458 juvenile Florida Pompano (approximately 150g each) were transferred into a clean/disinfected tank and given a 10 min freshwater dip treatment before entering their new holding tank. Before the move, 5 fish were euthanized and gill/ skin samples were examined at under the microscope. No signs of parasite or disease were observed. The following day, a 100ppm, 37% formalin treatment was administered for 1 hour. These treatments/ protocols are routinely conducted on all fish/ tanks at the University of Miami Experimental Hatchery as a form of prophylaxis. On Saturday, February 22, 2014, the Florida pompano were fed the usual rate of 3% of the total tank biomass, water quality parameters were taken and recorded, tank siphoned, and fish observed (both in the a.m. and p.m.). There were no signs of illness or parasites. The fish ate well and appeared to be good health. In the early morning of Sunday, February 23, two fish had died and the remaining fish appeared to be unhealthy, showing obvious symptoms of illness (erratic swimming). Gill and skin samples were taken of the recently deceased fish and it was concluded that *Brooklynella hostilis* was the cause for the tanks illness (Figure 1). The outbreak of the disease was extremely fast. Once the cause of death was determined, the UMEH followed its strict protocols and procedures to combat external parasites. The most common and usually effective method is treating the tank with 100 ppm, 37% formalin treatment for 1 hr., followed by a thorough siphoning. At final check, there were only 6 more dead fish removed from the tank. On Monday morning, February 24, 2014, all 450 fish remaining were dead, despite our efforts (Figures 2 and 3). Since then, all of our other fish have been given formalin treatments, moved, and tanks/equipment disinfected. No further parasites have been observed.

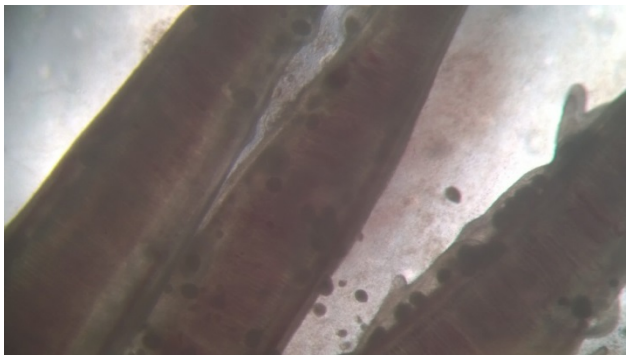

Figure 1. Gill and skin samples were taken of the recently deceased fish and it was conclude that *Brooklynella hostilis* was the cause for the tanks illness.

Figure 2. *Brooklynella hostilis* made its way into the tank and killed all 458 fish.

Figure 3. February 24, 2014, all 458 fish were dead.

Literature Cited

Lom, J., Nigrelli, R.F., 1970. *Brooklynella hostilis n. g., n. sp., a Pathogenic Cyrtophorine Ciliate in Marine Fishes*. Journal of Protozool. 17, 224–232.

Benetti, D.D., Alarcon, J., 2000. *General Prophylaxis and Quarantine of Marine Brood Fish*. Global Aquaculture Alliance: The Advocate. December 2000, 60-61.