

**United Soybean Board**  
**Final Report Form – Technical Bulletin**

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| <b>Project # and Title</b>                   | 1340-512-5261 Improving High Soy Feed Formulations<br>Supplemented with Taurine in US Marine Fish Feeds        |
| <b>Organization &amp;<br/>Project Leader</b> | Auburn University, School of Fisheries, Aquaculture and Aquatic<br>Sciences. Drs. Terry Hanson and Allen Davis |
| <b>Reporting Period</b>                      | Final Report covering March 1, 2013 through February 28, 2014                                                  |

**Introduction: Statement on the rationale and background for the studies**

The Florida pompano is one of several species of jacks that are considered highly prized food fish. They have a flakey texture and a mild flavor. Currently, they are reared in intensive indoor systems and outdoor cages in many areas of the world. Based on ongoing research this species performs well on soy based diets as long as nutritional and palatability needs are met. Previous USB funded projects have systematically evaluated nutrient restrictions that limit the inclusion of soy products in the feed. To date, we have been very successful in increasing the level of soy protein (solvent extracted meal and soy protein concentrate) in practical feed formulations for this species.

Further research efforts have been supported and coordinated with a range of funding agencies to allow leveraging of research dollars. Previous studies have included the use of poultry by-product meal and meat and bone meals as alternatives to fish meal in soy based diets. This research confirmed methionine and lysine were not deficient in high soy diets (~50% diet) but there was a clear response to taurine. The physiological characterization of deficiencies and the dietary requirement for taurine are currently underway. It is clear taurine is a limiting nutrient, even with supplements for lysine, methionine and taurine (as required), the removal of animal protein and/or fish meal results in depressed growth. This indicates that another nutrient is limiting in high soy diets when animal protein is reduced.

As part of this research we re-evaluated the amino acid profile of recent feed formulations to identify if there is a consistent amino acid or group of amino acids that are reduced. In the most recent diets glycine, valine and histidine were reduced as diets were shifted. These are not typically considered essential but all function as attractants. Consequently, evaluation of these or other possibly limiting amino acids is warranted.

Another way to improve production is through proper feed management. Without proper feed management economic returns from the feed may not be optimized. A preliminary study comparing one feeding to four feedings per day resulted in almost twice the final weight of the fish. This may be due to the feeding habits of pompano which grazes in the surf zone on small bivalves and other animals. Consequently the fish has evolved to have a relatively small stomach and quick digestive systems which may mean multiple feedings are required to obtain best performance. Hence, in order to help commercial producers improve growth rates and have efficient food conversion another component of this work was to evaluate increasing the number of feedings per day to determine if feed intake and growth are affected.

The objectives of this study were to: 1) evaluate if amino acids other than lysine, methionine and taurine are limiting in high soy diets with low levels of animal protein; and 2) determine the response of juvenile pompano to increasing numbers of feedings.

**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**

**Objective 1: To evaluate if amino acids other than lysine, methionine and taurine are limiting in high soy diets with low levels of animal protein.**

To demonstrate if there were amino acids other than taurine, lysine and methionine potentially limiting in high soy diets, a series of diets were formulated to contain high levels of soy (~65%) without animal protein. The plant based diet was run without supplements and with individual supplements of glycine, valine, histidine and a dry fish solubles. Additionally a reference diet containing animal protein was included for comparison. The diets were formulated to have similar proximate analyses (45% protein and 10% lipid) and meet the known nutrient requirements of this species. Proximate analyses of the diets were confirmed as well as the amino acid profiles, determined by University of Missouri Agricultural Experiment Station Chemical Laboratories (Columbia, MO, USA). Florida pompano, *T. carolinus* juveniles were purchased from Troutlodge Marine Farms LLC, (Proaquatix) Vero Beach, FL. The trial was conducted in a recirculating system with 32 culture tanks, water pump, supplemental aeration (using a central line, regenerative blower and air diffusers) as well as mechanical and biological filtration. Fifteen fish were stocked in each tank. Water quality parameters of temperature, dissolved oxygen, and salinity were monitored twice daily. Fish were fed to apparent satiation, to allow for increased or decreased intake depending on palatability characteristics of the diets. Feed was provided four times per day and the daily intake was recorded. At the termination of the 6 week experimental period, survival, final weight, and feed conversion ratio were determined. Group weights and individual weights were taken for the fish in each tank. Proximate analysis and amino acid profiles of the fish were determined by University of Missouri Agricultural Experiment Station Chemical Laboratories (Columbia, MO, USA). All data were analyzed by a one-way analysis of variance to determine significant ( $P < 0.05$ ) differences among the treatment means. When appropriate, Student-Neuman Keuls' multiple range test was used to distinguish significant differences between treatment means. Dunnett's t-test was used to contrast the diet without supplementation to each diet supplemented with amino acids. All statistical analyses were conducted using SAS system for windows (V9.3, SAS Institute, Cary, NC).

**Objective 2: Determine the response of juvenile Florida pompano to increasing numbers of feedings.**

Three growth trials were conducted at Claude Peteet Mariculture Center, Gulf Shores, AL to evaluate feed intake when 2, 4 and 6 feedings per day were offered. The fish origin, diet and fish sample analysis, water quality, growth performance and feed intake was determined and analyzed the same as in objective 1. The response to increasing the number of feedings was conducted in 3 different recirculating systems with different size fish stocked at different densities. Trial 1a stocked 0.33g fish at 150 fish/tank for 3 weeks, with fish weighed weekly.

Trial 1b stocked 13.14g fish at 15 fish/tank for 8 weeks and Trial 1c stocked 34.5g fish at 47 fish/tank. The fish in each tank were counted and weighed every two weeks. Trial 1a used a commercial crumble feed FF Starter (55% Protein, 15% lipid). Trial 1b and 1c used a commercial floating feed Finfish Silver (40% protein, 10% lipid) (Zeigler Bros. Inc.). The fish in trials 1a and 1b were fed to satiation either 2, 4 or 6 feedings per day over a 12 hour period. The daily feed intake for each tank was measured to calculate the percent body weight consumed. The fish in trial 1c were fed a fixed daily ration divided into 4 or 6 feedings per day.

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

### **Objective 1: To evaluate if amino acids other than lysine, methionine and taurine are limiting in high soy diets with low levels of animal protein.**

The analysis of the 6 test diets supplemented with various supplements reported actual protein levels ranging from 45.4-47.28% and lipid content ranging from 9.0-10.3%, on an as is basis. There were minimal differences in amino acid profiles of the diets, with higher values of the supplemented amino acid present in the respective diets. Albeit requirement data is only available for a few essential amino acids for this species, based on available literature as well as typical amino acid requirement for other species, there were no clear indications of deficiencies of essential amino acids. The analysis of the whole body samples did not show any differences in amino acid composition; however, the lipid content in the fish fed the reference diet and diet supplemented with histidine, were significantly lower than the others.

At the termination of the trials, there was reasonable growth, survival and feed conversion in all of the treatments. Survival of the fish was high in all of the treatments with no differences, ranging from 81.7-100%. The growth performance of the fish was consistent with a number of trials in which final weights and percent weight gain was reduced as animal protein is removed. The final weight of the fish ranged from 15.6 g in the valine supplemented diet to 20.8 g in fish offered the reference diet. Using pair wise contrasts we evaluated if there were differences between the plant based diets without any supplements and each of the other diets. Based on this test, the reference diet with animal meal performed significantly better but no other diet did. A similar result was found doing pair wise contrast using the reference diet as compared to all other diets. In this case diets supplemented with valine and histidine were significantly smaller whereas fish fed diets with glycine ( $P=0.0638$ ) and fish protein concentrate ( $P=0.0587$ ) were not different. These results indicated a small but not strong response to these supplements in terms of final weights.

The FCR was highest in the valine supplemented diet (2.31) and lowest in the reference diet (1.51). There were no significant differences in performance when Dunnett's comparison of the all plant diet without supplementation and each supplemented diet was conducted. There was no significant difference in feed intake for any of the diets suggesting there was not a palatability issue in the diets without animal protein or without supplemented attractants.

In general, these results indicate that there is a weak response to fish protein concentrate (dry fish solubles) and glycine albeit neither completely solved the problem of reduced performance i.e. they did not clearly produce an improvement in growth.

**Objective 2: Determine the response of juvenile Florida pompano to increasing numbers of feedings.**

There was high survival for all three trials, ranging from 85.1-100% with no significant differences between treatments. In trial 1a the fish fed 2 feedings per day were significantly smaller, 2.37g than the fish fed 4 or 6 feedings per day, 2.89 and 2.86g respectively albeit 4 or 6 feedings did not influence final weight. The fish fed 2 feedings per day consumed significantly less feed, 926g than the fish offered 4 or 6 feedings/day, which consumed 1063 and 1153g. The FCR was not significantly different. The results from the second trial (1b) were similar to that of the smaller fish (trial 1a) with the poorest performance from 2 feedings per day. Again the fish fed 4 or 6 times/day had final mean weights and weight gain that were similar; however, the FCR (1.62) was significantly lower (better) when fed 4 feedings per day. Analyzing the feed intake and the cost of the feed, the best FCR and best return in profit would be 4 feedings per day. Proximate composition of whole body samples of fish from Trial 1b showed no significant differences. Trial 1c was conducted to compare growth when fed a fixed daily ration (determined from the intake in satiation trials) divided into 4 or 6 feedings per day. After 30 days, there were no significant differences between 4 and 6 feedings in final weight, percent gain, feed, FCR or survival. The commercial floating pellet was analyzed and taurine content was 0.22%, less than our recommended supplemented level. Although growth and survival were good, additional taurine may have improved performance.

The water quality remained within acceptable levels for all of the trials. In trial 1a: temperature was  $24.6 \pm 2.6$  C, salinity  $31.3 \pm 0.5$  ppt, dissolved oxygen  $5.80 \pm 0.73$  mg/L, pH  $7.70 \pm 0.25$  and total ammonia nitrogen  $0.61 \pm 1.03$ . In trial 1b: temperature was  $26.9 \pm 1.5$  C, salinity  $31.3 \pm 3.3$  ppt, dissolved oxygen  $5.27 \pm 0.44$  mg/L, pH  $7.57 \pm 0.32$  and total ammonia nitrogen  $0.14 \pm 0.15$ . In trial 1c: temperature was  $28.5 \pm 1.7$  C, salinity  $27.8 \pm 1.2$  ppt, dissolved oxygen  $5.58 \pm 0.33$  mg/L, pH  $7.65 \pm 0.16$  and total ammonia nitrogen  $0.11 \pm 0.19$ .

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

The removal of animal meals for the production of plant based diets is a primary goal for the sustainability of aquaculture and promotes the use of land based plant proteins. Although the all plant protein diets had reduced growth performance, not all treatments were significantly different from the reference diet containing animal protein. Glycine and FPC (dry fish solubles containing a range of free amino acids) both showed positive improvements to some degree. The amino acids that were supplemented may not be indispensable but may serve as attractants or palatability enhancers to improve the acceptability of high soy diets. The level supplemented did not significantly increase feed intake albeit there were some improvement in growth. Results indicate there is a limiting nutrient or a group of nutrients which is not present in plant based feed formulations. The essential amino acid requirements for Florida pompano still need to be determined to aid in feed formulation but they do not appear to be limiting in the present formulations. It is also possible the amount of plant proteins introduced antinutritional factors that inhibit the digestion of nutrients. New varieties of soybeans and soybean meal may improve digestion and reduce antinutritional factors.

Results of the feeding trials indicate a clear benefit of increasing the number of feedings in all the sizes of fish tested. The fish fed 2 feedings per day were significantly smaller than the fish fed 4 or 6 feedings per day albeit 4 or 6 feedings did not influence final weight. With proper

feed management economic returns from the feed may be optimized. Analyzing the feed intake and the cost of the feed, the best FCR and best return in profit would be 4 feedings per day. This information will help commercial producers to improve growth rates and have an efficient food conversion.

### **Presentations**

“Update on nutrition research with Florida pompano at Claude Peteet Mariculture Center.” M. A. Rhodes, R. Cook, G. Salze, D. A. Davis and T. Hanson. Alabama Fisheries Association, Inc Annual Meeting. Lakepoint State Park, Feb 19-21, 2014.

“World feed considerations and opportunities.” D. A. Davis. 2014 Financing Aquaculture Roundtable. Atlanta, GA January 8-9, 2014.

“Response of Florida pompano to increasing numbers of feedings.” M. A. Rhodes, R. Cook, D. Yu, K. Oliveira, D. A. Davis and T. Hanson. Aquaculture America 2014, Seattle Washington USA, February 9-12, 2014.