United Soybean Board
Final Report Form – Technical Bulletin

Project # and Title	1440-512-5261 Improving High Soy Feed Formulations Supplemented with Taurine in US Marine Fish Feeds
Organization & Project Leader	Auburn University, Drs. Terry Hanson and Allen Davis
Reporting Period	Final Report covering March 1, 2014 through Feb 28, 2015

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.

In addition to select supplements (essential amino acids, fatty acids, vitamins and minerals) being used to improve a feed formulation, a feed can be improved by improving the overall digestibility of the feed. There are new varieties of soybeans (and subsequently soybean meal) and enzyme supplements that may favor improved digestion of soybean meal in marine species. Given the high inclusion levels of soybean meal and soy protein concentrates that we have been able to achieve, problems with non-starch polysaccharides or other compounds such as phytate could be a realistic restriction. Both are known to inhibit nutrient availability and have been demonstrated in poultry to reduce performance. We feel it is prudent to determine if the presence of indigestible carbohydrates or high levels of phytate may be responsible for reduced performance.

The objectives of this project were to 1. Determine if non-starch polysaccharides influence the performance of Florida pompano in soy based feed formulations by evaluating “commodity” soybean meal and soybean meal produced from genetically improved soybeans with reduced levels of NSP on the growth and nutrient digestibility of Florida pompano and determining if the supplementation of a mixture of carbohydras’s will improve high soy feed formulations in the Florida pompano. 2. Determine the response of Florida pompano to varying levels of phytase in a high soy diet.

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

1. Determine if non-starch polysaccharides influence the performance of Florida pompano in soy based feed formulations.

A series of 4 diets were formulated to determine the effect of carbohydrase (mixture of enzymes) supplementation on soybean use (standard and low oligosaccharide). 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).

	Basal	Basal +CHOase	Low NSP	Low NSP +CHOase
Poultry by product meal	15.00	15.00	15.00	15.00
Soybean meal solvent extracted	47.70	47.70	41.10	41.10
Soybean meal, low NSP				
Menhaden Fish Oil	4.81	4.81	4.81	4.81
Soy Oil	0.00	0.00	0.42	0.42
Corn Starch	4.54	6.60	12.82	12.78
Whole Wheat	17.50	17.50	17.50	17.50
Corn protein concentrate	6.30	6.30	6.30	6.30
ASA Trace Mineral premix	0.25	0.25	0.25	0.25
ASA Vitamin premix w/o choline	0.50	0.50	0.50	0.50
Choline chloride	0.20	0.20	0.20	0.20
Stay C 250 mg/kg using 35%	0.10	0.10	0.10	0.10
CaP-dibasic	2.10	0.00	0.00	0.00
Lecithin	0.50	0.50	0.50	0.50
Taurine	0.50	0.50	0.50	0.50
CHOase	0.00	0.04	0.00	0.04
Proximate analyses (as is)				
Crude Protein (%)	41.15	40.86	42.45	44.10
Crude Fat (%)	8.91	8.76	8.70	9.17
Crude Fiber (%)	3.12	3.20	2.15	2.31
Moisture (%)	5.65	6.68	5.14	5.60
Ash (%)	7.50	5.59	5.46	5.63
Phosphorus (%)	1.20	0.79	0.73	0.78

Florida pompano, *T. carolinus* juveniles were purchased from Troutlodge Marine Farms LLC, (Proaquatix) Vero Beach, FL. Fish were picked up and transported back to Claude Peteet Mariculture Center in Gulf Shores, AL on May 6th. They averaged 0.35 g at pickup. Fish were fed a commercial diet until appropriate size for growth trials. A 10 week growth trial was initiated on June 6th with 10.1 g average mean weight fish. The trial was conducted in a recirculating system with 12 culture tanks, water pump, supplemental aeration (using a central line, regenerative blower and air diffusers) as well as mechanical and biological filtration. Twenty fish were stocked in each tank. Water quality parameters of temperature, dissolved oxygen, and salinity were monitored twice daily. Feed was provided four times per day. At the

termination of the 10 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).

2. To determine the response of Florida pompano to varying levels of phytase in a high soy diet

A series of 4 diets were formulated to evaluate the effect of phytase supplementation on phosphorus and amino acid availability. A 9 week growth trial was initiated on May 30th with 8.0 g average mean weight fish. The growth trial was conducted as described above in objective 1.

	Basal+CaP	Basal	Phytase 500	Phytase 4000
Poultry by product meal	15.00	15.00	15.00	15.00
Soybean meal solvent extracted	47.70	47.70	47.70	47.70
Menhaden Fish Oil	4.78	4.78	4.78	4.78
Corn Starch	4.57	6.67	6.66	6.61
Whole Wheat	17.50	17.50	17.50	17.50
Corn protein concentrate	6.30	6.30	6.30	6.30
ASA Trace Mineral premix	0.25	0.25	0.25	0.25
ASA Vitamin premix w/o choline	0.50	0.50	0.50	0.50
Choline chloride	0.20	0.20	0.20	0.20
Stay C 250 mg/kg using 35%	0.10	0.10	0.10	0.10
CaP-dibasic	2.10	0.00	0.00	0.00
Lecithin	0.50	0.50	0.50	0.50
Taurine	0.50	0.50	0.50	0.50
Phytase	0.00	0.00	0.0007	0.055
Proximate analyses (as is)				
Crude Protein (%)	41.64	40.71	41.03	41.56
Crude Fat (%)	8.77	8.51	8.60	8.74
Crude Fiber (%)	3.38	2.99	3.05	2.86
Moisture (%)	5.46	7.55	6.89	6.46
Ash (%)	7.52	5.76	5.58	5.64

Leveraged work.

Previous to this year's work we were able to evaluate a number of attractants this year through funding from Salton Stall Kennedy work with shrimp we had access to a hydrolyzed squid meal which we feel has potential. Hence, the previous work with attractants has been extended to

help us elucidate the potential restrictions of an all plant diet for the Florida Pompano. A series of 4 diets were formulated to evaluate the inclusion of 5% of squid hydrolysate, poultry by product meal and a chemical attractant mix.

	Plant	Attractant	Poultry	Squid
Poultry by product meal	0.00	0.00	5.00	0.00
Soybean meal solvent extracted	35.80	35.80	35.80	35.80
Soy protein concentrate	28.00	28.00	22.90	23.00
Menhaden Fish Oil	8.88	8.88	8.28	8.36
Corn Starch	5.07	0.07	5.77	5.59
Whole Wheat	8.00	8.00	8.00	8.00
Corn protein concentrate	10.00	10.00	10.00	10.00
ASA Trace Mineral premix	0.25	0.25	0.25	0.25
ASA Vitamin premix w/o choline	0.50	0.50	0.50	0.50
Choline chloride	0.20	0.20	0.20	0.20
Stay C 250 mg/kg using 35%	0.10	0.10	0.10	0.10
CaP-dibasic	2.10	2.10	2.10	2.10
Lecithin	0.50	0.50	0.50	0.50
Taurine	0.60	0.60	0.60	0.60
Chemical Attractant Mix	0.00	5.00	0.00	0.00
Squid Hydrolysate (expressed dry)	0.00	0.00	0.00	5.00
Proximate analyses (as is)				
Crude Protein (%)	44.87	49.61	44.70	46.56
Crude Fat (%)	9.01	9.92	9.25	10.05
Crude Fiber (%)	2.88	3.09	2.85	2.82
Moisture (%)	8.52	7.16	8.23	5.50
Ash (%)	6.28	6.36	6.56	6.39

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

1. Determine if non-starch polysaccharides influence the performance of Florida pompano in soy based feed formulations.

The water quality remained in acceptable levels for the trial. Temperature was 27.8 ± 1.2 C, salinity 28.4 ± 3.7 ppt, dissolved oxygen 5.44 ± 0.5 mg/L, pH 7.3 ± 0.7 and total ammonia nitrogen 0.06 ± 0.07.
The proximate analysis of the diets was analyzed, protein ranged from 40.9-44.1% and lipid ranged from 8.7-9.2%. The Phosphorus analysis of the diets is complete, 1.20, 0.79, 0.73 and 0.78% for Diets 1-4 respectively.

After the 10 week trial, 100% survival in all tanks. The final mean weights ranged from 76.7 to 89.6g. The FCR ranged from 1.73 to 1.93, the low NSP soy diets were significantly lower FCR. The final mean weight was significantly different when comparing the commodity soybean vs

the low NSP soybean meal which produced the best response. There was no significant effect of carbohydrase on growth.

IW=10.13g	Treatment	Final weight (g)	Wt. Gain (%)	Survival (%)	FCR
	Basal	79.3	677.0[b]	100	1.93[a]
	Basal+Cho	76.7	660.0[b]	100	1.87[ab]
	LowNSP	89.6	790.6[a]	100	1.81[ab]
	LowNSP+Cho	88.1	767.3[a]	100	1.73[b]
	p value	0.0378	0.0061	-	0.0211
	PSE	1.4926	10.8055	-	0.0184

The apparent digestibility coefficients (ADC) including apparent dry matter digestibility (ADMD) and apparent energy digestibility (AED) for the basal diet, Basal+CHOase, LowNSP, LowNSP+CHOase were ADMD 46.1 ± 2.7, 51.8 ± 3.0, 48.9 ± 1.8, 46.7 ± 3.5 and AED 63.1 ± 2.7, 65.7 ± 0.7, 64.7 ± 2.5, 65.0 ± 4.6, respectively. The apparent protein digestibility values are currently being determined. The analysis of the whole fish and protein and phosphorus retention are below.

Whole Fish	Basal	Basal +CHOase	Low NSP	Low NSP +CHOase	p-value	PSE
Protein (%)	16.81	16.83	16.89	16.55	0.9065	0.3528
Moisture (%)	66.61	67.41	66.61	66.96	0.4466	0.3792
Lipid (%)	14.40	13.08	13.82	14.10	0.1599	0.3763
Fiber (%)	0.28	0.44	0.33	0.34	0.384	0.0601
Ash (%)	2.60	2.91	2.64	2.45	0.2056	0.1363
Phosphorus (%)	0.49	0.49	0.45	0.45	0.4982	0.0280
Protein Retention	21.44	22.29	23.37	20.97	0.2068	0.7655
Phosphorus Retention	20.05[b]	31.15[a]	33.18[a]	29.46[a]	0.0033	1.7529

2. To determine the response of Florida pompano to varying levels of phytase in a high soy diet

The water quality remained in acceptable levels for the trial. Temperature was 27.4 ± 1.5 C, salinity 25.5 ± 3.6 ppt, dissolved oxygen 5.84 ± 0.4 mg/L, pH 7.4 ± 0.7 and total ammonia nitrogen 0.03 ± 0.12.

The proximate analysis of the diets was analyzed, protein ranged from 40.7-41.6% and lipid ranged from 8.5-8.8%. The phytic acid content (g/100g) of the diets was determined, Diet 1 was 0.82, Diet 2 was 0.76, Diet 3 was 0.86 and Diet 4 was 0.72. The Phytate-P level (g/100g) was also determined, Diet 1 was 0.384, Diet 2 was 0.274, Diet 3 was 0.301 and Diet 4 was 0.306.

The trial was conducted for 9 weeks with average survival ranging from 98.3-100%. The final mean weights ranged from 64.7 to 72.5g. Fish offered the diet containing Phytase500 were the largest. FCR ranged from 1.81 to 1.96 with no significant differences.

IW=7.95g	Treatment	Final weight (g)	Wt. Gain (%)	Survival (%)	FCR
	Basal+P	68.7[ab]	764.7	98.33	1.96
	Basal-P	64.7[b]	716.1	98.33	1.95
	Phytase500	72.5[a]	808.2	100	1.81
	Phytase4000	68.4[ab]	761.0	100	1.93
	p value	0.0294	0.1365	0.5957	0.2493

PSE	0.7127	11.9671	0.5892	0.0268

The apparent digestibility coefficients (ADC) including apparent dry matter digestibility (ADMD) and apparent energy digestibility (AED) for the Basal+CaP, Phytase 500 and Phytase 4000 were ADMD 49.3 ± 2.0, 52.1 ± 4.3, 54.9 ± 3.3 and AED 67.9 ± 1.3, 64.9 ± 3.8, 66.2 ± 3.0, respectively. The apparent protein digestibility values are currently being determined. The analysis of the whole fish and protein and phosphorus retention are below.

Whole Fish	Basal+CaP	Basal	Phytase 500	Phytase 4000	p-value	PSE
Protein (%)	17.48	17.32	17.02	16.63	0.3764	0.3463
Moisture (%)	67.70	68.21	67.29	67.04	0.5373	0.5792
Lipid (%)	12.22	12.29	13.18	14.08	0.1277	0.5463
Fiber (%)	0.40	0.37	0.44	0.40	0.7971	0.0516
Ash (%)	3.30	2.66	2.88	2.64	0.3189	0.2623
Phosphorus (%)	0.48	0.46	0.48	0.44	0.3656	0.0201
Protein Retention	21.80	22.12	23.19	21.00	0.2246	0.6749
Phosphorus Retention	19.46[b]	28.11[a]	28.13[a]	26.54[a]	0.0069	1.8000

Leveraged work

The water quality remained in acceptable levels for the trial. Temperature was 27.3 ± 1.8 C, salinity 34.3 ± 3.8 ppt, dissolved oxygen 5.33 ± 0.55 mg/L, pH 7.30 ± 0.82 and total ammonia nitrogen 0.04 ± 0.06. Including 5% squid hydrolysate significantly improved growth and FCR; however the diets containing either poultry meal or a chemical attractant mix preformed similar to the all plant protein diet.

IW=22.30g	Treatment	Final weight	Weight gain (%)	Survival (%)	FCR	TGC
	Plant	89.52[b]	303.38[b]	71.11	2.13[b]	0.0868[b]
	Attractant	103.15[b]	364.92[b]	75.56	1.92[ab]	0.0982[b]
	Poultry	103.37[b]	359.03[b]	75.56	1.90[ab]	0.0977[b]
	Squid	135.00[a]	504.32[a]	82.22	1.65[a]	0.1206[a]
	p value	0.0016	0.0100	0.6288	0.0152	0.0042
	PSE	2.6018	15.5311	2.9397	0.0385	0.0022

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

In general the completed research adds to our understanding of the application of high soy feed formulations and possible nutrient limitations when marine fish are offered high soy feed formulations. Based on the present results lines of soybean meal with reduced levels of NSP have repeatedly improved the performance of the fish. However, the use of a mixture of enzymes to help digest typically undigested carbohydrates (NSP) did not result in an increase in performance. These results indicate that either the NSP or other nutrients linked to reductions in the NSP in the soybean meal improve the meal quality. These results indicate the selective breeding of soybean can influence nutritional quality of the subsequent meal and would allow for a higher inclusion rate or improved value of the product.

Another component of soybean meal is phytate which has been shown to influence the performance in other animals. Phytase is an enzyme that has been used to digest phytate in the animals stomach and has shown promise to increase performance. In early studies the objective

of the use of phytase has been to reduce phytate levels but recent research has indicated that higher levels to more completely digest phytate are beneficial. We are just now completing all of the biochemical work so the complete picture is not available. Based on current results, there are indications that phyatse did improve performance of the fish but is not clear if phosphorus bioavailability was impacted. Based on current results, this technique should be pursued as it would also allow for a reduction in phosphorus levels of the feed and possibly higher inclusion levels of soybean products or improved performance at a typical level.

Last year we evaluated a range of attractant in pompano feeds. We were able to leverage funds to continue this work as a "side project". For this work we evaluated the efficacy of several attractants in high soy low animal protein feeds. It is interesting to note that adding 5% poultry meal (additional animal protein) did not improve the high soy feed formulation indicating animal meal levels may not be the limiting factor. However, the addition of a hydrolyzed squid meal did improve performance indicating there could be benefits of this product in high soy feed formulations.

Presentations

"Improving high soy feed formulations for Florida pompano *Trachinotus carolinus* through enzyme supplementation." **Chuck Roe**, Melanie Rhodes, D. Allen Davis, Yangen Zhou, To Pham Thi Ha Van, Terry Hanson and Guillaume Salze. Aquaculture America 2015, New Orleans, LA, February 19-22, 2015.
"Improving high soy feed formulations for Florida pompano *Trachinotus carolinus* through enzyme supplementation." **Chuck Roe**, Melanie Rhodes, D. Allen Davis, Yangen Zhou, To Pham Thi Ha Van, Terry Hanson and Guillaume Salze. Alabama Fisheries Association, Inc Annual Meeting. Prattville, AL, February 11-12, 2015.
"Inclusion of amino acids and attractants in plant based diets to support Florida pompano *Trachinotus carolinus* growth." **Melanie Rhodes**, Igor Simone Tiagua Vicente, Guillaume Salze, D. Allen Davis, Terry Hanson. Aquaculture America 2015, New Orleans, LA, February 19-22, 2015.