United Soybean Board
Final Report Form – Technical Bulletin

Project # and Title	Project # : 1440-512-5261 Use of Soy-based Products in Practical Diets for Florida Pompano, *Trachinotus carolinus*. Phase III
Organization & Project Leader	University of Miami Dr. Daniel Benetti Dr. Jorge Suarez
Reporting Period	March 01, 2014- February 28, 2015

Introduction: Statement on the rationale and background for the studies

Florida pompano, *Trachinotus carolinus*, 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 species naturally require large amounts of fishmeal, which represents the primary protein source and the greatest cost component in feed formulations. Lack of adequate feeds for the growout of pompano remains a serious bottleneck preventing pompano aquaculture expansion to commercial scale.

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 using combinations of various soy protein products in order to develop cost-effective and environmentally-friendly diets.

Results of phases one and two of our research study, funded by United Soybean Board (USB), indicated that the Florida pompano has the ability to utilize soy-based products efficiently. However, the substitution of fishmeal in the diet limits components such as the sulfur amino acid, methionine, which will need to be supplemented. In order to satisfy the the dietary methionine requirement of this fish, the appropriate inclusion of methionine must be determined through experimentation.

The proposed goal of this study is to quantify the total sulfur amino acid requirement of adult

Florida pompano and determine the replacement value of cystine for methionine, ultimately optimizing the use of soy-based products in aquaculture feeds. Essentially, the main objective is 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. 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 feeds are used. We proposed to conduct innovative scientific research to address and resolve this problem. This proposal represents the third phase of a research study financed by United Soybean Board (USB) in 2012 and 2013.

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

The Florida pompano that will be used for the first objective of USB III, determining the total sulfur amino acid dietary requirement, were acquired from Troutlodge in Vero Beach, Florida as juveniles and raised to the appropriate experimental size (200 – 250 g) at the University of Miami Experimental Hatchery (Fig 1). The fish have been maintained in a 15 ton, outdoor, flow-through tank at UMEH and are being fed a high quality diet (Otohime) until the onset of the experimental trials. Biosecurity protocols have been in place to ensure the health of the fish. Additionally, the fish have undergone a prophylaxis treatment every two weeks.

The committee and the graduate student assigned to the project (Christina Belfranin), have invited **Dr. Delbert Gatlin** of Texas A&M University to her master's thesis committee. Dr. Gatlin is a renowned nutritionist in the field of aquaculture and the editor for nutrition in *Aquaculture*. He has accepted her invitation and made a trip to the University of Miami Experimental Hatchery to meet with the committee and the student to review the project protocol and add his valuable insight and contirnbution to the experimental procedure.

Six isonitrogenous and isoenergetic diets (D1, D2, D3, D4, D5 and D6) were formulated with six graded levels of methionine (0.5%,0.7%,0.90%,1.10%,1.30% and 1.50%).

All diets were manufactured at the Fish Technology Center (Bozeman, MT) by cooking extrusion. The manufacturing process was supervised by **Dr. Rick Barrows** of the USDA. All ingredients were ground to a particle size of <200 μm using an air-swept pulverizer (Model

18H, Jacobsen, Minneapolis, MN). The diets were processed using a twin-screw cooking extruder (DNDL-44, Buhler AG, Uzwil, Switzerland) with a 8-14 second exposure to 125-145^{o}C in the extruder barrel using a 5 mm die opening. Pressure at the die head varied from 32-47 bar, depending on test ingredient. Pellets were flash dried with a 4 M conveyor and cooling fan then collected and batch dried with a pulse bed drier (Buhler AG, Uzwil, Switzerland) for approximately 20 minutes at 98ºC resulting in final moisture levels less than 10% followed by cooling with forced air at ambient temperature or approximately 15 minutes. All added fish oil was top-coated on the dried and cooled feed using a vacuum coater (A.J. Mixing, Ontario, Canada). Diets were stored in plastic lined paper bags at room temperature until fed (Fig 2).
Five pompano from UMEH were randomly selected, frozen, ground, and homogenized before being lyophilized. The lyophilized pompano tissue was then sent to Texas A&M University for amino acid composition analyses.

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

In order to supplement the diets with the appropriate quantities of the various crystalline amino acids, the amino acid composition of the Florida pompano tissue was used as a reference. Five pompano from UMEH were randomly selected, frozen, grinded, and homogenized before being lyophilized. The lyophilized pompano tissue was then sent to Texas A&M University for amino acid composition analyses (Table 1).

Performed proximate composition analyses (dry matter, protein, lipid, energy, fiber, ash and amino acids) of the regular soybean meal, soy protein concentrate, fish meal and wheat flour (Table 2).

Six isonitrogenous and isoenergetic diets (D1, D2, D3, D4, D5 and D6) were formulated with six graded levels of methionine (0.5%,0.7%,0.90%,1.10%,1.30% and 1.50%). The six formulations all share equal inclusion levels of the four practical ingredients (fishmeal at 12.5%, regular soybean meal at 7.5%, soy protein concentrate at 7.5%, and wheat flour at 12%). These ingredients provide approximately 21.5% protein to the diets. The total inclusion of crystalline amino acids, based on the amino acid composition of the pompano, is approximately 19%. This will yield diets that contain approximately 40.5% crude protein, which is less than

the optimum crude protein inclusion of 48% for Florida pompano. This was done to guarantee the efficient use of dietary protein in the process of protein synthesis to attain accurate amino acid requirements. The different crystalline amino acids are at equal inclusion levels for all six diets except for Methionine and an Aspartic/Glutamic acid (50/50) mix. The inclusion of crystalline Methionine increases in approximate increments of 0.20% starting with 0.0% in Diet 1 up to 1.04% in Diet 6. As Methionine is increased, the Aspartic/Glutamic acid mix is decreased to assure isonitrogenous diets (Table 3).

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

Our Florida Pompano *Trachinotus carolinus* project focused on determining the total sulfur amino acid requirements in large fish, which consume higher amounts of food. To fulfill this objective, we had to extend the fattening period of the Pompano by eight months, until they reached an average weight of 250g. This situation caused a four-month delay in the planned chronogram. It is also important to mention that this project started with a delay of two months because the contract was signed in May 2014 and not in March 2014 as originally planned.

As previously outlined, we plan to perform a 6-month growth-performance study, analyze the data and produce a comprehensive final report. The data collection is scheduled to commence around mid-April and last until mid-October. We ask, therefore, that you allow us until December 2015 for the submission of the new, more detailed, version of the final report, which will include the total sulfur amino acid requirement of adults Florida Pompano, *Trachinotus Carolinus*.

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 product as nutritionists will be able to formulate feeds specifically for fish of a certain size range, diminishing the high operation costs associated with the feed.

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 pompano aquaculture by the industry (e.g. both Open Blue Sea Farms in Panama and Tropic Seafood in the Bahamas, two our most important industry partners, have expressed serious interest in pursusing commercial

aquaculture of this species, pending development of adequate feeds.

One of the most important expected result and anticipated product of this project is that the soybean industry will continue to invest in the future. A significant portion of the funding requested is intended to be used to fund graduate students to conduct innovative scientific research related to soybean utilization in aquafeeds and nutrition. The efforts of the US soybean industry and the partnership with our program is that it is and will continue to spark widespread interest in soybean research and nutrition and help forge a new generation of bright and talented group of qualified professionals. These professionals will doubtlessly contribute enormously to the development of novel technologies and generate great benefits to the soybean industry in the future.

Table 1. The amino acid (AA) profile (g/100g dry matter basis) in whole body of pompano (Florida pompano).

Amino Acid	Rep 1	Rep 2	Rep 3	Average
His	1.47	1.65	1.83	1.65
Tau	0.68	0.75	0.86	0.77
Ser	2.65	3.04	2.95	2.88
Arg	4.08	4.75	4.69	4.51
Gly	6.06	7.34	6.32	6.57
Asp	5.34	6.34	5.61	5.76
Glu	7.68	9.02	8.16	8.29
Thr	2.91	3.31	3.29	3.17
Ala	4.99	5.97	5.01	5.32
Pro	3.54	4.28	3.48	3.77
Lys+Tyr	6.62	7.89	7.15	7.22
Met	1.85	2.09	2.18	2.04
Val	3.47	3.96	3.77	3.73
Ile	2.97	3.39	3.31	3.23
Leu	5.05	5.74	5.63	5.47
Phe	2.70	3.08	3.50	3.10
Cys	0.15	0.17	0.18	0.16

Table 2. Proximate composition (g/100g as feed).

Proximate Components	Ingredients			
	Fishmeal	Regular Soybean Meal	Soy Protein Concentrate	Wheat Flour
Dry Matter	93.38	90.69	93.06	87.90
Crude Protein	79.58	48.23	66.42	11.19
Crude Lipid	10.01	3.41	1.00	1.76
Crude Fiber	<0.2	3.50	3.80	0.20
Ash	4.06	6.70	5.46	0.61
Energy (kJ/g)	23.15	18.17	19.19	16.23
Aminoacids				
Arginine	4.83	3.25	4.62	0.41
Histidine	1.87	1.24	1.74	0.23
Isoleucine	3.88	2.23	3.07	0.38
Leucine	7.36	3.69	5.23	0.73
Lysine	6.98	3.13	4.40	0.28
Methionine	2.53	0.71	0.96	0.17
Phenylalanine	3.71	2.44	3.40	0.54
Threonine	4.08	2.12	2.98	0.38
Tryptophan	1.01	0.70	0.97	0.13
Valine	4.45	2.31	3.17	0.47
Alanine	4.93	2.08	2.90	0.34
Aspartic acid	7.97	5.44	7.63	0.49
Cystine	0.94	0.69	0.90	0.24
Glutamic acid	12.51	8.74	12.49	3.58
Glycine	3.66	2.02	2.83	0.39
Proline	3.62	2.50	3.54	1.22
Serine	4.00	2.69	3.77	0.59
Tyrosine	3.10	1.59	2.17	0.30
Taurine	0.20	0.02	<0.01	<0.01

Table 3. Composition of experimental diets for determining methionine requirements for Florida pompano (g/100 g DM).

Ingredients	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
Fishmeal	12.50	12.50	12.50	12.50	12.50	12.50
Regular SBM	7.50	7.50	7.50	7.50	7.50	7.50
SPC	7.50	7.50	7.50	7.50	7.50	7.50
Wheat Flour	12.00	12.00	12.00	12.00	12.00	12.00
Fish Oil	13.73	13.73	13.73	13.73	13.73	13.73
CL-Choline 60%	0.23	0.23	0.23	0.23	0.23	0.23
Lecithin	1.00	1.00	1.00	1.00	1.00	1.00
Vitamin/Mineral	1.00	1.00	1.00	1.00	1.00	1.00
Alphacel	25.055	25.055	25.055	25.055	25.055	25.055
Others	0.275	0.275	0.275	0.275	0.275	0.275
Crystalline Amino Acids						
Arginine	1.49	1.49	1.49	1.49	1.49	1.49
Histidine	0.51	0.51	0.51	0.51	0.51	0.51
Isoleucine	1.03	1.03	1.03	1.03	1.03	1.03
Leucine	1.62	1.62	1.62	1.62	1.62	1.62
Lysine	2.01	2.01	2.01	2.01	2.01	2.01
Methionine	0.00	0.22	0.42	0.62	0.82	1.04
Cystine	0.00	0.00	0.00	0.00	0.00	0.00
Asp/Glu 50/50 Mix	1.04	0.82	0.62	0.42	0.22	0.00
Phenylalanine	0.90	0.90	0.90	0.90	0.90	0.90
Tyrosine	0.17	0.17	0.17	0.17	0.17	0.17
Threonine	0.98	0.98	0.98	0.98	0.98	0.98
Tryptophan	0.43	0.43	0.43	0.43	0.43	0.43
Valine	1.24	1.24	1.24	1.24	1.24	1.24
Taurine	0.46	0.46	0.46	0.46	0.46	0.46
Serine	0.66	0.66	0.66	0.66	0.66	0.66
Glycine	3.20	3.20	3.20	3.20	3.20	3.20
Alanine	2.24	2.24	2.24	2.24	2.24	2.24
Proline	1.23	1.23	1.23	1.23	1.23	1.23
Total	100.00	100.00	100.00	100.00	100.00	100.00

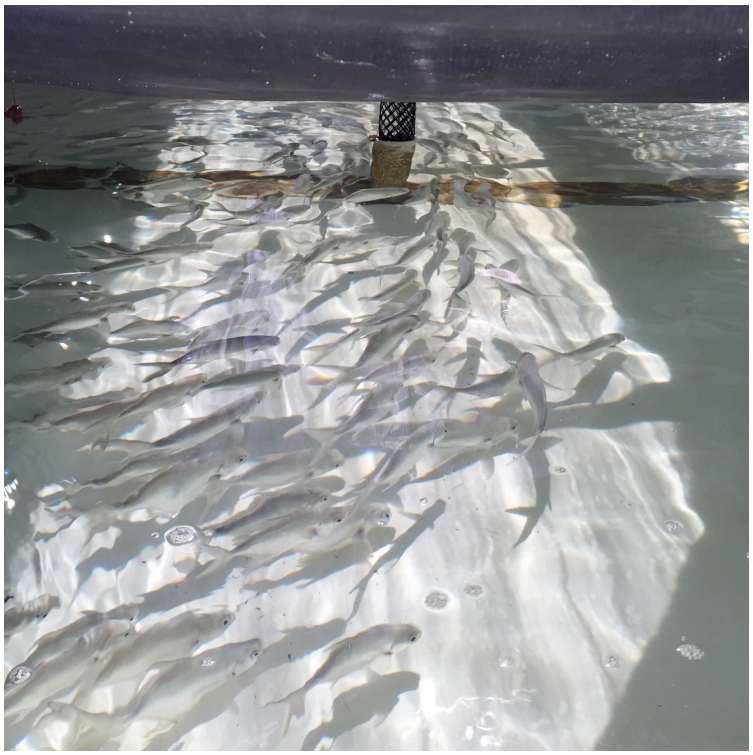

Figure 1. Florida pompano during growout at UMEH for methionine requirement experimental trials.

Figure 2. Manufacturing of pelleted diets at the Fish Technology Center (Bozeman, MT). Photo courtesy of Dr. Rick Barrows.