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Project Title:	Improving High Soy Feed Formulations Supplemented with Taurine in US Marine Fish Feeds
Organization:	Auburn University
Principal Investigator Name:	Drs. Terry Hanson and Allen Davis

Project Status - What key activities were undertaken and what were the key accomplishments during the life of this project? Please use this field to clearly and concisely report on project progress. The information included should reflect quantifiable results (expand upon the KPIs) that can be used to evaluate and measure project success. Technical reports, no longer than 4 pages, may be included in this section.

Based on the previous research with Florida pompano, *Trachinotus carolinus*, we have successfully demonstrated an increased use of soybean meal and soy protein concentrate in formulated feeds for this species. With the present information on nutrient limitation, we can replace the fish meal with other animal proteins at equivalent levels of inclusion but have been limited to a minimum of ~15% inclusion.

The objectives of this project were to:

1. Evaluate the use of PepSoyGen (Nutraferma) which is a functional soy protein manufactured using fermentation processes.
2. Identify the minimal level of inclusion of fishmeal based on current knowledge of nutrient supplements.
3. Compare squid meal and squid hydrolysate as a nutrient source and palatability enhancer in our soy based diet.

1. Evaluate the use of PepSoyGen (Nutraferma) which is a functional soy protein manufactured using fermentation processes.

Soybean meal has been considered and studied as one of the suitable protein sources to replace the use of fish meal in fish diets and demand is expected to continue to increase. Therefore, proper processing and innovation are urgently needed to overcome the problem of using soybean meal, such as palatability issue, digestibility and bioavailability of minerals in the diet. Recent innovation has been developed by incorporating *Aspergillus* spp and *Bacillus* spp in the meal known as PepSoyGen^{R} (PSG, Nutraferma Inc. South Dakota). Although, PepSoyGen may not contain enough methionine to meet the dietary requirement, it may have potential advantage as a functional ingredient which may have probiotic type effects to improve the digestibility and growth performance of aquatic animals. Therefore, the aim of this study was to examine the effect of PepSoyGen inclusion in soy based feed formulations of Florida pompano *Trachinotus carolinus*. In this experiment, we used a high soy feed which contained 15% poultry by product meal in the formulation. To demonstrate if fermented soybean is able to improve the soy based diets, increasing levels of fermented soybean (0, 50%, 75% and 100%) were used to replace the commodity soy bean meal used in the basal formulation (Table 1)

Diets were prepared by mixing pre-ground dry ingredients and menhaden fish oil in a food mixer (Hobart, Troy, OH, USA) for 15 minutes. Boiling water was then blended into the mixture to attain a consistency appropriate for pelleting. The moist mash from each diet was passed through a 3 mm die in a meat grinder, and the pellets were dried in a forced air drying oven (<50 ^{o}C) to a moisture content of less than 10%. Diets were stored at -20 ºC, and prior to use each diet were ground and sieved to an appropriate size. The diets were formulated to have similar proximate analyses (40% protein and 8% lipid). Proximate analysis of the diets was confirmed (Table 1) and amino acid profile of the diets were determined (Table 2).

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 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.

Routine system maintenance such as siphoning of solids and partial water exchanges, were conducted as needed. Water quality parameters of temperature, dissolved oxygen, pH and salinity were monitored twice daily using a multi-probe YSI ProPlus meter. Survival, final weight, thermal growth coefficient and feed conversion ratio were determined at the end of the 8-week experimental period.

Fish were fed to satiation, feed was provided four times per day and the daily intake was recorded. At the termination of each study, group weights and individual weights were taken for the fish in each tank. The percent survival per tank was calculated. The percent weight gained was calculated for each treatment as Gain = (final mean weight-initial mean weight)/initial mean weight) *100. Feed conversion ratio (FCR=dry weight of feed offered/wet weight gain) were calculated at the end of the feeding trials. 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 use of soy protein concentrate diet with poultry by product meal and each diet that was supplemented. All statistical analyses were conducted using SAS system for windows (V9.3, SAS Institute, Cary, NC).

Amino acid profile analyses were performed based on standard methods and conducted by the ESCL Missouri Laboratory (Columbia, MO). While proximate and mineral analysis, include: total Sulfur, Phosphorus, Potassium, Magnesium, Calcium, Sodium, Iron, Manganese, Copper and Zinc conducted by Midwest Laboratories (Omaha, NE). For hematocrit analysis, fresh blood was transferred to micro capillary tubes immediately after sampling, and centrifuged (LW Scientific M248 min, 14,800 ×g) for determination of the packed-cell volume (PCV). Blood smears were prepared for later analysis and were stained with a modified Wright's stain (Wescor Aerospray 7150 Slide Stainer, ELItech Biomedical Systems, Logan, UT). Serum samples collected after the end of trial were analyzed for total protein, glucose, cholesterol, bilirubin, and bile acid concentrations, as well as liver enzyme activities including alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) expressed in units per liter. All measurements were performed using an automated chemistry analyzer (Cobas C311, Roche Diagnostics, Indianapolis, IN). Total protein concentration was determined by the biuret reaction (Total Protein Gen. 2, Roche Diagnostics). Glucose concentration was directly related to the rate of NADPH formation catalyzed by the sequential action of the enzymes Hexokinase and Glucose-6-phosphate dehydrogenase in the presence of NADP (Glucose HK, Roche Diagnostics). Cholesterol concentration was directly proportional to the color intensity of a dye formed by a series of reactions catalyzed by cholesterol esterase, cholesterol oxidase and peroxidase (Cholesterol Gen. 2, Roche Diagnostics). Bilirubin was measured by the Diazo method (Total Bilirubin Special, Roche Diagnostics). Total bile acids concentration was determined from the rate of formation of thio-NADH from bile acids catalyzed by the enzyme 3-alpha hydroxysteroid dehydrogenase in the presence of excess NADH (Diazyme Total Bile Acids Assay Kit, Diazyme Laboratories, Poway, CA). Liver and intestine histology were also performed in order to investigate abnormalities due to the dietary effect. For this purposes, Histological liver and intestine samples fixed in formaldehyde were embedded in paraffin, cut at 5 µm, and stained with hematoxylin & eosin or periodic acid–Schiff (PAS) according to the standard protocols.

Table 1. Composition of plant based diets with minimum level of fish meal inclusion

Composition	Basal	50% PSG	75% PSG	100% PSG
Poultry by product meal	15.00	15.00	15.00	15.00
Soybean Meal	**47.21**	**23.54**	**11.68**	0.00
Fermented Soybean	0.00	**20.60**	**30.90**	**41.07**
Empyreal 75	6.30	6.30	6.30	6.30
Menhaden Fish Oi	**4.74**	**4.90**	**4.97**	**5.05**
Corn Starch	0.70	3.72	5.24	6.70
Whole wheat	22.00	22.00	22.00	22.00
Trace Mineral premix	0.25	0.25	0.25	0.25
ASA Vitamin premix	0.50	0.50	0.50	0.50
Choline chloride	0.20	0.20	0.20	0.20
Stay C 35%	0.10	0.10	0.10	0.10
CaP-dibasic	2.00	1.90	1.87	1.85
Lecithin	0.50	0.50	0.50	0.50
Taurine	**0.50**	**0.49**	**0.49**	**0.48**
Proximate analyses (as is)				
Crude Protein	42.35	41.60	41.69	41.89
Moisture	4.75	6.09	6.43	6.51
Crude Fat	9.96	9.32	9.59	9.58
Crude Fiber	2.62	3.23	3.01	2.79
Ash	6.74	6.57	6.49	6.40

Table 2. Amino Acid analysis of practical diets formulated at Auburn University analyzed at University of Missouri Agricultural Experiment Station Chemical Laboratories (Columbia, MO, USA).

Component	Basal	50% PSG	75% PSG	100% PSG
Taurine	0.73	0.69	0.67	0.66
Hydroxyproline	0.31	0.35	0.30	0.38
Aspartic Acid	3.71	3.71	3.72	3.80
Threonine	1.47	1.48	1.49	1.51
Serine	1.78	1.83	1.80	1.88
Glutamic Acid	7.53	7.53	7.47	7.64
Proline	2.47	2.37	2.38	2.39
Lanthionine	0.03	0.04	0.05	0.04
Glycine	2.14	2.17	2.13	2.19
Alanine	2.15	2.17	2.17	2.20

Cysteine	0.59	0.57	0.57	0.57
Valine	1.97	1.96	1.98	2.02
Methionine	0.74	0.74	0.75	0.72
Isoleucine	1.78	1.77	1.80	1.81
Leucine	3.51	3.49	3.53	3.56
Tyrosine	1.52	1.48	1.28	1.53
Phenylalanine	2.01	1.99	2.00	2.02
Hydroxylysine	0.09	0.08	0.08	0.07
Ornithine	0.00	0.01	0.01	0.00
Lysine	2.20	2.09	2.08	2.13
Histidine	0.95	0.94	0.94	0.95
Arginine	2.57	2.51	2.41	2.52
Tryptophan	0.47	0.46	0.50	0.45

2. Identify the minimal level of inclusion of fishmeal based on current knowledge of nutrient supplement

Fish meal has been used as the main protein source in aquaculture diets for several reasons; include the relatively high protein content, appropriate amino acid profile and apparent digestibility as well as highly palatable to most species of farmed fish. However, due to the sustainability and cost issues, the role of fish meal in fish diets is likely to shift over the next decade from the primary source of dietary protein to that of a minimal source of dietary protein. In addition, reducing the use of fish meal in feed formulation will also able improve the feed efficiency as the prices of fish meal have risen in the past three decades and are likely to increase further with continued demand. In previous research with Florida pompano, *Trachinotus carolinus*, our lab has successfully demonstrated an increased use of soybean meal and soy protein concentrate in formulated feeds for this species. Increases in the inclusion level of soy products has been primarily driven by identifying limiting nutrients to supplement to the diets as fishmeal is removed. With the present information on nutrient limitations we can replace the fish meal with other animal proteins at equivalent levels of inclusion but have been limited to a minimum of 15% inclusion. Clearly, taurine is the first limiting amino acid, whereas lysine and methionine can be limiting in some formulations. Based on previous research we have made good progress towards reducing the animal protein content and possibly eliminate it producing an all plant based protein diet. However, as the level of fish meal is reduced we find something is limiting performance of the fish when animal protein is reduced. To demonstrate if the replacement of fish meal to the minimum level affects the growth performance of Florida pompano, two trials were preformed Trial 1, formulation and proximate composition of diets containing 15%, 12%, 9%, and 6% level of fish meal (Table 3) with amino acid composition in Table 4 and Trial 2 diets containing 12%, 6%, 3% and 0% level of fish meal (Table 5) and amino acid composition in Table 6. The methods for making diets, the fish origin and trial conditions were the same as in objective 1.

Table 3. Composition of plant based diets with minimum level of fish meal inclusion

	15% FM	12% FM	9% FM	6% FM
Menhaden Fishmeal	**15.00**	**12.00**	**9.00**	**6.00**
Soybean meal	**46.60**	**46.60**	**46.60**	**46.60**
Soy Protein Concentrate	0	**3.08**	**6.14**	**9.21**
Empyreal 75l	8.00	8.00	8.00	8.00
Menhaden Fish Oil	**5.00**	**5.29**	**5.58**	**5.87**
Corn Starch	3.85	3.14	2.45	1.75
Whole wheat	18.00	18.00	18.00	18.00
ASA Trace Mineral premix	0.25	0.25	0.25	0.25
ASA Vitamin premix	0.50	0.50	0.50	0.50
Choline chloride	0.20	0.20	0.20	0.20
Stay C 35%	0.10	0.10	0.10	0.10
CaP-dibasic	1.50	1.80	2.10	2.40
Lecithin	0.50	0.50	0.50	0.50
Methionine		0.02	0.04	0.06
Taurine	**0.50**	**0.52**	**0.54**	**0.56**
Proximate analyses (as is)				
Phosphorus	1.32	1.20	1.23	1.25
Crude Protein	39.82	38.50	39.50	41.32
Moisture	6.41	9.61	8.09	8.17
Crude Fat	9.69	8.24	8.48	8.29
Crude Fiber	2.89	2.55	2.65	2.88
Ash	7.86	7.56	7.13	6.93

Table 4. Amino Acid analysis of practical diets formulated at Auburn University analyzed at University of Missouri Agricultural Experiment Station Chemical Laboratories (Columbia, MO, USA).

Component	**15% FM**	**12% FM**	**9% FM**	**6% FM**
Taurine	0.73	0.61	0.70	0.65
Hydroxyproline	0.22	0.18	0.38	0.12
Aspartic Acid	3.61	3.68	3.71	3.98
Threonine	1.41	1.42	1.41	1.48
Serine	1.57	1.55	1.60	1.70
Glutamic Acid	7.14	7.06	7.31	7.68
Proline	2.43	2.25	2.42	2.48
Lanthionine	0.00	0.00	0.00	0.00
Glycine	1.92	1.87	1.86	1.84
Alanine	2.17	2.09	2.06	2.03
Cysteine	0.51	0.52	0.54	0.57
Valine	1.93	1.92	1.94	2.01
Methionine	0.73	0.73	0.75	0.74
Isoleucine	1.68	1.68	1.72	1.81

Leucine	3.44	3.36	3.42	3.51
Tyrosine	1.23	1.02	1.28	1.36
Phenylalanine	1.90	1.91	1.94	2.04
Hydroxylysine	0.08	0.07	0.07	0.05
Ornithine	0.03	0.05	0.03	0.03
Lysine	2.13	2.11	2.09	2.20
Histidine	0.97	0.95	0.97	1.01
Arginine	2.32	2.26	2.41	2.56
Tryptophan	0.51	0.50	0.52	0.56

Table 5. Composition of plant based diets with partial and total replacement of fish meal

	12% FM	6% FM	3% FM	0% FM
Menhaden Fishmeal	**12.00**	**6.00**	**3.00**	**0.00**
Soybean meal	**46.60**	**46.60**	**46.60**	**46.60**
Soy Protein Concentrate	2.89	8.98	12.01	15.05
Empyreal 75	8.00	8.00	8.00	8.00
Menhaden Fish Oil	5.02	5.47	5.70	5.93
Corn Starch	**3.60**	**2.38**	**1.73**	**1.13**
Whole wheat	18.00	18.00	18.00	18.00
ASA Trace Mineral premix	0.25	0.25	0.25	0.25
ASA Vitamin premix	0.50	0.50	0.50	0.50
Choline chloride	0.20	0.20	0.20	0.20
Stay C 35%	0.10	0.10	0.10	0.10
CaP-dibasic	1.80	2.40	2.75	3.05
Lecithin	0.50	0.50	0.50	0.50
Methionine	0.02	0.06	0.02	0.06
Taurine	0.52	0.56	0.58	0.59
Proximate analyses (as is)				
Crude Protein	41.72	41.88	43.16	42.50
Moisture	7.59	7.78	6.07	6.39
Crude Fat	9.63	8.08	8.73	8.74
Crude Fiber	2.82	2.82	2.97	3.18
Ash	7.18	6.73	6.70	6.50
Phosphorus	1.10	1.08	1.08	1.09

Table 6. Amino Acid analysis of practical diets formulated at Auburn University analyzed at University of Missouri Agricultural Experiment Station Chemical Laboratories (Columbia, MO, USA).

	12% FM	6% FM	3% FM	0% FM
Taurine	0.71	0.71	0.73	0.71
Hydroxyproline	0.15	0.19	0.15	0.08
Aspartic Acid	3.75	3.83	4.02	4.02
Threonine	1.49	1.47	1.52	1.52
Serine	1.71	1.75	1.83	1.84
Glutamic Acid	7.62	7.74	8.03	8.09
Proline	2.57	2.53	2.54	2.55
Lanthionine	0.00	0.00	0.00	0.00
Glycine	1.91	1.73	1.74	1.67
Alanine	2.21	2.08	2.08	2.07
Cysteine	0.52	0.55	0.57	0.57
Valine	2.05	2.03	2.11	2.03
Methionine	0.71	0.72	0.73	0.71
Isoleucine	1.81	1.82	1.88	1.88
Leucine	3.77	3.72	3.80	3.85
Tyrosine	1.52	1.46	1.53	1.56
Phenylalanine	2.12	2.14	2.22	2.24
Hydroxylysine	0.11	0.09	0.08	0.08
Ornithine	0.03	0.03	0.02	0.02
Lysine	2.24	2.19	2.27	2.22
Histidine	1.00	1.00	1.03	1.02
Arginine	2.47	2.49	2.62	2.60
Tryptophan	0.51	0.52	0.56	0.53

3. Novel soy protein concentrate and soy products are the leading candidates for alternate protein ingredients. Based on the previous research with Florida pompano, *Trachinotus carolinus*, we have successfully demonstrated an increased use of soybean meal and soy protein concentrate in formulated feeds for this species. With the present information on nutrient limitation, we can replace the fish meal with other animal proteins at equivalent levels of inclusion but have been limited to a minimum of 15% inclusion. Therefore, in this experiment, we use 15% of poultry by product meal as the control and novel soy protein concentrate was used to replace poultry by product meal (PBM, 15% diet) on an iso-nitrogenous basis. To demonstrate if there are palatability or amino acids profile issue, a series of diets were formulated to contain with graded levels of squid meal or squid hydrolysates (1%, 2% and 4%) replacing the novel soy protein concentrate (NutriVance^{TM}) without the use of animal protein, proximate analysis of the diets was confirmed (Table 7) and amino acid profile of the diets were determined (Table 8).

Table 7. Composition of plant based diets with novel soy protein concentrate and squid products.

	PBM	NV	1%SH-NV	2%SH-NV	4%SH-NV	1%SM-NV	2%SM-NV	4%SM-NV

Poultry by product meal[1]	15.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Soybean Meal[2]	47.21	47.21	47.21	47.21	47.21	47.21	47.21	47.21
Soy protein concentrate[3]	0.00	**14.80**	**13.65**	**12.51**	**10.22**	**13.68**	**12.56**	**10.32**
Squid hydrolysates [4]	0.00	0.00	**1.00**	**2.00**	**4.00**	0.00	0.00	0.00
Squid meal[5]	0.00	0.00	0.00	0.00	0.00	**1.00**	**2.00**	**4.00**
Corn protein concentrate[6]	6.30	8.00	8.00	8.00	8.00	8.00	8.00	8.00
Menhaden Fish Oil[7]	4.74	6.37	6.35	6.33	6.29	6.33	6.30	6.23
Corn Starch	0.70	0.47	0.66	0.84	1.21	0.69	0.83	1.19
Whole wheat	22.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00
ASA Trace Mineral premix[9]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
ASA Vitamin premix[10]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Choline chloride[8]	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Stay C 35%[11]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
CaP-dibasic [9]	2.00	3.10	3.08	3.06	3.02	3.04	3.05	3.00
Lecithin [9]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Taurine[8]	**0.50**	**0.50**	**0.50**	**0.50**	**0.50**	**0.50**	**0.50**	**0.50**
Lysine[8]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Proximate analyses (as is)								
Crude Protein (%)	42.60	41.49	41.15	39.94	42.23	41.76	42.63	40.81
Crude Fat (%)	7.29	8.36	9.31	11.02	7.66	8.05	5.91	10.87
Crude Fiber (%)	9.56	8.28	7.95	8.53	8.94	9.08	10.29	8.04
Moisture (%)	2.75	3.24	2.91	2.88	3.08	2.98	3.17	3.13
Ash (%)	6.49	6.44	6.37	6.16	6.39	6.29	6.46	6.22

Table 8. Amino Acid analysis of practical diets formulated at Auburn University analyzed at University of Missouri Agricultural Experiment Station Chemical Laboratories (Columbia, MO, USA).

Component	PBM	NV	1%SH-NV	2%SH-NV	4%SH-NV	1%SM-NV	2%SM-NV	4%SM-NV
Taurine	0.65	0.59	0.61	0.63	0.75	0.59	0.62	0.61
Hydroxyproline	0.34	0.10	0.05	0.05	0.01	0.05	0.04	0.08
Aspartic Acid	3.91	4.04	3.87	3.96	4.07	4.02	4.19	3.96
Threonine	1.52	1.46	1.42	1.45	1.52	1.48	1.55	1.47
Serine	1.71	1.69	1.63	1.67	1.71	1.74	1.79	1.67
Glutamic Acid	7.72	7.90	7.64	7.72	7.93	8.02	8.20	7.63
Proline	2.61	2.49	2.43	2.48	2.57	2.61	2.61	2.40
Lanthionine	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Glycine	2.19	1.71	1.69	1.66	1.78	1.72	1.82	1.74
Alanine	2.18	2.00	1.97	1.95	2.09	2.09	2.15	2.02
Cysteine	0.59	0.59	0.57	0.58	0.61	0.58	0.61	0.56
Valine	2.08	2.04	1.97	2.01	2.07	2.05	2.14	2.00
Methionine	0.71	0.65	0.64	0.66	0.71	0.66	0.71	0.67
Isoleucine	1.82	1.85	1.80	1.84	1.89	1.86	1.93	1.81
Leucine	3.57	3.65	3.56	3.59	3.75	3.81	3.89	3.59

Tyrosine	1.36	1.34	1.35	1.38	1.40	1.44	1.46	1.17
Phenylalanine	2.05	2.12	2.04	2.07	2.12	2.15	2.20	2.07
Hydroxylysine	0.08	0.04	0.05	0.05	0.06	0.04	0.05	0.05
Ornithine	0.03	0.03	0.03	0.03	0.06	0.03	0.03	0.05
Lysine	2.22	2.12	2.04	2.08	2.16	2.08	2.21	2.11
Histidine	1.00	1.02	0.98	0.99	1.01	1.01	1.05	0.99
Arginine	2.61	2.53	2.47	2.52	2.55	2.56	2.66	2.48
Tryptophan	0.58	0.58	0.55	0.55	0.58	0.56	0.56	0.51

1. The water quality remained in acceptable levels for the trial. The parameters, temperature was 28.6 ± 1.6 C, salinity 31.2 ± 3.9 ppt, dissolved oxygen 5.9 ± 0.5 mg/L, pH 8.1 ± 0.4, total ammonia nitrogen 0.13 ± 0.24 mg/L, nitrite 18.3 ± 5.6 mg/L, and nitrate 7.1 ± 5.9 mg/L.

At the end of the growth trial, final weight, percentage weight gain, thermal growth coefficient, feed conversion ratio, and feed intake were not affected by the dietary treatment (Table 9). Based on the results of present study, fermented soy can be used in the soy based feed formulations of juvenile Florida pompano replacing up to 100% of commercial soy supplemented with 15% poultry by product meal without compromising the growth performance, blood profile and serum biochemistry of fish. Interestingly, significantly less fiber was observed with 100% replacement of commercial soy.

Table 9. Growth performance of juvenile pompano (initial weight 13.05 g) after 8 weeks in response to diets with minimal inclusion of fish meal.

	Final Weight (g)	Weight Gain (%)	FCR	TGC	Survival (%)	Feed Intake (g/fish)
Basal	72.44	324.81	1.72	0.1016	93.3	94.99
50% PSG	68.25	301.68	1.78	0.0960	78.3	89.75
75% PSG	73.48	335.18	1.54	0.1025	80.0	85.78
100% PSG	69.10	309.95	1.58	0.0979	73.3	82.33
P-value	0.7531	0.7857	0.3119	0.7788	0.0027	0.0791
PSE	3.98	26.43	0.09	0.005	2.5	3.002

2. The water quality remained in acceptable levels for both trials. For the first trial parameters, temperature was 27.1 ± 2.0 C, salinity 33.7 ± 2.6 ppt, dissolved oxygen 5.5 ± 0.6 mg/L, pH 7.8 ± 0.3, total ammonia nitrogen 0.13 ± 0.24 mg/L, nitrite 19.9 ± 7.5 mg/L, and nitrate 57.7 ± 48.5 mg/L. For the second trial parameters, temperature was 28.7 ± 1.2 C, salinity 33.0 ± 2.6 ppt, dissolved oxygen 5.5 ± 0.4 mg/L, pH 7.8 ± 0.2, total ammonia nitrogen 0.05 ± 0.06 mg/L, nitrite 21.9 ± 4.1 mg/L, and nitrate 74.8 ± 29.8 mg/L.

At the end of the first 8 week growth trial, final weight, percentage weight gain, thermal growth coefficient and feed intake were affected by the dietary treatment, but no significant differences in food conversion ratio and survival rate (Table 10). Administration of 15% fish meal provided the higher final weight compared to other treatments

Table 10. Growth performance of juvenile pompano (mean initial weight 13.05 g) after 8 weeks in response to diets with minimal inclusion of fish meal (Basal: 15% fish meal; 12%FM: 12% fish meal; 9% FM: 9% fish meal; 6%: 6% fish meal; PWG: Percentage weigh gain; TGC: Thermal growth coefficient; FI: feed intake, and SR: survival rate).

	Final Weight (g)	Weight Gain (%)	FCR	TGC	Survival (%)	Feed Intake (g/fish)
15% FM	70.34a	432.74a	1.69	0.1183a	98.3	96.39
12% FM	70.73a	444.14a	1.68	0.1188a	100	97.07
9% FM	66.33ab	411.98ab	1.77	0.1129ab	91.7	94.51
6% FM	62.04b	376.45b	1.86	0.1069b	95	90.74
P-value	0.0177	0.0202	0.0445	0.0208	0.2272	0.1947
PSE	1.636	12.256	0.041	0.002	2.764	2.013

At the end of the second growth trial, final weight, percentage weight gain, thermal growth coefficient and feed intake were affected by the dietary treatment, but no significant differences in food conversion ratio and survival rate (Table 11). Administration of 15% fish meal provided the higher final weight compared to other treatments.

Table 11. Growth performance of juvenile pompano (mean initial weight 18.45 g) after 8 weeks in response to diets with minimal inclusion of fish meal (12%FM: 6%: 6% fish meal; 3%: 3% fish meal, 0%FM: 0% fish meal; PWG: Percentage weigh gain; TGC: Thermal growth coefficient; FI: feed intake, and SR: survival rate).

	Biomass (g/tank)	Final Weight (g)	Weight Gain (%)	FCR	TGC	Survival (%)	Feed Intake (g/fish)
12% FM	1970.5	100.2a	442.4	1.70	0.1230	98.3	138.5a
6% FM	1858.3	92.9ab	405.6	1.78	0.1163	100	132.8b
3% FM	1837.5	91.9ab	393.2	1.81	0.1145	100	132.6b
0% FM	1733.2	88.1b	382.9	1.83	0.1118	98.3	128.1b
P-value	0.0518	0.0260	0.2056	0.3644	0.1092	0.5957	0.0014
PSE	48.618	2.2011	18.767	0.0516	0.0054	1.1785	1.1389

3. The water quality remained in acceptable levels for the trials. The parameters, temperature was 26.6 ± 2.0 C, salinity 30.1 ± 2.8 ppt, dissolved oxygen 5.9 ± 0.6 mg/L, pH 8.0 ± 0.2, total ammonia nitrogen 0.16 ± 0.26 mg/L, nitrite 19.0 ± 10.8 mg/L, and nitrate 31.3 ± 23.1 mg/L.

Table 12. Growth performance and contrast values of juvenile pompano (initial weight 7.68 g) after 8 weeks in response to diets supplemented with novel soy concentrate and squid products. PBM: 15% Poultry Based Meal; SBM-NV: Soy Based meal-Nutrivance; 1%SH-NV: 1 % Squid hydrolysates+ Nutrivance; 2%SH-NV: 2 % Squid hydrolysates+Nutrivance; 4%SH-NV: 4 % Squid hydrolysates+ Nutrivance; 1%SM-NV: 1 % Squid meal+ Nutrivance; 2%SM-NV: 2 % Squid meal+ Nutrivance, and 4%SH-NV: 4 % Squid meal+ Nutrivance.

	Biomass (g)	Final Weight (g)	Weight Gain (%)	FCR	TGC	Survival Rate (%)	Feed Intake (g/fish)
PBM	702.3a	38.60a	411.3a	1.42b	0.0903a	91.25	43.99a
SBM-NV	405.7bc	28.17bcd	267.6bc	1.69ab	0.0677bc	73.75	32.54bc
1%SH-NV	408.2bc	23.65cd	202.8c	2.09ab	0.0562c	85.00	32.05bc
2%SH-NV	515.2bc	32.84abc	331.1ab	1.48b	0.0784ab	78.75	36.54bc
4%SH-NV	541.9ab	35.17ab	349.3ab	1.44b	0.0822ab	77.50	38.93b
1%SM-NV	365.3c	21.58d	186.2c	2.29a	0.0522c	85.00	31.32c
2%SM-NV	431.2bc	27.25bcd	257.3bc	1.69ab	0.0660bc	80.00	32.11bc
4%SM-NV	512.3bc	30.16abcd	287.0bc	1.67ab	0.0717abc	86.25	36.09bc
P-value	<0.0001	0.0003	0.0001	0.0066	0.0001	0.3956	0.0001
PSE	28.120	1.8458	21.129	0.1220	0.0037	4.0686	1.2579

Did this project meet the intended Key Performance Indicators (KPIs)? List each KPI and describe progress made (or not made) toward addressing it, including metrics where appropriate.

1. The project was successfully completed.
2. The data will be used as part of a Doctoral of Science Dissertation and we will also develop both industry based publications and scientific reviewed publications describing the results.
3. The results and continued work on plant based feed formulations continue the support of high inclusion of soy products in aquaculture diets with the following ingredient replacement strategies:
   - Fermented soy can be used in the soy based feed formulations of juvenile Florida pompano replacing up to 100% of commercial soy supplemented with 15% poultry by-product meal without compromising the growth performance, blood profile and serum biochemistry of fish.
   - Fish meal replacement trials demonstrated no significant difference in the FCR, survival and percentage weight gain between fish fed the diets in which the minimum level of fish meal (15%) was substituted by different levels of soy protein concentrates, whereas the final weight and feed intake decrease with increasing level of fish meal substitution.
   - The present findings showed that fish maintained on the diet containing 15% PBM were larger and had the lowest FCR. Similar performance was obtained in fish maintained on the diet containing 4% squid hydrolysates. The results demonstrate that diets containing 100% plant based protein sources have a limitation and low levels of animal based proteins are required to improve the growth performance of Florida pompano juvenile.

Expected Outputs/Deliverables - List each deliverable identified in the project, indicate whether or not it was supplied and if not supplied, please provide an explanation as to why.

1. Publications are being developed and are expected to be published in the near future.
2. Three abstracts were submitted and will be presented at Aquaculture America 2017 in San Antonio, TX between Feb. 19-22, 2017.

- Assessing the use of fermented soy protein in soy based feed formulations on the hematological, histological, growth, serum and nutritional value of Florida pompano *Trachinotus carolinus*
- Identifying the minimal level of fishmeal inclusion in high soy feed formulations for Florida pompano *Trachinotus carolinus*
- Towards the better use of soy based diet for Florida pompano *Trachinotus carolinus*: Use of novel soy protein concentrate and squid products.

Describe any unforeseen events or circumstances that may have affected project timeline, costs, or deliverables (if applicable.)

None

What, if any, follow-up steps are required to capture benefits for all US soybean farmers? Describe in a few sentences how the results of this project will be or should be used.

The correct supplementation of soy protein concentrates and fermented soy products in soy based diet formulation for Florida pompano is needed to reduce the use of fish meal and feed cost formulation. At the end of the growth trials, results show reduced growth performance when 100% of animal proteins are replaced with plant based proteins. However, animal proteins such as poultry by-product meal and/or squid hydrolysates, which are less expensive than fish meal can be included at low levels without compromising the growth performance, blood profile and serum biochemistry of fish.

List any relevant performance metrics not captured in KPI's.

None