United Soybean Board Domestic Programs
Report Form

Project # and Title	Project # 1440-512-5261 Use of soy-based products in practical diets for white seabass (*Atractoscion nobilis*), California yellowtail (*Seriola lalandi*) and striped bass (*Morone saxatilis*)
Reporting Period	3/1/14-2/28/15

Introduction

The overall objective of this project is to build demand for U.S. soy in aquaculture markets by developing soy based feeds for White seabass (WSB; *Atractoscion nobilis*) and California yellowtail (YT; *Seriola lalandi*) and striped Bass (SB; *Morone saxatilis*).

WSB and YT are highly valued commercial and sport fish in southern California and are considered excellent food fish. SB is a highly valued sport fish along the east and west coasts of the United States and is considered an excellent food fish. WSB commonly occur from northern Baja California, Mexico to Point Conception, California, USA. WSB are currently cultured by Hubbs- SeaWorld Research Institute (HSWRI) for stock enhancement. The hatchery in Carlsbad, California is capable of producing an excess of fingerlings required for the stock enhancement program and there is great potential and interest in the commercial culture of the species in offshore net cages. Within the existing stock enhancement project, WSB are cultured to an average size of 20-25 cm using a commercially available diet. YT are in the family carangidae or jacks – a group that typically requires high levels of high quality fish-based protein in the diet. HSWRI has cultured YT to market size in offshore cages in northern Baja California, Mexico. Commercial culture of hybrid SB (*Morone saxatilis* x *Morone chrysops*) is widespread throughout the U.S. However, aquaculture of pure SB is limited. HSWRI has cultured SB on an experimental scale, worked with cage farmers in Baja Mexico and has test-marketed seawater-reared SB in various U.S. markets with very positive feedback.

Great potential exists to expand commercial culture of all three species in both northern Baja California, Mexico and Southern California. The use of alternate ingredients such as soy protein and lipid could greatly improve profitability, while simultaneously addressing issues associated with the long term sustainability of fish meal and fish oil resources. A good opportunity exists to demonstrate the effectiveness of diets based on soy protein in the rapidly developing offshore aquaculture industry in this region.

Research funded by RFP Numbers SB8461 & SB 9463 conducted in 2008 and 2009 consisted of a series of growth trials with WSB and YT. Initial trials were designed to develop a basic practical diet that could then be utilized to identify upper limits for the inclusion of soy products and minimal levels of fish meal.

Project Status

Using the initial formulations we identified practical levels for lipid and dietary protein. This was then followed by studies designed to evaluate the replacement of fish meal with soy products and identify limiting nutrients. Hence, we have been able to develop a practical research diet formulated to contain 40% protein and 10% lipid for WSB and 48% protein and 14% lipid for YT. Using the developed diet, we have identified that we can reduce fish meal from 55% of the diet to around 10-20% of the diet for both species using soy as the replacement protein. We identified taurine as a limiting amino acid and methionine as potentially limiting for both species.

Research funded by RFP Number SB0463 in 2010 was designed to determine the actual requirement for taurine, determine if methionine is limiting and if so determine the actual requirement for both WSB and YT. Two WSB trials were run testing taurine levels from 0-1.6% of the diet. Combining the data sets, the dietary requirement was estimated at 0.99%. A YT trial testing taurine inclusion from 0.32-1.50% showed no differences between treatments with regards to performance indicating that a taurine inclusion rate of 0.32% is adequate. Moreover, a 2009 trial showed greatly reduced growth and feed efficiency when feeding 0.20% taurine, indicating that the taurine requirement lies between 0.20 and 0.32% of the diet. Finally, a YT trial testing methionine inclusion from 0.0-0.24% showed no significant difference in performance between treatments indicating that in these feeds methionine was not limiting.

Research funded by RFP Number SB1463 in 2011 included a long term (10 month) grow-out trial with both WSB and YT. In each of these trials a soy-based diet performed equal to or better than commercial diets. Laboratory trials included a trial that showed WSB require a methionine supplementation of at least 0.2% in soy based diets (12% fish meal). We ran a trial showing that attractants did not improve palatability in fish meal free diets for YT. Previous research has shown a greater level of fish oil-sparing is possible with saturated fatty acid-rich, hydrogenated soy oil (SFA) than with C18 polyunsaturated fatty acid-rich, standard soy oil (STD). To confirm this, and to assess whether dietary supplementation with DHA (22:6n-3) would influence the results, we assessed the performance of juvenile WSB fed diets containing fish oil, or SFA or STD with or without the addition of an algal DHA supplement. The SFA-based feeds yielded equivalent growth performance regardless of DHA supplementation; growth performance was impaired among fish fed the STD, but the addition of DHA corrected this effect. In 2011 HSWRI also started utilizing the same methodology used on WSB and YT in to develop practical soy-based diets for SB. In the fall of 2011 we ran two trials with SB. The first trial tested graded levels of soy protein replacing fish meal from 40% to 0% and the second tested various combinations of supplements at 0% fish meal. Palatability problems in diets below 20% fish meal resulted in the early termination of these trials.

Research funded by RFP Number SB2463 in 2012 included the initiation of a commercial scale grow out trial with WSB testing a high soy diet developed through this research vs. a high fish meal commercial diet. We ran a trial showing that, as with YT, the use of attractants does not improve palatability in diets for WSB. We ran a trial with WSB to further evaluate the replacement of fish oil with soy oil supplemented with long chain-polyunsaturated fatty acids (LC-PUFA) and to confirm previous results. This trial showed that SFA soy oil performed similarly to a fish oil control with regards to growth and fillet fatty acid profile and that SFA soil oil-based diet supplemented with DHA increased fillet enrichment over a fish oil-based diet. We also ran a trial to evaluate the importance of LC-PUFA for YT which showed the essentiality of DHA and ARA in soy oil-based feeds. We also ran a trial to further evaluate the protein requirement of YT showing that growth plateaued at 44% protein. With SB we tested graded levels of either soybean meal or soy protein concentrate replacing fish meal from 40% to 20% and the second tested an attractant and taurine individually and in combination in a 30% fish meal diet. We saw no palatability problems with any of these diets nor did we find any significant differences between any of the treatments. Furthermore we ran the diets from the two trials run in 2011 (40-0% fish meal) on the same fish to test whether or not larger fish would find lower fish meal diets more palatable and found the same results. The SB did not find diets below 20% fish meal palatable.

Research funded by RFP 1340-512-5216 in 2013 included trials to refine dietary protein requirement for WSB (40%) and lipid requirement for YT (12%) and trials indicating that YT and WSB can utilize carbohydrates as energy. We also showed that soybean oil is an effective replacement for fish oil in diets for YT and showed that fermented soybean meal can be used to effectively replace a portion of fish meal and soybean meal in diets for WSB and YT. A commercial-scale grow-out trial showed WSB performed similarly on a soy-based vs. a fish meal-based diet over 15 months. SB continued to be challenging with several trials completed. Results suggested that strain and cohort differences can affect feeding response and performance.

In 2014 we planned to optimize the carbohydrate component in high soy diets for WSB and determine the taurine requirement of YT. We also planned to further assess the value of hydrogenated soybean meal in diets for YT as well as fermented soybean meal and its associated microbes in diets for WSB and YT. We continued grow out trials with a high soy feed for WSB. We planned to compare feeding response and growth performance of SB from outside of CA on diets similar to those that proved problematic for CA strain SB. The results of this work is presented below, including our research on the use of fine mesh netting for nursery production of WSB.

Objective 1 – Laboratory Trials to evaluate the utilization of various carbohydrate sources as energy in reduced lipid high soy feed formulations for WSB and YT (HSWRI/Auburn).

In an 8 week trial, WSB were fed a series of five low FM diets with various carbohydrate sources as well as a series of four diets with the addition of enzymes and soy fiber. WSB performed best on a diet (Starch + fiber) with soy fiber (Table 2). Fish fed diets with wheat and rice grew slower than other treatments. Poorest growth was observed in WSB fed a diet with sorghum. The addition of CHOase and phytase to the diet with wheat did not improve performance over the basal diet. There was a weak correlation between growth rates and proportion of gelatinized starch in the diet. This indicates that the growth response is not just due to different carbohydrate sources.

Table 1. Feed formulations and proximate composition of diets for WSB for Objective 1.

Ingredient g/100g as is	Starch	Wheat	Corn	Rice	Sorghum	Wheat + CHOase	Wheat + Phy	Wheat + CHOase + Phy	Starch + fiber
Menhaden Fishmeal-5303111409	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Soy concentrate - soycomil	37.20	32.90	34.93	35.04	35.06	32.90	32.90	32.90	37.20
Empyreal 75 - cargil	12.40	10.97	11.64	11.68	11.69	10.97	10.97	10.97	12.40
Menhaden Fish Oil	4.28	3.89	3.33	3.57	3.42	3.89	3.89	3.89	4.28
Corn Starch	30.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	30.00
Whole wheat	0.00	25.00	0.00	0.00	0.00	25.00	25.00	25.00	0.00
Whole corn	0.00	0.00	25.00	0.00	0.00	0.00	0.00	0.00	0.00
Whole rice	0.00	0.00	0.00	25.00	0.00	0.00	0.00	0.00	0.00
Sorghum	0.00	0.00	0.00	0.00	25.00	0.00	0.00	0.00	0.00
Trace Mineral premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
ASA_Vitamin premix w/o choline	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Choline chloride	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Stay C 35%	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
CaP-dibasic	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Lecithin (soy commercial)	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Taurine	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Quantom blue phytase	0.00	0.00	0.00	0.00	0.00	0.00	0.014	0.014	0.00
CHOase-Rovabio Excel LC	0.00	0.00	0.00	0.00	0.00	0.04	0.00	0.04	0.00
Soy Fiber	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.50
Cellufill	1.97	8.09	5.96	5.56	5.67	8.05	8.08	8.04	0.47
Total	100.0	100.00	100.0	100.00	100.00	100.00	100.00	100.00	100.00
Proximate composition					g/100g as is				
Moisture	7.65	7.19	7.79	6.83	8.05	8.06	7.87	7.69	8.53
Dry matter	92.35	92.81	92.21	93.17	91.95	91.94	92.13	92.31	91.47
Protein	42.70	42.80	42.00	42.80	43.00	42.30	41.90	42.80	42.70
Fat	6.55	6.72	6.48	6.44	6.38	6.98	6.60	6.91	7.02
Fiber	6.00	5.10	4.70	4.70	4.70	4.80	5.00	6.70	4.50
Ash	4.99	5.10	5.91	5.93	6.04	5.78	6.01	6.06	5.78
Starch (% gelatinized)	44.10	67.35	41.84	49.77	39.89	69.01	64.35	67.35	48.26
Starch (total)	24.94	18.07	20.17	21.84	19.83	17.36	16.97	17.46	24.18

Table 2. Performance of WSB in trial for Objective 1.

Diet	Initial Wt (g)	Final Wt (g)	% Gain	% Survival	FCR
Starch	3.0	13.6[c]	350[c]	93	2.3[c]

Wheat	3.0	16.1[b]	440[b]	95	1.9[c]
Corn	3.0	6.3[d]	108[d]	87	7.4[a]
Rice	3.0	14.9[bc]	394[bc]	88	2.0[c]
Sorghum	3.0	9.4[e]	212[e]	82	4.0[b]
Wheat + CHOase	3.1	15.4[b]	398[bc]	82	1.9[c]
Wheat + Phy	3.0	15.2[b]	409[b]	92	2.0[c]
Wheat + CHOase + Phy	3.1	16.5[b]	440[b]	97	1.8[c]
Starch + fiber	3.0	18.8[a]	534[a]	85	1.6[c]

In an 8 week trial YT, were fed a series of five diets with various carbohydrate sources. YT performed best on a diet with wheat but, unlike WSB, diets with rice and corn starch, produced statistically similar results (Table 4). As with WSB the diet with sorghum produced the poorest results. Additionally, there was a strong correlation between growth rates and percent gelatinization of the diet. This indicates that the growth response is not just due to different carbohydrate sources.

Table 3. Feed formulations and proximate composition of diets for YT for Objective 1.

Ingredient g/100g as is	Starch	Wheat	Corn	Rice	Sorghum
Menhaden Fishmeal-5303111409	35.80	35.80	35.80	35.80	35.80
Soy concentrate - soycomil	20.85	17.31	18.98	19.07	19.09
Empyreal 75 - cargil	10.42	8.66	9.49	9.53	9.55
Menhaden Fish Oil	2.38	2.02	1.50	1.73	1.59
Corn Starch	28.00	5.00	5.00	5.00	5.00
Whole wheat	0.00	23.00	0.00	0.00	0.00
Whole corn	0.00	0.00	23.00	0.00	0.00
Whole rice	0.00	0.00	0.00	23.00	0.00
Sorghum	0.00	0.00	0.00	0.00	23.00
Trace Mineral premix	0.25	0.25	0.25	0.25	0.25
ASA_Vitamin premix w/o choline	0.50	0.50	0.50	0.50	0.50
Choline chloride	0.20	0.20	0.20	0.20	0.20
Stay C 35%	0.10	0.10	0.10	0.10	0.10
CaP-diebasic	0.50	0.50	0.50	0.50	0.50
Lecithin (soy commercial)	0.50	0.50	0.50	0.50	0.50
Taurine	0.25	0.25	0.25	0.25	0.25
Cellufill	0.24	5.91	3.93	3.57	3.67
Total	100.00	100.00	100.00	100.00	100.00
Proximate composition			g/100g as is		
Moisture	5.82	6.34	6.61	6.28	10.82
Protein	45.2	45.8	45.1	45	43.7
Fat	7.96	7.41	7.62	7.85	7.11
Fiber	1.78	2.74	3.1	2.38	3.06
Ash	9.44	9.78	9.41	9.5	9.01
% gelatinized	52.42	63.94	43.02	46.29	32.33
Starch (gelatinized)	13.02	10.78	8.35	9.79	6.23
Starch (total)	24.84	16.86	19.41	21.15	19.27

Table 4. Performance of YT in trial for Objective 1.

Diet	Initial Wt (g)	Final Wt (g)	% Gain	% Survival	FCR
Starch	15.8	178.5[ab]	1033[ab]	98	1.4[b]
Wheat	15.7	188.6[a]	1100[a]	97	1.4[b]

Corn	15.8	156.1[bc]	890[bc]	98	1.5[a]
Rice	15.8	174.6[abc]	1009[ab]	100	1.4[b]
Sorghum	15.8	141.2[c]	795[c]	97	1.6[a]

Objective 2 – Laboratory Trial to optimize the carbohydrate (energy) component in high soy diets for WSB (HSWRI/Auburn).

In an 8 week trial, WSB were fed a series of nine diets. The first six diets were formulated with an increasing level of carbohydrates (4.6-28.5% starch) and decreasing lipid (13.2-3.7%), with equivalent digestible energy levels. The last three diets were formulated without filler, with starch content ranging 16.4-26.8% and lipids 19.3-7.8% (Table 5). There was limited effect on the growth response as long as the dietary lipid-to-starch ratio was neither extremely low nor high (Table 6). This indicates that WSB are tolerant to carbohydrates and able to effectively utilize them as an energy source.

Table 5. Feed formulations and proximate composition of diets for WSB for Objective 2.

Ingredient g/100g as is	1	2	3	4	5	6	7	8	9
Menhaden Fishmeal-5303111409	14.50	14.50	14.50	14.50	14.50	14.50	14.50	14.50	14.50
Soybean meal solvent extracted	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00
Soy concentrate - soycomil	15.70	15.70	15.70	15.70	15.70	15.70	15.70	15.70	15.70
Empyreal 75 - cargil	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Menhaden Fish Oil	9.77	7.97	6.17	4.37	2.57	0.77	9.77	4.37	15.74
Corn Starch	0.00	5.00	10.00	15.00	20.00	25.00	16.08	21.48	10.11
Whole wheat	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Trace Mineral premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
ASA_Vitamin premix w/o choline	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Choline chloride	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Stay C 35%	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
CaP-diebasic	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80
Lecithin (soy refined, USB)	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Methionine	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Taurine	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Cellufill	16.08	12.88	9.68	6.48	3.28	0.08	0.00	0.00	0.00
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Proximate composition				g/100g as is					
Protein	40.18	41.19	40.59	40.96	40.82	41.35	40.38	40.98	41.32
Moisture	3.83	4.23	4.83	5.63	5.67	6.24	5.16	5.84	4.86
Fat	13.18	11.21	9.70	7.67	5.90	3.74	13.19	7.81	19.30
Fiber	12.42	9.76	8.83	6.76	4.51	2.26	2.29	2.66	2.34
Ash	6.87	6.77	6.88	6.75	6.87	6.89	6.85	6.88	6.80
Starch	4.60	8.63	25.70	18.77	24.52	28.46	22.26	28.80	16.43

Table 6. Performance of WSB in trial for Objective 2.

Diet	Initial Wt (g)	Final Wt (g)	% Gain	% Survival	FCR
1	4.1	18.0[d]	340[d]	95	1.6[bc]
2	4.1	20.2[c]	396[bcd]	87	1.6[bc]
3	4.1	20.8[c]	410[bcd]	83	1.6[bc]
4	4.0	21.6[bc]	437[abc]	93	1.6[bc]
5	4.1	20.3[c]	402[bcd]	90	1.7[ab]

6	4.2	20.1^{c}	385^{cd}	93	1.8^{a}
7	4.1	24.7^{a}	507^{a}	95	1.5^{c}
8	4.2	23.2^{ab}	459^{ab}	92	1.6^{bc}
9	4.1	23.9^{a}	487^{a}	92	1.5^{c}

Table 7. Whole body proximate composition (g/100g as is) of WSB in trial for Objective 2.

Trt#	Crude Protein*	Moisture	Crude Fat	Crude Fiber	Ash
1	14.65^{c}	80.05^{a}	1.40^{c}	0.31^{a}	3.37
2	14.58^{c}	80.21^{a}	1.53^{c}	0.21^{ab}	3.15
3	14.98^{abc}	79.68^{a}	1.60^{c}	0.26^{ab}	3.26
4	14.92^{bc}	80.01^{a}	1.35^{c}	0.25^{ab}	3.28
5	15.32^{abc}	80.22^{a}	1.10^{d}	0.24^{ab}	3.36
6	15.33^{abc}	80.32^{a}	0.84^{e}	0.22^{ab}	3.35
7	15.80^{ab}	78.02^{c}	2.73^{b}	0.17^{b}	3.19
8	15.88^{a}	79.13^{a}	1.61^{c}	0.18^{b}	3.24
9	15.28^{abc}	76.84^{b}	4.33^{a}	0.18^{b}	3.07

Objective 3 – Laboratory Trial to determine the taurine requirement of YT (HSWRI/Auburn).

In an 8 week trial, YT were fed a series of eight FM free diets formulated with an increasing level of supplemental taurine (0.10-1.02%; Table 8). Survival was greatly reduced at the lowest levels of dietary taurine and growth performance plateaued before the upper range of dietary taurine was reached (Table 9). Regression analyses indicated that the taurine requirement is 0.24-0.28%. Protein efficiency begins to decrease above 0.72% dietary taurine and protein deposition decreased slightly as taurine approaches the upper end (1.02%) of dietary taurine (Figure 1).

Table 8. Feed formulations and proximate composition of diets for YT for Objective 3.

	% dietary taurine							
Ingredient g/100g as is	0.10	0.18	0.22	0.27	0.31	0.39	0.63	1.02
Menhaden Fishmeal	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Poultry by product meal	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00
Soybean meal solvent extracted	19.10	19.10	19.10	19.10	19.10	19.10	19.10	19.10
Soy concentrate – soycomil	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Empyreal 75 – cargil	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00
Menhaden Fish Oil	8.10	8.10	8.10	8.10	8.10	8.10	8.10	8.10
Whole wheat	22.00	22.00	22.00	22.00	22.00	22.00	22.00	22.00
Trace Mineral premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
ASA_Vitamin premix w/o choline	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Choline chloride	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Stay C 35%	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
CaP-diebasic	1.80	1.80	1.80	1.80	1.80	1.80	1.80	1.80
Lecithin (soy commercial)	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Methionine	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18
Taurine	0.00	0.05	0.10	0.15	0.20	0.30	0.50	0.90

Cellufill	1.77	1.72	1.67	1.62	1.57	1.47	1.27	0.87
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Proximate composition				g/100g as is				
Protein	43.25	42.45	42.16	42.87	42.52	43.48	44.52	46.88
Moisture	9.07	10.84	10.71	10.13	9.98	9.32	7.40	5.66
Fat	11.85	11.60	11.59	11.60	11.54	12.00	12.23	13.02
Fiber	3.31	2.99	2.83	3.42	2.92	2.87	3.37	2.95
Ash	6.70	6.68	6.78	6.66	6.73	6..82	6.66	7.10
CP-Tau	43.15	42.27	41.94	42.60	42.21	43.09	43.89	45.86
Taurine	0.10	0.18	0.22	0.27	0.31	0.39	0.63	1.02

Table 9. Performance of YT in trial for Objective 3.

% Taurine	Initial Wt (g)	Final Wt (g)	% Gain	% Survival	FCR
0.10	4.9	19.9	309	60	2.7
0.18	4.9	40.4	724	55	1.8
0.22	4.9	83.6	1604	100	1.1
0.27	4.9	89.5	1726	95	1.1
0.31	4.9	87.8	1709	100	1.1
0.39	5.0	96.9	1857	100	1.1
0.63	4.9	100.0	1951	100	1.0
1.02	4.9	93.0	1797	78	1.1

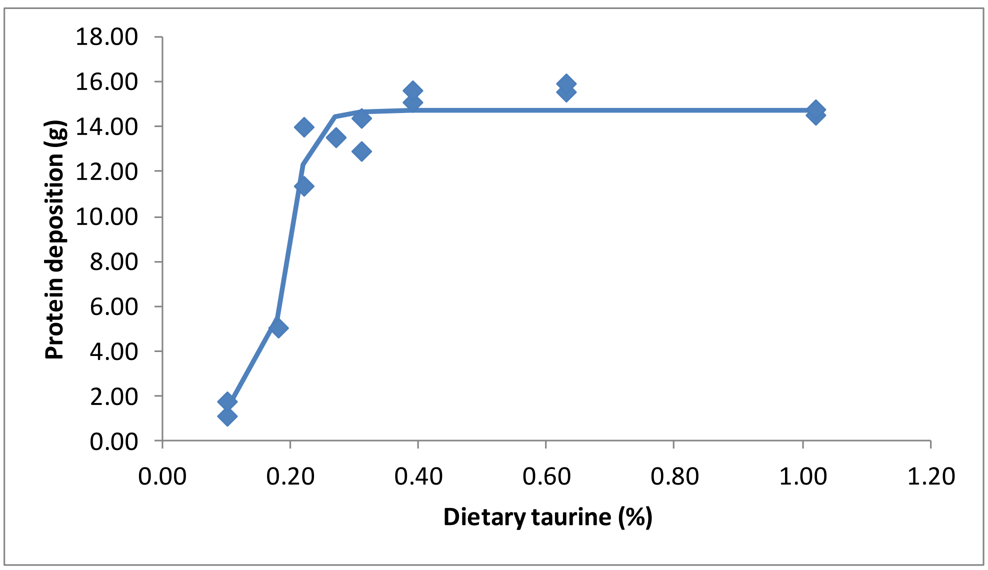

Figure 1. Protein deposition of YT in trial for Objective 3.

Objective 4 – Further assess the value of hydrogenated and partially hydrogenated soybean oils in feeds for YT (HSWRI/SIU).

In a 7 week trial, YT were fed a series of six fish oil diets with increasing (0-100%) levels of fully hydrogenated and decreasing (100-0%) levels of partially hydrogenated soybean oil in combination along with a fish oil control (Table 10). Growth performance was slightly lower in YT fed the 0% fish oil diets but the combination of soybean oils did not have an effect (Table 11). Although this is somewhat contrary to previous results in this species, we have observed reduced performance among other fish fed hydrogenated soybean oil in the past. We believe this is primarily due to minor differences in the digestibility of fish oil vs. soybean oil, a subject to be addressed in the 2015 collaborative research between HSWRI and SIUC. Analysis of tissue fatty acid composition is ongoing.

Table 10. Feed formulations and proximate composition of diets for YT for Objective 4.

	FO CONTROL	0 FULL: 100 PARTIAL	20 FULL: 80 PARTIAL	40 FULL: 60 PARTIAL	60 FULL: 40 PARTIAL	80 FULL: 20 PARTIAL	100 FULL: 0 PARTIAL
Ingredient				g/kg, as fed basis			
Menhaden fish meal	473.70	473.70	473.70	473.70	473.70	473.70	473.70
Soy protein concentrate	240.00	240.00	240.00	240.00	240.00	240.00	240.00
Menhaden fish oil	95.10	0.00	0.00	0.00	0.00	0.00	0.00
Fully hydrogenated soybean oil	0.00	0.00	15.10	30.20	45.20	60.30	75.40
Partially hydrogenated soybean oil	0.00	75.40	60.30	45.20	30.20	15.10	0.00
Corn starch	79.50	79.50	79.50	79.50	79.50	79.50	79.50
Wheat flour	50.00	50.00	50.00	50.00	50.00	50.00	50.00
Corn gluten meal	32.50	32.50	32.50	32.50	32.50	32.50	32.50
Soy lecithin	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Carboxymethyl cellulose	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Choline chloride	6.00	6.00	6.00	6.00	6.00	6.00	6.00
Vitamin premix	1.20	1.20	1.20	1.20	1.20	1.20	1.20
Mineral premix	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Stay C	1.00	1.00	1.00	1.00	1.00	1.00	1.00
ARA concentrate	0.00	0.50	0.50	0.50	0.50	0.50	0.50
DHA concentrate	0.00	10.30	10.30	10.30	10.30	10.30	10.30
EPA concentrate	0.00	8.90	8.90	8.90	8.90	8.90	8.90
Proximate composition			g/kg, dry matter basis (except dry matter)				
Dry matter	954 ± 0	953 ± 0	952 ± 0	948 ± 0	954 ± 0	953 ± 0	951 ± 0
Protein	542 ± 5	545 ± 0	552 ± 5	554 ± 3	552 ± 4	549 ± 7	544 ± 3
Lipid	167 ± 1	161 ± 4	165 ± 2	158 ± 1	166 ± 2	166 ± 1	164 ± 1
Ash	116 ± 0	116 ± 0	116 ± 1	117 ± 1	117 ± 1	117 ± 1	117 ± 0

Table 11. Performance of YT in trial for Objective 4.

Performance parameter	FO CONTROL	0 FULL: 100 PARTIAL	20 FULL: 80 PARTIAL	40 FULL: 60 PARTIAL	60 FULL: 40 PARTIAL	80 FULL: 20 PARTIAL	100 FULL: 0 PARTIAL
Survival (%)	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Initial weight (g)	10.90	11.00	10.80	10.80	11.00	10.90	10.90
Final weight (g)	103.5[a]	91.4[b]	89.5[b]	94.3[b]	89.6[b]	94.0[b]	93.5[b]
Weight gain (%)	849[a]	733[b]	728[b]	770[b]	714[b]	760[b]	757[b]
FCR (dry matter basis)	1.27[b]	1.38[a]	1.37[a]	1.32[ab]	1.35[ab]	1.29[ab]	1.31[ab]
SGR (% body weight/d)	4.32[a]	4.08[b]	4.06[b]	4.16[b]	4.03[b]	4.14[b]	4.13[b]

Feed intake (% body weight/d)	6.71	6.72	6.66	6.64	6.48	6.46	6.52
HSI	0.75	0.96	0.92	0.74	0.75	0.54	0.88
VSI	6.80	7.43	7.33	6.67	7.56	6.90	7.29

Objective 5 – Laboratory Trials to evaluate the inclusion of fermented soybean meal and/or the microbes associated with it in diets for YT and WSB (HSWRI/SIU).

In 8 week trials, YT and WSB were fed a series of eight diets including a high and a low fish meal series with graded levels of the probiotic Natufermen (Table 12 and 14). The low FM series for both species also included a traditional and a fermented soy (PepSoyGen) control. The same microbes in Natufermen are used to produce PepSoyGen. Each series also included a FM control. Although in most cases, the data were too variable to detect significant differences among treatments, growth was generally best among WSB and YT fed diets containing greater amounts of fish meal (Table 13 and 15). The inclusion of microbial material - either in the context of control or reduced fish meal feeds - seemed to have relatively little effect. Analysis of blood chemistry and gut histology results is ongoing.

Table 12. Feed formulations and proximate composition of diets for YT for Objective 4.

Ingredient	40% FM Control	40% FM + 10% Nf	40% FM + 50% Nf	40% FM + 100% Nf	20% FM SBM	20% FM PSG	20% FM SBM + 10% Nf	20% FM SBM + 50% Nf	20% FM SBM + 100% Nf
	g/kg, as fed basis								
Menhaden fish meal	400.0	400.0	400.0	400.0	200.0	200.0	200.0	200.0	200.0
Soybean meal	180.0	180.0	180.0	180.0	350.0	0.0	350.0	350.0	350.0
PepSoyGen	0.0	0.0	0.0	0.0	0.0	462.5	0.0	0.0	0.0
Corn protein concentrate	0.0	0.0	0.0	0.0	104.1	50.0	104.1	104.1	104.1
Soy protein concentrate	0.0	0.0	0.0	0.0	120.0	70.0	120.0	120.0	120.0
Wheat flour	156.3	156.3	156.3	156.3	47.8	37.7	47.8	47.8	47.8
Poultry byproduct meal	63.2	63.2	63.2	63.2	0.0	0.0	0.0	0.0	0.0
Menhaden fish oil	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0
Soybean oil	22.4	22.4	22.4	22.4	42.9	45.3	42.9	42.9	42.9
Blood meal	70.0	70.0	70.0	70.0	0.0	0.0	0.0	0.0	0.0
Dicalcium phosphate	0.0	0.0	0.0	0.0	24.6	22.9	24.6	24.6	24.6
Taurine	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0
Methionine	2.0	2.0	2.0	2.0	2.6	3.5	2.6	2.6	2.6
Lysine	2.0	2.0	2.0	2.0	3.8	3.9	3.8	3.8	3.8
Choline	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0
Vitamin premix	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2
Mineral	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0

premix									
Stay C	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Carboxymethyl cellulose	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0
Cellulose (inert filler)	10.0	9.6	7.9	5.8	10.0	10.0	9.6	7.9	5.8
Natufermen	0.0	0.4	2.1	4.2	0.0	0.0	0.4	2.1	4.2
Proximate composition	g/kg, dry matter basis (except dry matter)								
Dry matter	96 ± 0	95 ± 0	95 ± 0	95 ± 0	96 ± 0	96 ± 0	96 ± 0	96 ± 0	95 ± 0
Protein	513 ± 4	501 ± 1	503 ± 1	506 ± 3	522 ± 2	505 ± 3	525 ± 1	523 ± 2	523 ± 1
Lipid	142 ± 2	142 ± 1	146 ± 0	146 ± 1	148 ± 1	140 ± 2	142 ± 1	149 ± 1	151 ± 1
Ash	106 ± 7	107 ± 7	107 ± 4	116 ± 3	107 ± 1	104 ± 6	107 ± 9	99 ± 5	107 ± 3

Table 13. Performance of YT in trial for Objective 4.

Performance parameter	40% FM Control	40% FM + 10% Nf	40% FM + 50% Nf	40% FM + 100% Nf	20% FM SBM	20% FM PSG	20% FM SBM + 10% Nf	20% FM SBM + 50% Nf	20% FM SBM + 100% Nf
Survival (%)	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Initial weight (g)	26.2	25.9	26.0	26.0	25.7	25.8	25.8	25.9	26.2
Final weight (g)	143.6	143.4	151.7	147.5	127.0	137.8	122.9	129.3	126.8
Weight gain (%)	449.0	453.0	483.0	468.0	394.0	433.0	376.0	400.0	385.0
FCR (dry matter basis)	1.7	1.7	1.6	1.7	1.8	1.7	1.9	1.8	1.9
SGR (% body weight/d)	3.1	3.1	3.2	3.2	2.9	3.0	2.8	2.9	2.9
Feed intake (% body weight/d)	6.0	5.9	5.9	6.0	5.9	5.9	6.0	5.9	5.9
HSI	0.7	0.8	0.8	0.7	0.7	0.8	0.8	0.7	0.7

Table 14. Feed formulations and proximate composition of diets for WSB for Objective 4.

Ingredient	g/kg, as fed basis								
	48% FM Control	48% FM + 10% Nf	48% FM + 50% Nf	48% FM + ±100% Nf	12% FM SBM	12% FM PSG	12% FM SBM + 10% Nf	12% FM SBM + 50% Nf	12% FM SBM + 100% Nf
Menhaden fish meal	480.0	480.0	480.0	480.0	120.0	120.0	120.0	120.0	120.0
Soybean meal	100.0	100.0	100.0	100.0	250.0	0.0	250.0	250.0	250.0
PepSoyGen	0.0	0.0	0.0	0.0	0.0	250.0	0.0	0.0	0.0
Corn protein concentrate	36.7	36.7	36.7	36.7	156.4	154.6	156.4	156.4	156.4
Soy protein concentrate	80.0	80.0	80.0	80.0	200.0	180.0	200.0	200.0	200.0
Wheat flour	181.6	181.6	181.6	181.6	78.5	97.7	78.5	78.5	78.5
Menhaden fish oil	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0
Soybean oil	21.4	21.4	21.4	21.4	48.3	48.8	48.3	48.3	48.3
Dicalcium phosphate	0.0	0.0	0.0	0.0	35.9	36.2	35.9	35.9	35.9
Taurine	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0
Methionine	0.6	0.6	0.6	0.6	3.0	3.3	3.0	3.0	3.0
Lysine	2.5	2.5	2.5	2.5	10.8	12.3	10.8	10.8	10.8

Choline	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0
Vitamin premix	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2
Mineral premix	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Stay C	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Carboxymethyl cellulose	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0
Cellulose	3.0	2.8	1.9	0.7	3.0	3.0	2.8	1.9	0.7
Natufermen	0.0	0.2	1.1	2.3	0.0	0.0	0.2	1.1	2.3
Proximate composition			g/kg, dry matter basis (except dry matter)						
Dry matter	97 ± 0	97 ± 0	97 ± 0	97 ± 0	97 ± 0	97 ± 0	96 ± 0	96 ± 0	96 ± 0
Protein	498 ± 2	499 ± 3	510 ± 3	500 ± 1	497 ± 2	492 ± 1	498 ± 3	497 ± 2	495 ± 3
Lipid	148 ± 2	149 ± 1	141 ± 1	151 ± 1	145 ± 2	148 ± 0	144 ± 1	148 ± 1	142 ± 1
Ash	117 ± 8	121 ± 3	117 ± 4	127 ± 2	91 ± 1	95 ± 4	89 ± 3	99 ± 3	96 ± 0

Table 15. Performance of WSB in trial for Objective 4.

Performance parameter	48% FM Control	48% FM + 10% Nf	48% FM + 50% Nf	48% FM + 100% Nf	12% FM SBM	12% FM PSG	12% FM SBM + 10% Nf	12% FM SBM + 50% Nf	12% FM SBM + 100% Nf
Survival (%)	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Initial weight (g)	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0
Final weight (g)	39.2	39.8	39.9	38.8	36.3	36.5	36.1	38.6	37.1
Weight gain (%)	337.0	345.0	344.0	330.0	302.0	307.0	302.0	328.0	313.0
FCR (dry matter basis)	1.22[a]	1.20[a]	1.21[a]	1.25[ab]	1.34[b]	1.31[ab]	1.35[b]	1.27[ab]	1.28[ab]
SGR (% body weight/d)	3.0	3.0	3.0	3.0	2.8	2.9	2.8	3.0	2.9
Feed intake (% body weight/d)	4.0	4.0	4.0	4.0	4.1	4.1	4.2	4.1	4.0
HSI	2.44[ab]	2.74[a]	2.44[ab]	2.39[ab]	1.84[b]	1.81[b]	1.82[b]	1.93[ab]	1.87[b]

Objective 6 – Growout trials to evaluate soy based formulations for WSB over an extended period of time (HSWRI/SIU/Auburn).

A grow out trial is underway through mid 2015. Results will be reported next year when they are available.

Objective 7 – Laboratory trials to evaluate the effects of temperature on acceptance and utilization of soy-based feeds by SB. (HSWRI/Auburn).

This trial was to be conducted on CA strain of SB. California producers failed to produce SB this year, so we were unable to complete this objective.

Objective 8 – Laboratory Trials* to *evaluate the effects of SB strain differences on the acceptance and utilization of soy-based feeds. (HSWRI/Auburn).

As stated above, growers in California were unable to produce fingerlings and we were unable to obtain more than one strain of SB from other parts of the U.S. Therefore, we were not able complete this objective in this research cycle.

Objective 9 – Laboratory Trials using successful techniques developed in the first two objectives to further evaluate effects of inclusion rate of soy on performance of SB. (HSWRI/Auburn).

In a 7 week trial, SB from the eastern U.S. were fed a series of nine diets. Four of the diets (Series 1) were formulated with a graded replacement of FM (from 32 to 0%) using soy-based meals and a 12% inclusion of PBM. Three additional zero FM diets like those in Series 1 were formulated to be deficient in methionine and/or taurine (Series 2). Finally, a diet high in PBM and a diet with no PBM were formulated for testing (Series 3; Table 16). Performance of SB generally decreased with decreasing FM among the Series 1 diets (Table 17). There were no differences in performance with any of the diets "deficient" in methionine or taurine. Performance on the high PBM diet was similar to the higher FM formulations and lowest on the 0% PBM meal diet indicating that PBM may enhance palatability or other nutritional properties of these diets. This strain of SB performed much better than California strains fed similar diets in previous research even producing reasonable growth on diets that California fish would not accept.

Table 16. Feed formulations and proximate composition of diets for SB for Objective 9.

Ingredient g/100g as is	FM32% Soy12 %	FM16% Soy24 %	FM8% Soy30 %	FM0% Soy36 %	FM0% Soy36 %-M	FM0% Soy36 %-T	FM0% Soy36 %-M-T	PBM35 % Soy12 %	FM0% Soy45 %
Menhaden Fishmeal	32.00	16.00	8.00	0.00	0.00	0.00	0.00	8.00	0.00
Poultry by product meal	12.00	12.00	12.00	12.00	12.00	12.00	12.00	34.89	0.00
Soybean meal solvent extracted	5.10	10.35	12.97	15.54	15.54	15.54	15.54	5.10	19.65
Soy concentrate -soycomil	6.63	13.46	16.86	20.20	20.20	20.20	20.20	6.63	25.55
Empyreal 75 - cargil	4.59	9.32	11.67	13.99	13.99	13.99	13.99	4.59	17.69
Menhaden Fish Oil	6.00	7.20	7.81	8.41	8.41	8.41	8.41	4.46	10.16
Corn Starch	10.83	7.58	5.64	3.76	3.86	4.71	4.81	10.23	0.86
Whole wheat	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00
ASA Trace Mineral premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
ASA Vitamin premix w/o choline	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Choline chloride	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Stay C 35%	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
CaP-diebasic	0.00	1.20	2.10	3.00	3.00	3.00	3.00	3.00	3.00
Lecithin (soy commercial)	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Methionine				0.10		0.10		0.10	0.10
Taurine	0.80	0.85	0.90	0.95	0.95			0.95	0.95
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Proximate composition					g/100g as is				

Protein	42.18	42.84	42.34	43.81	43.00	43.52	42.83	41.59	41.52
Moisture	9.17	9.05	8.93	6.01	9.25	5.13	5.96	7.82	7.62
Fat	12.36	12.10	11.79	11.80	11.31	11.69	11.56	10.81	12.23
Crude	1.83	2.38	2.71	2.84	2.81	2.79	2.86	2.46	2.97
Ash	8.47	7.54	7.27	7.62	7.14	7.47	7.58	10.40	6.95

Table 17. Performance of SB in trial for Objective 9.

Trt#	Initial Wt (g)	Final Wt (g)	% Gain	% Survival	FCR
FM32% Soy12%	5.7	17.2[a]	204[a]	100	2.1[b]
FM16% Soy24%	5.7	16.9[a]	198[ab]	100	2.1[b]
FM8% Soy30%	5.7	15.8[ab]	179[abc]	98	2.3[b]
FM0% Soy36%	5.6	15.0[ab]	168[bc]	98	2.4[b]
FM0% Soy36%-M	5.6	14.3[b]	155[c]	97	2.5[b]
FM0% Soy36%-T	5.8	15.6[ab]	170[bc]	92	2.3[b]
FM0% Soy36%-M-T	5.5	14.1[b]	154[c]	93	2.5[b]
PBM35% Soy12%	5.7	16.1[ab]	183[abc]	98	2.3[b]
FM0% Soy45%	5.7	12.2[c]	115[d]	92	3.1[a]

Table 18. Whole body proximate composition (g/100g as is) of SB in trial for Objective 9.

Diet	Protein	Moisture	Fat	Fiber	Ash
FM32% Soy12%	16.39	68.60[abc]	11.23[abc]	0.34	3.49
FM16% Soy24%	16.36	68.85[abc]	11.11[abc]	0.36	3.48
FM8% Soy30%	16.46	68.02[c]	12.09[a]	0.37	3.41
FM0% Soy36%	15.82	68.65[abc]	11.72[abc]	0.30	3.44
FM0% Soy36%-M	16.25	69.54[ab]	10.74[c]	0.37	3.43
FM0% Soy36%-T	15.93	68.65[abc]	11.53[abc]	0.33	3.58
FM0% Soy36%-M-T	16.08	68.59[bc]	11.82[ab]	0.31	3.54
PBM35% Soy12%	16.21	68.32[c]	11.84[ab]	0.36	3.52
FM0% Soy45%	15.86	69.76[a]	10.82[bc]	0.34	3.36

We conducted a second 11 week feeding trial using the same fish from the trial described above and similar diets in order to determine if larger fish had better acceptability of the soy-based diets (Table 19). The second trial was initiated after rearing them for several months on a commercial feed. The fish showed a poorer feeding response than they did in the previous trial. The performance results were generally similar among treatments (Table 20). The commercial feed did not yield statistically better growth than the other FM diets but it did yield the fastest growth. These results indicate that diet acceptance and utilization can vary among strains and sizes of SB.

Table 19. Feed formulations and proximate composition of diets for SB in second trial for Objective 9.

Ingredient g/100g as is	FM32% Soy12 %	FM16% Soy23 %	FM8% Soy29 %	FM0% Soy 29%	FM0% Soy 29%-T	FM0% Soy 29%-L	FM0% Soy 29%-M	FM0% Soy 29% Fiber
Menhaden Fishmeal	32.00	16.00	8.00	0.00	0.00	0.00	0.00	32.00
Poultry by product meal	12.00	12.00	12.00	12.00	12.00	12.00	12.00	12.00
Soybean meal solvent extracted	5.09	10.14	12.61	15.11	15.11	15.11	15.11	5.09
Soy concentrate - soycomil	6.62	13.18	16.40	19.64	19.64	19.64	19.64	6.62

Empyreal 75 - cargil	4.59	9.13	11.35	13.60	13.60	13.60	13.60	4.59
Menhaden Fish Oil	6.63	7.90	8.53	9.17	9.17	9.17	9.17	6.63
Corn Starch	10.22	6.60	4.84	3.03	3.98	3.03	3.03	8.72
Whole wheat	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00
Trace Mineral premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
ASA_Vitamin premix w/o choline	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Choline chloride	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Stay C 35%	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
CaP-dibasic	0.00	1.70	2.60	3.50	3.50	3.50	3.50	0.00
Lecithin (soy commercial)	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Lysine	0.00	0.30	0.50	0.65	0.65	0.00	0.65	0.00
Methionine	0.00	0.10	0.20	0.30	0.30	0.30	0.00	0.00
Glutamic acid	0.00	0.00	0.00	0.00	0.00	0.65	0.30	0.00
Taurine	0.80	0.90	0.91	0.95	0.00	0.95	0.95	0.80
Soy Fiber	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.50
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Calculated composition (as-is)								
Crude protein	42.00	42.00	42.00	42.00	42.00	42.00	42.00	42.00
Lysine	2.67	2.63	2.66	2.63	2.63	1.98	2.63	2.67
meth+cys	1.54	1.50	1.53	1.56	1.56	1.56	1.26	1.54
Meth	1.08	0.99	1.00	1.00	1.00	1.00	0.70	1.08
Taurine	1.01	1.03	1.00	1.01	0.06	1.01	1.01	1.01
Total lipid	12.00	12.00	12.00	12.00	12.00	12.00	12.00	12.00
n-3 HUFA	2.37	2.33	2.31	2.29	2.29	2.29	2.29	2.37
Carbohydrates	27.13	27.05	27.02	26.95	27.65	26.95	26.95	26.03
Phosphorous	1.42	1.40	1.40	1.40	1.40	1.40	1.40	1.42

Table 20. Performance of SB in second trial for Objective 9.

Trt#	Initial Wt (g)	Final Wt (g)	% Gain	% Survival	FCR
FM32% Soy12%	54.5	106.4	95^{ab}	95	2.1
FM16% Soy23%	55.5	106.6	93^{ab}	95	2.1
FM8% Soy29%	57.5	108.3	88^{ab}	95	2.1
FM0% Soy 29%	57.5	103.7	81^{b}	98	2.3
FM0% Soy 29%-T	58.3	104.7	80^{b}	93	2.3
FM0% Soy 29%-L	59.5	104.7	76^{b}	90	2.4
FM0% Soy 29%-M	59.1	111.2	89^{ab}	93	2.1
FM0% Soy 29% Fiber	58.4	107.9	85^{b}	98	2.3
Commercial	57.1	125.4	120^{a}	100	1.8

Objective 10 - Test the effectiveness of small mesh copper netting in an offshore cage with juvenile WSB during warmer water months and at commercial stocking densities. (HSWRI)

For this objective, we tested the efficacy of small copper mesh netting for nursery production of marine fish during summer months and at commercial stocking densities as means of reducing dependency of culturing these fish in coastal areas.

Figure 2 – Cages being deployed.

Figure 3 – Cages being stocked with fish.

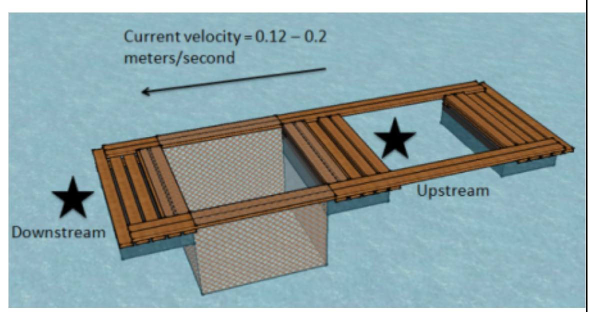

Figure 4 - Diagram of water sampling locations used to test for upstream/downstream copper

All work was conducted at the Hubbs-SeaWorld Research Institute's Leon Raymond Hubbard, Jr. Marine Fish Hatchery (CLD) and the adjacent Agua Hedionda Lagoon (AHL) in Carlsbad California. Three sets of two-cage rafts were used each containing one copper-alloy cage and one standard nylon net. These rafts were constructed in 2013 for use in a previous lower stocking density study also funded by the United Soybean Board. In 2013, Copper-alloy cages were constructed of 6.35mm mesh were assembled on land and deployed using a crane aboard a floating barge (Figure 2). Care was taken to use only copper-alloy hardware for the assembly of the copper-alloy cages and to not allow for any other metals to come in contact with the material. Trex composite material was used as a platform for each of the rafts and Styrofoam-filled HDPE dock floats were used for buoyancy. Nylon nets each required a pendant weight (approximately 10kg ea) at each of the four bottom net corners in order to keep the cage from collapse during ebbing and flowing tides. No such weights were necessary for the copper-alloy cages due to the rigidity of the material. Semi-rigid plastic mesh lids were made for all cages to prevent bird predation. Cage rafts were moored to existing WSB juvenile cages within the AHL. Once installed, each cage provided a total of 4 m^3 of water volume.

In July 2014, each cage was stocked with 1,200 WSB juveniles weighing an average of 3g each (Figure 3). During the 12-week trial, juveniles were initially fed with Otohime EP1 diet (1mm), transitioned onto Otohime EP2 diet (2mm) 10 days into the study, then onto 3mm EWOS marine juvenile diet 22-days into the study, next onto EWOS "small" marine fish diet 53-days into the study and finally onto EWOS "medium" marine fish diet 75-days into the study. Daily temperature and dissolved oxygen readings were taken in each cage and mortalities were removed whenever present. Weight and length (SL and TL) measures were taken from 30 fish in each cage at weeks 4 and 8. A final sampling of 40 fish from each cage was taken at the termination of the trial. Fish from each cage were also processed for liver copper-concentration analysis. Finally, at the end of the trial, a subset of fish reared in copper-alloy cages were transferred to land-based raceway tanks, reared for an additional 4 weeks and processed for liver copper-concentration analysis.

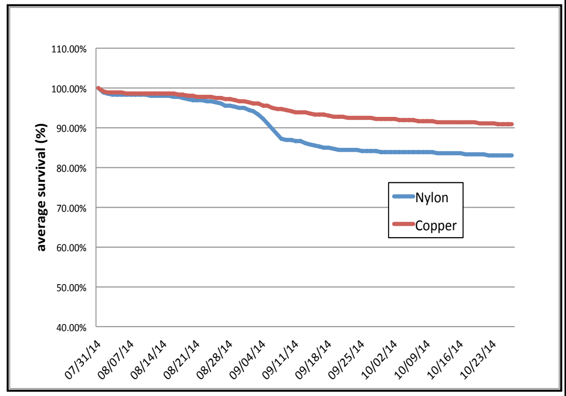

Figure 5. Average survival of juvenile WSB reared in copper-alloy and nylon mesh cages

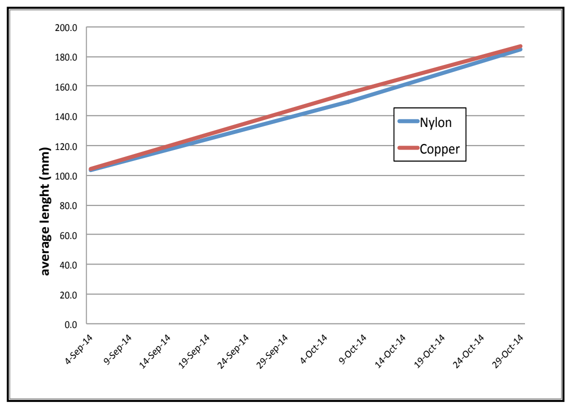

Figure 6. Average length increase of juvenile WSB reared in copper-alloy and nylon mesh cages

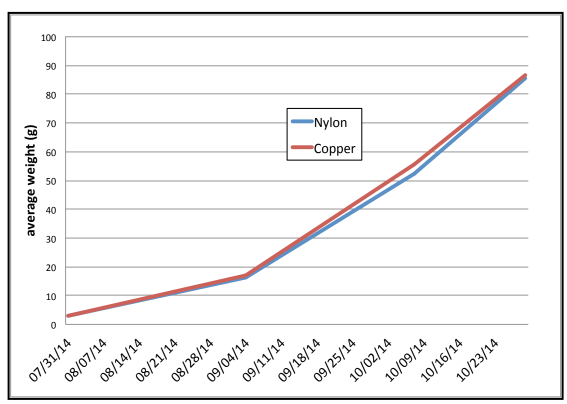

Figure 7. Average weight increase of juvenile WSB reared in copper-alloy and nylon mesh cages

Water samples for copper concentration analysis were collected 1 meter upstream (n=3) and downstream (n=3) of a copper cage during a 0.12-0.2 meter/second incoming tide (Figure 3). Samples were collected using methods described by EPA 1669. Samples were sent to Calscience Environmental Laboratories and were analyzed for total and dissolved copper using methods described by EPA 1640.

Juvenile performance – there was no significant difference in average survival between the two treatments at the end of the study. Fish from both treatments exhibited acceptable levels of survival >80% (Figure 5). No significant differences were found in growth or feed conversion ratios (FCR) (Figure 6-8). Due to higher culture water temperatures, tank-reared fish, however, did exhibit a significantly higher weight gain than either cage treatment.

Cage performance – The most obvious difference between cage types was the almost complete absence of biofouling on the copper-alloy material through the study period; the nylon material, on the other hand, exhibited relatively rapid growth of biofouling. Despite the relatively high final fish density of 21.5 grams of fish per liter of cage volume, no obvious water quality differences were detected between cage treatments. Regardless, fouled nylon nets were swapped out for clean nets at week 8 of this study.

Results from upstream/downstream seawater total or dissolved copper concentration analyses showed no significant differences (Figure 9). Moreover, neither upstream nor downstream sample concentrations approached the California chronic saltwater copper standard of 3.1 µg/L.

Other observations – No significant differences in liver copper-concentrations were detected between copper and nylon treatments or 4-weeks post transfer to land-based raceway tanks (Figure 10).

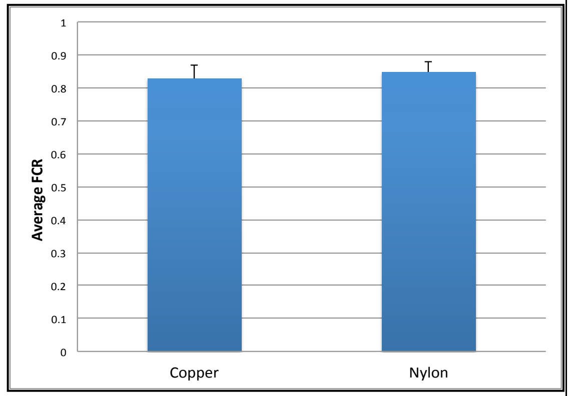

Figure 8. Average food conversion rates of juvenile WSB reared in copper-alloy and nylon mesh cages

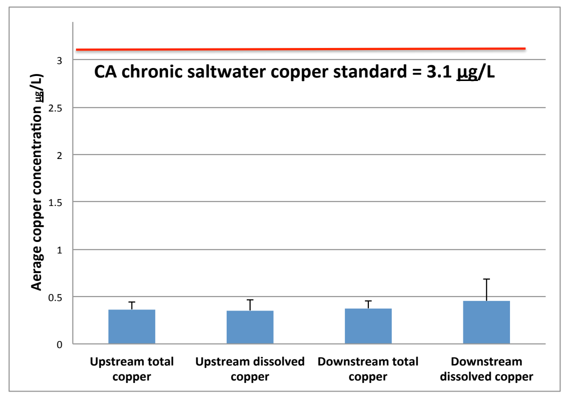

Figure 9. Dissolved seawater copper concentrations upstream and downstream of the cages.

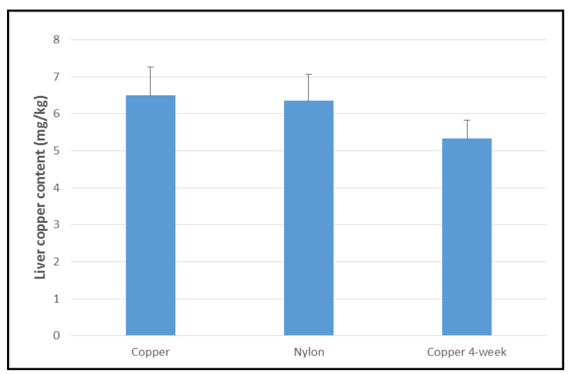

Figure 10. Liver copper concentrations from WSB reared in copper and nylon cages for three months; including WSB moved to land-based raceway tanks for one month post-study.