United Soybean Board
Final Report Form – Technical Bulletin

Project # and Title	Project #2463 Use of soy-based products in practical diets for white seabass, California yellowtail and striped bass
Organization & Project Leader	Organization: Hubbs-SeaWorld Research Institute Project Leader: Mark Drawbridge
Reporting Period	1/1/2012-12/31/2012

Introduction: Statement on the rationale and background for the studies

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*). Both species are highly valued commercial and sport fish in southern California and are 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. HSWRI is currently culturing YT to market size in offshore cages in northern Baja California, Mexico. Great potential exists to expand commercial culture in both northern Baja California, Mexico and Southern California. YT are in the family carangidae or jacks – a group that typically requires high levels of high quality fish-based protein in the diet. 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. 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 the influence of 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 requirement appears to be between 0.4 and 0.6% of the diet depending on the model used to estimate the requirement. A YT trial testing taurine inclusion from 0.25-1.25% showed no differences between treatments with regards to performance indicating that a taurine inclusion rate of 0.25% is adequate. 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 C_{18} 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.

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

Study 1 – Long term grow out trial with WSB (HSWRI/Auburn, HSWRI Carlsbad).

WSB were grown for 10 months, beginning in August 2012, comparing two commercial feeds with the best performing soy-based diet from our laboratory trials.

Study 2 – Long term grow out trial with YT (HSWRI/Auburn, HSWRI San Diego).

YT were grown for 10 months, beginning in June 2012, comparing a commercial feed with the best performing soy-based diet from our laboratory trials.

Study 3 – Commercial Growout Trial (HSWRI/Auburn/Pacifico Aquaculture, Baja California, Mexico)

Commercial farmers of Pacifico Aquaculture are growing WSB from 20g to a market size of ~600g using the best soy-based diet from our laboratory trials thus far. This trial started in May 2012 and is on-going.

Study 4 – Evaluation of Attractants in WSB feeds (HSWRI/Auburn, HSWRI Carlsbad).

An 8 week trial was run with WSB testing inclusion of rates of 1% and 2% of both squid and krill meal in 6% FM diets.

Study 5 – Further Evaluation of Replacement of Fish Oil with Soy Oil Supplemented with LC-PUFA in WSB feeds (HSWRI/SIU, HSWRI Carlsbad).

An 8 week trial was run with WSB to confirm the results of the previous trial assessing soy oils in WSB feeds. Diets were formulated with SFA and STD soy oil with and without DHA supplementation and compared to a fish oil control.

Study 6 – Evaluation of the Relative Importance of LC-PUFA's in YT Feeds (HSWRI/SIU, HSWRI Carlsbad).

An 8 week trial was run with YT to determine the relative importance of ARA, EPA and DHA in meeting the n-3 and n-6 LC-PUFA requirements by supplementing the fat base with these various LC-PUFA concentrates.

Study 7 – Further Evaluation of YT Protein Requirement (HSWRI/Auburn, HSWRI San Diego).

An 8 week trial was run with YT testing protein levels from 40-48% (40-47% soy) in increments of 2%.

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

Study 1 – Long term grow out trial with WSB (HSWRI/Auburn, HSWRI Carlsbad).

WSB were grown for 10 months, beginning in August 2012, comparing two commercial feeds with the best performing soy-based diet from our laboratory trials. The 43% soy, 12% fish meal formula (Table 1) outperformed the two commercial feeds, Skretting Marine Grower and Star Milling Trout (Table 2) with regards to growth and FCR (Table 3).

Table 1. Study 1 (WSB) and Study 2 (YT) diet formulations for long term trials (g/100g as is).

Ingredient (g/100g as is)	WSB	YT
Fishmeal-menhaden	12.00	20.00
Soybean meal solvent extracted	26.00	0.00
Soy protein concentrate ADM	17.00	41.30
Menhaden Fish Oil	6.60	10.20
Whole wheat	25.95	15.25
US Fish and wildlife trace mineral	0.10	0.10
US Fish and wildlife vitamin	0.40	0.40
Choline chloride	0.20	0.20
Stay C 35%	0.10	0.10
CaP-diebasic	1.80	1.00
Lecithin	1.00	1.00
Corn protein concentrate	8.00	10.00
Taurine	0.70	0.30
Mold inhibitor	0.15	0.15
Total	100.00	100.00

Table 2. Proximate composition of feeds used for WSB and YT growout trials.

% as is	USB-WSB	Skretting	Star	USB-YT
Moisture	7.9	8.9	9.2	8.5
Crude Protein	41.5	51.6	39.4	49.7
Crude Fat	7.8	13.0	8.4	12.9
Fiber	4.7	3.6	3.7	5.7
Ash	6.7	8.0	7.1	7.2

Table 3. Growth performance of WSB over 10 months fed a soy based diet vs. two commercial feeds.

Diet	Initial WT (g)	Final WT (g)	WT Gain (%)	Survival (%)	FCR
Skretting Marine Grower	53.6	326.5	272.9	92.1	1.99
Star Milling	52.9	241.5	188.6	85.7	2.52
USB	53.3	375.7	322.4	95.2	1.89

Study 2 – Long term grow out trial with YT (HSWRI/Auburn, HSWRI San Diego).

YT were grown for 10 months, beginning in June 2012, comparing a commercial feed with the best performing soy-based diet from our laboratory trials. The 41% soy, 20% fish meal diet (Table 1) performed as well as the Skretting Marine Grower (Table 2) with regards to Growth and FCR (Table 4).

Table 4. Growth performance of YT over 10 months fed a soy based diet vs. a commercial feed.

Diet	Initial WT (g)	Final WT (g)	WT Gain (%)	Survival (%)	FCR
Skretting Marine Grower	336.4	1056.6	720.2	92.8	3.23
USB	339.9	1153.0	813.1	88.2	3.20

Study 3 – Commercial Growout Trial (HSWRI/Auburn/Pacifico Aquaculture, Baja California, Mexico)

Commercial farmers of Pacifico Aquaculture are growing WSB from 20g to a market size of ~600g using the best soy-based diet from our laboratory trials thus far vs. EWOS Dyna Seabream diet. The USB diet was made by Ziegler Bros. and follows roughly the same formula as the soy-based diet used in the WSB growout trial described in Study 1 (Table 1). This trial started in May 2012 and is on-going. Thus far the fish that are being fed the EWOS diet are larger in size but we are seeing good performance in the group being fed the soy-based diet as well (Table 5). Comparing the cost to produce a kg of fish on each feed the two diets seem to match up quite well thus far given the lower cost of the soy-based feed.

Table 5. Growth performance of WSB at Pacifico Aquaculture fed a soy based diet (USB Soy - S) vs. a commercial feed (EWOS - E) over the first 8 months of a commercial grow out trial including cost/kg of production (FCR x $/kg feed).

	Avg wt (g)		**Wt Gain (%)**		**FCR**		**Total Survival (%)**		**Feed price($/kg)**		**Prod. cost ($/kg growth)**	
Date	**E**	**S**	**E**	**S**	**E**	**S**	**E**	**S**	**E**	**S**	**E**	**S**
25-Jul-12	60	60	161	161			100	100	1.85	1.41		
11-Sep-12	92	78	300	239	1.76	2.95	99	100			3.26	4.15
31-Oct-12	177	147	670	539	1.19	1.24	99	99			2.20	1.74
4-Jan-13	248	188	978	717	1.48	2.10	99	99			2.74	2.95

Study 4 – Evaluation of Attractants in WSB feeds (HSWRI/Auburn, HSWRI Carlsbad).

An 8 week trial was run with WSB testing inclusion of rates of 1% and 2% of both squid and krill meal in 6% fish meal diets (Table 6). There was no significant enhancement in performance from diets with squid or krill meal added as an attractant at either concentration (Table 7). This leads us to believe that the fish found the basal formulation palatable enough without the addition of an attractant. Attractants may be beneficial in formulations with less than 6% fish meal.

Table 6. USB 2012 WSB Trial 1 Attractants

Ingredient (g/100g as is)	No Attractant	1% Squid	2% Squid	1% Krill	2% Krill
Menhaden fishmeal	6.00	6.00	6.00	6.00	6.00
Soybean meal solvent extracted	25.00	25.00	25.00	25.00	25.00
Soy concentrate -soycomil	23.00	21.80	20.60	22.10	21.20
Empyreal 75 - Cargill	5.50	5.50	5.50	5.50	5.50
Menhaden fish oil	7.31	7.29	7.26	7.23	7.14
Corn starch	1.84	2.06	2.29	1.82	1.81
Whole wheat	26.00	26.00	26.00	26.00	26.00
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	1.80	1.80	1.80	1.80	1.80
Lecithin (soy refined, USB)	1.00	1.00	1.00	1.00	1.00
Methionine	0.30	0.30	0.30	0.30	0.30
Taurine	1.20	1.20	1.20	1.20	1.20
Squid meal	0	1.00	2.00	0	0
Krill meal	0	0	0	1.00	2.00
Total	100.00	100.00	100.00	100.00	100.00

Table 7. USB 2012 WSB Trial 1 Attractants

Attractant	Initial WT (g)	Final WT (g)	WT Gain (%)	Survival (%)	FCR
No Attractant	4.5	27.8^{ab}	520^{ab}	88	1.22
1% Squid	4.6	29.8^{a}	552^{a}	88	1.18
2% Squid	4.6	26.1^{b}	468^{b}	77	1.22
1% Krill	4.5	29.4^{a}	548^{a}	85	1.23
2% Krill	4.5	28.7^{a}	537^{ab}	90	1.21

Study 5 – Further Evaluation of Replacement of Fish Oil with Soy Oil Supplemented with LC-PUFA in WSB feeds (HSWRI/SIU, HSWRI Carlsbad).

An 8 week trial was run with WSB replacing fish oil using the ideal soy lipid identified in 2011 (SFA soy oil) in conjunction with LC-PUFA supplementation to meet the estimated fatty acid requirements. Diets were formulated with SFA and STD soy oil with and without DHA supplementation and compared to a fish oil control (Table 8). Growth results support our original hypothesis (Table 9). Specifically, SFA soy with or without DHA supplementation was equivalent to the FO control relative to growth performance, but growth using STD soy was significantly impaired. Supplementing the STD soy with DHA improved the growth results somewhat, but did not totally restore performance. Tissue analysis also confirmed previous results, indicating that SFA soy has little effect on fillet fatty acid profile, whereas STD soy significantly alters tissue composition: unless the DHA supplement is added to STD soy oil, fillets of fish fed this oil contain significantly less DHA than those fed fish oil; conversely, fish fed unsupplemented SFA soy oil are equivalent to those fed fish oil, and those fed the DHA supplemented-SFA soy exhibit significant DHA enrichment beyond the levels associated with feeding fish oil. Taken together, these results strongly support the use of SFA soy oil as a lipid source in WSB feeds; whereas STD soy oil can be used, greater caution must be exerted in order to ensure fatty acid deficiency does not develop.

Table 8. WSB 2012 soy lipids

Ingredient (g/100g as is)	FISH Oil Only	SFA Soy Only	STD Soy Only	SFA Soy + DHA	STD Soy +DHA
Menhaden fish meal	40.00	40.00	40.00	39.84	39.84
Soy protein concentrate	19.00	19.00	19.00	19.00	19.00
Corn starch	11.88	11.88	11.88	11.88	11.88
Wheat flour	15.00	15.00	15.00	11.24	11.24
Menhaden fish oil	7.10	0.00	0.00	0.00	0.00
Standard soybean oil	0.00	0.00	7.10	0.00	5.24
SFA-enriched soybean oil	0.00	7.10	0.00	5.24	0.00
Soy lecithin	1.00	1.00	1.00	1.00	1.00
Carboxymethylcellulose	2.00	2.00	2.00	2.00	2.00
Choline chloride	0.60	0.60	0.60	0.60	0.60
Stay-C	0.20	0.20	0.20	0.20	0.20
Vitamin premix	0.12	0.12	0.12	0.12	0.12
Mineral premix	0.10	0.10	0.10	0.10	0.10
Corn gluten meal	3.00	3.00	3.00	3.00	3.00
Algal DHA meal	0.00	0.00	0.00	5.78	5.78
Total	100.00	100.00	100.00	100.00	100.00

Table 9. WSB 2012 soy lipids

Diet	Initial WT (g)	Final WT (g)	WT Gain (%)	Survival (%)	FCR
FO Only	4.0	27.4[bc]	578[ab]	100	1.40[abc]
SFA Soy	4.2	31.2[a]	645[a]	100	1.31[c]
STD Soy	4.0	24.5[c]	507[c]	100	1.51[a]
SFA + DHA	4.1	29.7[ab]	626[a]	97	1.35[bc]
STD Soy + DHA	4.1	26.1[c]	543[bc]	98	1.46[ab]

Study 6 – Evaluation of the Relative Importance of LC-PUFA's in YT Feeds (HSWRI/SIU, HSWRI Carlsbad).

An 8 week trial was run with YT to determine the relative importance of ARA, EPA and DHA in meeting the n-3 and n-6 LC-PUFA requirements by supplementing the fat base with these various LC-PUFA concentrates (Table 10a,b). Growth results confirm the importance and apparent essentiality of DHA (22:6n-3) and ARA (20:4n-6): compared to a fish oil-based control feed, soy oil-based feeds yielded inferior growth performance unless supplemented with DHA and ARA (Table 11). Although there appeared to be some additional benefit in providing EPA (20:5n-3) as well, DHA and ARA appear to be the primary drivers of LC-PUFA essentiality in this species. Results clearly demonstrate that unless appropriate levels of LC-PUFAs are added to the diet, soy oil-based feeds are not sufficient to support growth of YT. Tissue analysis is complete and statistical analysis of results is nearing completion.

Table 10a. YT 2012 Soy Lipids

Ingredient (g/100g as is)	FO Control	SO Control	50% ARA	100% ARA	50% EPA	100% EPA
Menhaden fish meal	47.37	47.37	47.37	47.37	47.37	47.37
Soy protein concentrate	24.00	24.00	24.00	24.00	24.00	24.00
Menhaden fish oil	9.51	0.00	0.00	0.00	0.00	0.00
Soybean oil	0.00	9.51	9.37	9.24	8.48	7.45
Corn starch	7.95	7.95	7.95	7.95	7.95	7.95
Wheat feed flour	5.00	5.00	5.00	5.00	5.00	5.00
Corn gluten meal	3.25	3.25	3.25	3.25	3.25	3.25
Soy lecithin	1.00	1.00	1.00	1.00	1.00	1.00
Carboxymethyl cellulose	1.00	1.00	1.00	1.00	1.00	1.00
Choline chloride	0.60	0.60	0.60	0.60	0.60	0.60
Vitamin premix	0.12	0.12	0.12	0.12	0.12	0.12
Mineral premix	0.10	0.10	0.10	0.10	0.10	0.10
Stay C	0.10	0.10	0.10	0.10	0.10	0.10
ARA concentrate	0.00	0.00	0.14	0.27	0.00	0.00
DHA concentrate	0.00	0.00	0.00	0.00	1.03	0.00
EPA concentrate	0.00	0.00	0.00	0.00	0.00	2.06
TOTAL	100.00	100.00	100.00	100.00	100.00	100.00

Table 10b. YT 2012 Soy Lipids

Ingredient (g/100g as is)	50% DHA	100% DHA	50% ARA/DHA	100% ARA/DHA	50% ARA/EPA /DHA	100% ARA/EPA /DHA
Menhaden fish meal	47.37	47.37	47.37	47.37	47.37	47.37
Soy protein concentrate	24.00	24.00	24.00	24.00	24.00	24.00
Menhaden fish oil	0.00	0.00	0.00	0.00	0.00	0.00
Soybean oil	8.66	7.82	8.53	7.54	7.54	6.37
Corn starch	7.95	7.95	7.95	7.95	7.95	7.95
Wheat feed flour	5.00	5.00	5.00	5.00	5.00	5.00
Corn gluten meal	3.25	3.25	3.25	3.25	3.25	3.25
Soy lecithin	1.00	1.00	1.00	1.00	1.00	1.00

Carboxymethyl cellulose	1.00	1.00	1.00	1.00	1.00	1.00
Choline chloride	0.60	0.60	0.60	0.60	0.60	0.60
Vitamin premix	0.12	0.12	0.12	0.12	0.12	0.12
Mineral premix	0.10	0.10	0.10	0.10	0.10	0.10
Stay C	0.10	0.10	0.10	0.10	0.10	0.10
ARA concentrate	0.00	0.00	0.14	0.27	0.05	0.08
DHA concentrate	0.85	1.69	0.85	1.69	1.03	1.17
EPA concentrate	0.00	0.00	0.00	0.00	0.89	1.90
TOTAL	100.00	100.00	100.00	100.00	100.00	100.00

Table 11. YT 2012 Soy Lipids

Soy Lipid Diet	Initial WT (g)	Final WT (g)	WT Gain (%)	Survival (%)	FCR
FO Control	5.1	43.0[bc]	746.9[bc]	82	1.53[bcd]
SO Control	5.0	31.9[d]	532.3[d]	87	1.84[a]
50% ARA	5.0	31.6[d]	533.1[d]	91	1.78[ab]
100% ARA	5.1	31.3[d]	509.6[d]	91	1.87[a]
50% EPA	5.1	35.7[d]	604.9[cd]	87	1.68[abc]
100% EPA	5.1	33.1[d]	544.4[d]	89	1.81[ab]
50% DHA	5.1	32.9[d]	544.4[d]	87	1.79[ab]
100% DHA	5.1	36.2[dc]	604.7[cd]	91	1.65[abc]
50% ARA/DHA	5.1	34.2[d]	569.2[d]	87	1.73[abc]
100% ARA/DHA	5.1	44.4[b]	773.9[b]	98	1.47[cde]
50% ARA/EPA/DHA	5.1	59.9[a]	1079.7[a]	100	1.19[e]
100% ARA/EPA/DHA	5.3	58.2[a]	1000.7[a]	100	1.26[de]

Study 7 – Further Evaluation of YT Protein Requirement (HSWRI/Auburn, HSWRI San Diego).

An 8 week trial was run with YT testing protein levels from 40-48% (40-47% soy) in increments of 2% (Table 12). Growth began to plateau at 44% and there was little improvement at 46 and 48% protein (Table 13). Whole fish were collected and were analyzed for proximate composition and nutrient retention. The best PER was seen at 42% protein. This reduction in targeted protein levels will facilitate the use of soy products.

Table 12. YT 2012 Protein

	Protein/lipid				
Ingredient (g/100g as is)	48/14	46/13	44/12	42/11	40/10
Menhaden fish meal	30.00	28.74	27.53	26.24	25.01
Soybean meal solvent extracted	22.30	21.36	20.47	19.50	18.59

Soy concentrate -soycomil	25.00	23.95	22.94	21.87	20.84
Empyreal 75 - Cargill	2.00	1.92	1.84	1.75	1.67
Menhaden fish oil	9.38	8.53	7.68	6.84	6.00
Corn starch	8.97	12.90	16.69	20.70	24.55
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	0.00	0.25	0.50	0.75	1.00
Lecithin (soy refined, USB)	1.00	1.00	1.00	1.00	1.00
Taurine	0.30	0.30	0.30	0.30	0.30
Total	100.00	100.00	100.00	100.00	100.00

Table 13. YT 2012 Protein

Diet (P/L)	Initial WT (g)	Final WT (g)	WT Gain (%)	Survival (%)	FCR	PER
48/14	16.1	213.3[a]	1225.5[a]	100.0	1.19[b]	1.76[b]
46/13	16.2	213.0[a]	1217.9[a]	100.0	1.18[b]	1.84[ab]
44/12	16.3	208.1[a]	1179.6[a]	98.7	1.18[b]	1.93[ab]
42/11	16.1	190.2[b]	1081.0[b]	82.7	1.17[b]	2.05[a]
40/10	16.1	166.7[c]	933.3[c]	100.0	1.43[a]	1.76[b]

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

Results of our research continue to be very encouraging with regards to the development and commercialization of soy-based diets for WSB and YT. Long term growout trials have shown that soy-based diets perform equal to or better than commercial formulations and commercial trial that is currently underway is showing that a soy-based feed is competitive with a commercial feed. This information will be valuable to show the feed manufacturing industry that soy based diets are cost effective for these species. Feed manufacturers can also use the data to identify the maximum level of inclusion of soybean meal and soy oil in production diets. The project results will also target fisheries managers interested in stock replenishment of marine finfish, as well as commercial farmers who collectively will benefit from access to economically and environmentally sustainable diets. California has already legislated a mandate to "minimize the use of fish meal…", and this research clearly demonstrates how that may be practically accomplished.