Use of Soy-based Products in Practical Diets for White Seabass

2008 Year-End Report Submitted to the United Soybean Association

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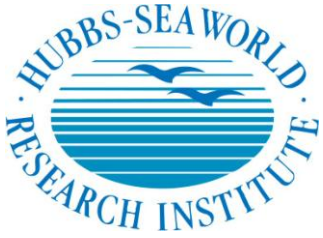

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Final Report

Project Title: USB 8463: Use of Soy-Based Products in Practical Diets for White Seabass

PROGRESS

Performance measure:
The ultimate goal of the project will be to advance practical diet development for this white seabass (WSB). This is important for both the culture of this species for stock enhancement as well as the great potential to expand the culture of this species commercially. The specific objectives of this research will be to: 1) Evaluate the response of WSB to a modification of the existing commercial diet being feed to WSB, 2) Evaluate the response of WSB to a diet with increasing levels of soy protein concentrate, 3) Evaluate the response of WSB to a diet with increasing levels of soy oil as a fish oil replacement.

Progress:
This research was conducted with juvenile WSB over three 56 day growth trials during the summer and fall of 2008.

Background:
White seabass (*Atractoscion nobilis*) are a 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. This species is currently cultured by Hubbs SeaWorld Research Institute (HSWRI) under contract by the California Department of Fish and Game for stock enhancement in southern California waters as the Ocean Resources Enhancement and Hatchery Program (OREHP). The hatchery in Carlsbad, California is capable of producing an excess of fingerlings required for the stock enhancement program and there is great potential in the near future for the commercial culture of the species particularly in offshore net cage systems to utilize the readily available supply of fingerlings. Within the existing stock enhancement project, WSB are typically cultured to an average size of 20-25 cm prior to release. Fish are reared to this size on commercially available diets. Many other species in the same family (Sciaenidae) are cultured worldwide and some, such as red drum (*Sciaenops ocellatus*), are successfully reared on diets utilizing soy protein. WSB is considered a prime candidate to be reared on practical diets utilizing soy based protein as well. Use of alternative sources of protein such as soy based protein will promote sustainability of the culture of WSB.

Materials and methods:
Three growth trials were conducted in a recirculating system at HSWRI's marine fish hatchery in Carlsbad, CA. Each trial was conducted over a 56 day culture period. The first trial was designed to provide initial data on the efficacy of several practical diets. Three diets and a commercial reference diet were evaluated. The research diets were 42% protein 12% lipid with varying protein sources: fish meal (FM), FM + solvent extracted soybean meal (SBM) or FM + soy protein concentrate (SPC). The second trial was designed to evaluate varying levels of FM replaced with SPC. The basal diet contained 40% FM and 24.6% SBM as the primary protein sources. The FM was then reduced to 30, 20 and 15% of the diet using SPC as the replacement protein. Soy-based protein ranges from 29-67% of total protein in these diets. A third trial was conducted to evaluate various lipid levels in soy-based diets. These diets were formulated with 42% protein (44% of total protein is soy–based) with lipid levels increasing from 10-18%.

Diets were prepared by mixing pre-ground dry ingredients and menhaden fish oil in a food mixer (Hobart, Troy, OH) 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° C) to a moisture content of less than 10%. Diets were stored at -20° C, and prior to use each diet were ground and sieved to an appropriate size. Diets were analyzed for proximate composition by the New Jersey Feed Lab (P.O. Box 06650, Trenton, NJ, 08650).

The culture system utilized for these studies was a semi-closed recirculating system consisting of 40, 60L square culture tanks, water pump, supplemental aeration (provided using a central line, regenerative blower, and air diffusers) as well as mechanical and biological filtration. A small amount (<2L/min) of ozonated seawater was continuously added to the system for water exchange. Water temperature was controlled with a heat exchanger (Aqua Logic Inc., San Diego, CA). Tanks were siphoned daily to remove solids. Temperature, dissolved oxygen and salinity were measured daily and pH was measured biweekly using a Hach HQ 40d multi probe meter. Total ammonia-nitrogen was determined biweekly using the salicilate method.

Every two weeks and at the termination of each trial fish were counted and weighed. Feed conversion ratio (FCR) was calculated at the end of the feeding trials as the dry weight of feed offered divided by the wet weight gain of the fish.

The first growth trial was conducted with juvenile fish (3.5 g mean initial weight) stocked at a rate of 15 fish per tank (60 L) using four replicate tanks per dietary treatment. To provide initial data on the efficacy of practical diets utilizing soy protein a series of three diets with FM or FM with either SBM or SPC and a commercial reference diet were evaluated (Table 1). Test diets included: 1) FM 59%, 2) FM 40% - SPC 19%, 3) FM 40% - SBM 25%. All test diets were formulated to have similar proximate analyses with 42% protein and 12% lipid and the commercial diet had 50.8% protein and 13.3% lipid (Table 2).

The second growth trial used juvenile fish (16.8 g mean initial weight) stocked at a rate of 10 fish per tank using for replicate tanks per dietary treatment. In order to evaluate substitution of FM with SBM or a combination of SBM and SPC on an equal protein basis a series of 4 diets were produced (Table 3). Test diets included: 1) FM 40% - SBM 24.6%, 2) FM 30% - SBM 24.6% - SPC 10.3%, 3) FM 20% - SBM 24.6% - SPC 20.5%, 4) FM 15% SBM - 24.6% - SPC 25.6%. All diets were formulated to have similar proximate analyses with 42% protein and 12% lipid. Proximate analyses as well as amino acid composition of the test diets are presented in Table 4.

The third growth trial used juvenile fish (6.3 g mean initial weight) stocked at a rate of 15 fish per tank (60L) using for replicate tanks per dietary treatment. In order to evaluate various levels of lipid in soy based diets a series of five diets were produced. The test diets were formulated with 30% FM, 24% SBM and 10% SPC (44% of total protein soy-based) with an increasing level of lipid (Table 5). All diets were formulated to have 42% protein and 10,12,14,16 or 18% lipid (Table 6).

Statistical analysis:
All data were subjected to a one-way analysis of variance to determine significant ($P \leq 0.05$) differences among the treatment means. Student-Neuman Keuls' multiple range test was used to distinguish significant differences between treatment means. All statistical analyses were conducted using SAS system for windows, (SAS Institute, Cary, NC).

Results and Discussion:

The first growth trial was designed to provide initial data on the efficacy of practical diets utilizing soy protein. In this trial an all FM diet was compared to two diets in which approximately 30% of the FM was replaced with either SBM or SPC. A commercial reference diet typically used to rear this fish was also included for initial comparisons. The growth trial was conducted with juvenile fish having an initial weight of 3.5 g reared over a 56 day culture period. During the trial water quality was maintained within acceptable limits for this species and fish were in good health (Table 7).

Production parameters for the first growth trial included final weight, biomass gained, survival, and feed conversion ratio (FCR) (Table 8). Performance of diets as final weight and biomass gained (%) was significantly ($P<0.05$) lower from inclusion of either SBM or SPC. However there was no significant difference between soy-based diets and the commercial reference diet. FCR showed a similar, significant tendency. Results indicate that growth and survival of WSB fed these diets performed adequately when compared to the FM and reference diet. These results also confirm the efficacy of the basal diet as model for further research.

The second growth trial was designed to evaluate the efficacy of substituting FM with increasing levels of soy-based protein from 24.6% to 50.2% inclusion. The growth trial was conducted with juvenile fish having an initial weight of 16.8 g reared over a 56 day culture period. During the trial water quality was maintained within acceptable limits for this species and fish were in good health (Table 7).

Production parameters for the second growth trial included final weight, biomass gained, survival, and FCR (Table 9). Final weight and biomass gained (%) had a negative correlation and FCR had a positive correlation, all of which were significant ($P<0.05$), to the inclusion rate of soy-based protein. Given the incremental reduction of performance of the fish, the results probably indicate a limiting nutrient or palatability problem with the higher levels of inclusion. Consequently, limiting nutrients, combination with other ingredients and palatability problems should be investigated. Although reductions in growth were observed survival and FCR were acceptable especially when one considers the results of the third growth trial.

The third growth trial was designed to evaluate a range, 10-18%, of lipid levels in soy based diets. The growth trial was conducted with juvenile fish having an initial weight of 6.3 g reared over a 56 day culture period. During the trial water quality was maintained within acceptable limits for this species and fish were in good health (Table 7).

Production parameters for the third growth trial included final weight, biomass gained, survival, and FCR (Table 10). Results indicate that final weight and biomass gained (%) are higher and FCR better at the lowest (10%) lipid level. This tendency was significant ($P<0.05$) for biomass gained and FCR. Whole body proximate analysis indicated that body lipid increased with increasing dietary lipid (Table 11). Whole body protein was highest in fish fed the diet with the lowest (10%) lipid level. The increase in lipid deposition probably indicates that energy relative to the available protein was adequate even in the lower lipid diets. Hence, it is unlikely that the high lipid diets that have been utilized are required for WSB.

Results from these initial trials on WSB are encouraging in that a considerable portion of the protein can be replaced with soy-based protein. Reductions in growth were observed as FM was reduced. This response appears primarily due to nutrient imbalances or palatability problems which can be corrected as opposed to an allergic response. Test results indicate that the 42% protein diet may contain higher levels of protein than are required. Hence, prior to further

optimizing of soy products, further evaluation should be done to determine if a lower level of dietary protein is acceptable. Once the optimal protein level is determined for this species, combinations of soy-based protein with other protein sources and/or supplements should be evaluated to maximize the inclusion of SBM and SPC.

Table 1. Diet formulations for WSB for initial evaluation of practical soy-based diets.

Ingredient	(g/100g)		
	Fishmeal	FM40-SC	FM40-SE
Fishmeal	59.0	40.0	40.0
Soybean meal solvent extracted	0.0	0.0	25.0
Soyprotein concentrate ADM (63% P)	0.0	19.0	0.0
Menhaden Fish Oil	5.3	7.1	7.0
Corn Starch	14.7	12.9	6.9
Whole wheat	16.0	16.0	16.0
ASA Trace Mineral premix	0.25	0.25	0.25
ASA Vitamin premix w/o choline	0.50	0.50	0.50
Choline chloride	0.20	0.20	0.20
Stay C 35%	0.10	0.10	0.20
Lecithin (soy refined, MP Biomedical)	1.00	1.00	1.00
Corn Gluten meal	3.00	3.00	3.00
Total	100.00	100.00	100.00

Table 2. Proximate composition of diets for initial evaluation of soy-based diets for WSB.

	Fishmeal	FM40-SC	FM40-SE	Commercial
Moisture	8.12	11.00	6.47	7.99
Protein	43.3	42.0	44.4	50.8
Fat	11.29	11.00	12.19	13.33
Fiber	0.32	0.98	1.19	1.48
Ash	14.75	12.13	11.34	9.12

Table 3. Diet formulations for WSB, evaluating replacement of FM with soy-based protein.

Ingredient	**(g/100g)**			
	FM40-SE	FM30	FM20	FM15
Fishmeal	40.0	30.0	20.0	15.0
Soybean meal solvent extracted	24.6	24.6	24.6	24.6
Soyprotein concentrate ADM (63% P)	0.0	10.3	20.5	25.6
Menhaden Fish Oil	7.0	7.9	8.9	9.3
Corn Starch	7.9	6.6	4.7	3.6
Whole wheat	16.0	16.0	16.0	16.0
ASA Trace Mineral premix	0.25	0.25	0.25	0.25
ASA Vitamin premix w/o choline	0.50	0.50	0.50	0.50
Choline chloride	0.20	0.20	0.20	0.20
Stay C 35%	0.10	0.10	0.10	0.10
CaP-diebasic MP Biomedical	0.00	0.00	0.80	1.40
Lecithin (soy refined, MP Biomedical)	1.00	1.00	1.00	1.00
Corn Gluten meal	2.50	2.50	2.50	2.50
Total	100.00	100.00	100.00	100.00

Table 4. Proximate composition, protein digestibility and amino acid profile of diets for WSB evaluating replacement of FM with soy-based protein.

	FM40-SE	FM30	FM20	FM15
Moisture	8.54	10.31	9.52	8.77
Protein	42.9	41.6	42.2	42.8
Fat	12.11	12.34	12.33	12.56
Fiber	1.10	1.76	2.23	1.91
Ash	10.15	8.49	7.67	7.43
% Protein Digestible[1]	86.71	82.93	79.86	79.21
Amino Acid				
Methionine	0.95	0.87	0.79	0.80
Cystine	0.44	0.46	0.49	0.54
Lysine	2.89	2.85	2.71	2.85
Phenylalanine	0.29	0.35	0.48	0.36
Leucine	2.68	2.42	2.56	2.48
Isoleucine	1.36	1.29	1.49	1.46
Threonine	1.79	1.66	1.65	1.67
Valine	2.05	1.94	2.09	2.07
Histidine	0.99	1.06	1.09	1.10
Arginine	3.11	2.84	2.43	2.63
Glycine	3.17	2.77	2.55	2.42
Aspartic Acid	4.46	4.34	4.49	4.72
Serine	2.02	1.88	2.00	2.04
Glutamic Acid	6.64	7.16	7.40	7.87
Proline	2.31	2.31	2.44	2.57
Hydroxyproline	0.51	0.42	0.34	0.28
Alanine	3.66	3.44	3.48	3.30
Tyrosine	0.76	0.79	0.90	0.82
Total	40.07	38.86	39.37	39.98

[1]Using .0002% pepsin solution

Table 5. Diet formulations for WSB, evaluating various lipid levels in soy-based diets.

Ingredient	**(g/100g)**				
	44-10	44-12	44-14	44-16	44-18
Fishmeal	30.0	30.0	30.0	30.0	30.0
Soybean meal solvent extracted	25.0	25.0	25.0	25.0	25.0
Soyprotein concentrate ADM (63% P)	10.0	10.0	10.0	10.0	10.0
Menhaden Fish Oil	5.9	7.9	9.9	11.9	13.9
Corn Starch	8.5	6.5	4.5	2.5	0.5
Whole wheat	16.0	16.0	16.0	16.0	16.0
ASA 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
Lecithin (soy refined, MP Biomedical)	1.00	1.00	1.00	1.00	1.00
Corn Gluten meal	2.50	2.50	2.50	2.50	2.50
Total	100.00	100.00	100.00	100.00	100.00

Table 6. Proximate composition of diets evaluating various lipid levels in soy-based diets for WSB.

	44-10	44-12	44-14	44-16	44-18
Moisture	4.93	4.96	6.37	7.07	6.39
Protein	44.0	44.0	44.1	43.4	43.3
Fat	10.98	12.66	14.73	16.54	18.43
Fiber	1.48	2.11	1.88	1.98	1.74
Ash	11.72	10.80	9.46	9.07	9.16

Table 7. Water quality parameters for WSB reared in semi-closed recirculating systems for the three growth trials.

Parameter	Average ± Standard Deviation		
	Trial 1	Trial 2	Trial 3
Temperature (º C)	18.0 ± 0.8	18.1 ± 0.5	18.1 ± 0.3
Dissolved Oxygen (mg/L)	7.7 ± 0.3	7.4 ± 0.2	7.1 ± 0.2
pH	7.8 ± 0.1	7.8 ± 0.1	7.8 ± 0.1
Salinity (ppt)	34.4 ± 0.6	34.5 ± 0.7	34.2 ± 0.4
TAN (mg/L)	0.05 ± 0.03	0.03 ± 0.03	0.02 ± 0.03

Table 8. Response of WSB to initial evaluation of practical soy-based diets. Values with different superscripts are significantly different (P<0.05).

Diet	Initial wt (g)	Final wt (g)	% Gain	% Surv	FCR
Fishmeal	3.4	17.2 [a]	401.8 [a]	96.7	1.04 [a]
FM40-SC	3.5	15.4 [b]	344.8 [b]	98.3	1.17 [b]
FM40-SE	3.5	14.1 [c]	307.2 [b]	93.3	1.23 [b]
Commercial	3.4	14.9 [bc]	332.4 [b]	98.3	1.20 [b]
PSE	0.112	0.342	11.662	2.041	0.018
P value	0.9886	0.0002	0.0007	0.3096	<0.0001

Table 9. Response of WSB to diets with increasing levels of soy-based protein. Values with different superscripts are significantly different (P<0.05).

Diet	Initial wt (g)	Final wt (g)	% Gain	% Surv	FCR
FM40-SE	16.9	36.5 [a]	116.6 [a]	100.0	1.53 [a]
FM30	16.8	33.4 [b]	99.2 [b]	100.0	1.77 [b]
FM20	16.8	29.2 [c]	74.3 [c]	97.5	2.22 [c]
FM15	16.0	27.1 [d]	69.0 [c]	95.0	2.42 [c]
PSE	0.211	0.619	4.242	2.795	0.077
P value	0.0531	<.0001	<.0001	0.5519	<.0001

Table 10. Response of WSB to soy-based diets with various lipid levels. Values with different superscripts are significantly different (P<0.05).

Diet	Initial wt (g)	Final wt (g)	% Gain	% Surv	FCR
44-10	6.2	19.9	222.3 [a]	96.7	1.24 [a]
44-12	6.2	18.8	201.5 [b]	98.3	1.30 [ab]
44-14	6.4	18.6	190.3 [b]	100.0	1.36 [b]
44-16	6.4	18.9	196.6 [b]	96.7	1.33 [ab]
44-18	6.2	18.9	205.7 [b]	98.3	1.31 [ab]
PSE	0.1553	0.4922	4.9675	1.6907	0.0230
P value	0.7142	0.4387	0.0046	0.5732	0.0336

Table 11. Proximate composition of whole WSB fed soy-based diets with various lipid levels.

Diet	Moisture	Protein	Fat	Ash
44-10	79.46	15.9	2.21	3.30
44-12	78.21	14.7	2.62	3.80
44-14	79.85	14.4	2.62	3.09
44-16	78.24	14.9	2.73	3.83
44-18	77.94	14.5	2.94	4.30
PSE	0.6392	0.3714	0.1622	0.3046
P value	0.1791	0.0574	0.0727	0.0993