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[Article]

Development of a Practical Soy-Based Diet for White Seabass

DAVE JIRSA*
Hubbs-SeaWorld Research Institute, 2595 Ingraham Street, San Diego, California 92109, USA

D. ALLEN DAVIS
Department of Fisheries and Allied Aquacultures, Auburn University, 315 Swingle Hall, Auburn, Alabama 36849, USA

MARK DRAWBRIDGE
Hubbs-SeaWorld Research Institute, 2595 Ingraham Street, San Diego, California 92109, USA

Abstract.—Two feeding trials were conducted to begin development of a practical soy-based diet for white seabass *Atractoscion nobilis*. The first trial was designed to provide initial data on the efficacy of practical soy-based diets. Three diets and a commercial reference diet were evaluated. Research diets were 42% protein and 12% lipid, with varying protein sources: fish meal (FM), FM plus solvent-extracted soybean meal (SBM), or FM plus soy protein concentrate (SPC). Final weight (14.1–17.2 g), percent weight gain (307.2–401.8%), and feed conversion ratio (FCR; 1.0–1.2) followed similar trends, with fish offered the FM-based diet significantly outperforming the other dietary treatment groups. There was no significant difference between soy-based diets and the commercial reference diet, and no significant differences in survival due to dietary treatments were observed. The second trial was designed to evaluate varying levels of FM replacement with SPC in a series of four diets containing 42% protein and 12% lipid. The basal diet contained 40% FM and 24.6% SBM as primary protein sources. The FM was then reduced to 30, 20, and 15% of the diet using SPC as the replacement protein. Final weight (27.1–36.5 g), percent weight gain (69.0–116.6%), and FCR (1.5–2.4) followed similar trends, with performance decreasing as FM level was reduced. In general, each incremental reduction in FM resulted in significant reductions in final weight and percent weight gain and significant increases in FCR. Results from these initial trials on white seabass are encouraging because the poor response was most likely due to a nutrient imbalance or palatability problem, which can be corrected, as opposed to an allergic response. Given that the open formulations performed similarly to the commercial feed, these simple formulations can be used as a starting point for the development of practical diets for white seabass.

The white seabass *Atractoscion nobilis* is a highly valued commercial and sport fish in southern California and is considered an excellent food fish. White seabass commonly occur from northern Baja California, Mexico, to Point Conception, California (Thomas 1968). Adult white seabass inhabit the nearshore zone over rocky bottoms and kelp beds (Young 1973), reaching a length of 1.5 m and a weight of 38 kg (Miller and Lea 1972). Juveniles and age-0 fish are found in embayments and shallow water along the open coast (Allen and Franklin 1988, 1992).

White seabass have been cultured by the Hubbs-SeaWorld Research Institute (HSWRI) under contract by the California Department of Fish and Game for stock enhancement in southern California waters as part of the Ocean Resources Enhancement and Hatchery Program (OREHP) since 1986. The HSWRI's marine fish hatchery in Carlsbad, California, has been in operation since 1995 and is capable of producing an excess of fingerlings required for the stock enhancement program. There is potential for the commercial culture of the species—particularly in offshore net-cages—to utilize the surplus supply of fingerlings. Within the existing stock enhancement project, white seabass are typically cultured to an average length of 20–25 cm prior to release. During this period, they are reared on commercially available diets formulated for other species.

Protein is one of the most expensive ingredients in diets for marine aquaculture, which have traditionally utilized fish meal as the primary protein source. It is increasingly important to find an alternative protein source because the supply of fish meal is limited and inconsistent and demand is increasing. Plant proteins, particularly soy proteins, have the potential to provide the aquaculture industry with a sustainable protein source that is less expensive and has more opportunities for expanded production. There has been no previous research to determine the feasibility of replacing fish meal with soy protein in practical diets

* Corresponding author: djirsa@hswri.org

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for white seabass. However, many other species in the same family (Sciaenidae) are cultured worldwide, and some (e.g., red drum *Sciaenops ocellatus*) have been reared on soy-based diets (Reigh and Ellis 1992; Davis et al. 1995; McGoogan and Gatlin 1997; Davis and Arnold 2004). Although white seabass do well on commercial feeds, nutrient requirements must be well understood if this species is to be cultured efficiently. The goal of this research was to begin development of a practical soy-based diet for white seabass by comparing several soy-based diets with the commercial diet currently used to produce white seabass.

Methods

General methods.—Two growth trials of 56 d in duration were conducted in a recirculating system at the HSWRI's marine fish hatchery in Carlsbad. Juvenile white seabass reared from eggs spawned by captive, wild-caught broodstock were used for the trials.

The first trial was designed to provide initial data on the efficacy of several practical diets, and the second trial was designed to evaluate varying levels of fish meal replacement with soy protein. Diets were prepared by mixing preground dry ingredients and menhaden fish oil in a food mixer (Hobart, Troy, Ohio) for 15 min. 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 were ground and sieved to an appropriate size prior to use. Diets were analyzed for proximate composition by the New Jersey Feed Laboratory (Trenton, New Jersey).

The experimental system was a semiclosed recirculating system consisting of forty 60-L, square culture tanks, a water pump, supplemental aeration (provided using a central line, regenerative blower, and air diffusers), and mechanical and biological filtration. A small amount (<2 L/min) of ozonated seawater was continuously added to the system for water exchange. Water temperature was controlled using a titanium heat exchanger. 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 multiprobe meter (Hach Company, Loveland, Colorado). Total ammonia nitrogen was measured twice weekly using the salicylate method (Hach Company).

Every 2 weeks and at the termination of each trial, fish were counted and group weighed. Feed conversion ratio (FCR) was calculated at the end of the feeding trials (FCR = [dry weight of feed offered]/[wet weight gain of the fish]).

TABLE 1.—Practical diet formulations (g/100 g of diet, as-is basis) for white seabass trial 1 to evaluate a fish-meal-based (FM) diet or diets containing soy protein concentrate (SPC) or solvent-extracted soybean meal (SBM).

Ingredient	FM	FM40-SPC	FM40-SBM
Menhaden FM[a]	59.0	40.0	40.0
SBM, solvent extracted[b]	0.0	0.0	25.0
SPC, ADM (63% P)[c]	0.0	19.0	0.0
Menhaden fish oil[a]	5.3	7.1	7.0
Corn starch[d]	14.65	12.85	6.85
Whole wheat	16.0	16.0	16.0
ASA trace mineral premix[e]	0.25	0.25	0.25
ASA vitamin premix without choline[f]	0.50	0.50	0.50
Choline chloride[d]	0.20	0.20	0.20
Stay C, 35%[g]	0.10	0.10	0.20
Lecithin (soy refined, MP Biomedicals)[d]	1.00	1.00	1.00
Corn gluten meal[h]	3.00	3.00	3.00
Total	100.00	100.00	100.00

[a] Omega Protein, Inc., Reedville, Virginia.
[b] De-hulled, solvent-extracted SBM, Faithway Feed Co., Inc., Guntersville, Alabama.
[c] Soycomil P, SPC, 63% protein (P), Archer Daniels Midland (ADM), Decatur, Illinois.
[d] MP Biomedicals, Inc., Solon, Ohio.
[e] American Soybean Association (ASA) Premix (g/100 g of premix): cobalt chloride, 0.004; cupric sulfate pentahydrate, 0.250; ferrous sulfate heptahydrate, 4.0; manganous sulfate anhydrous, 0.650; potassium iodide, 0.067; sodium selenite, 0.010; zinc sulfate heptahydrate, 13.193; α cellulose, 81.826.
[f] ASA Premix (g/kg of premix): thiamin HCl, 0.5; riboflavin, 8.0; pyridoxine HCl, 5.0; Ca-pantothenate, 20.0; niacin, 40.0; biotin, 0.040; folic acid, 1.80; cyanocobalamin, 0.002; vitamin A acetate (500,000 international units [IU]/g) 2.40; vitamin D3 (400,000 IU/g), 0.50; DL-α-tocopheryl acetate, 80.0; α cellulose, 834.258.
[g] Stay C (L-ascorbyl-2-polyphosphate, 35% Active C), Roche Vitamins, Inc., Parsippany, New Jersey.
[h] Grain Processing Corporation, Muscatine, Iowa.

Trial 1.—The first growth trial was conducted with juvenile fish (mean ± SD initial weight = 3.5 ± 0.2 g) stocked at a rate of 15 fish/tank using four replicate tanks per dietary treatment. To provide initial data on the efficacy of practical diets utilizing soy protein, a commercial reference diet and a series of three test diets with fish meal or fish meal plus either solvent-extracted soybean meal (SBM) or soy protein concentrate (SPC) were evaluated (Table 1). Test diets included (1) 59% fish meal, (2) 40% fish meal plus 19% SPC, and (3) 40% fish meal plus 25% SBM. All test diets were formulated to have similar proximate analyses with 42% protein and 12% lipid, whereas the commercial diet had 50.8% protein and 13.3% lipid (Table 2).

Trial 2.—The second growth trial used juvenile fish (mean ± SD initial weight = 16.8 ± 0.6 g) stocked at a rate of 10 fish/tank, with four replicate tanks per dietary

TABLE 2.—Proximate composition (g/100 g of diet, as-is basis)[a] of diets used in an initial evaluation of soy-based diets for white seabass. See table 1 for diet codes and descriptions.

Component	FM	FM40-SPC	FM40-SBM	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

[a] Analyses conducted by New Jersey Feed Laboratory, Trenton, New Jersey.

treatment. Test diets included (1) 40% fish meal plus 24.6% SBM, (2) 30% fish meal plus 24.6% SBM and 10.3% SPC, (3) 20% fish meal plus 24.6% SBM and 20.5% SPC, and (4) 15% fish meal plus 24.6% SBM and 25.6% SPC (Table 3). All diets were formulated to have similar proximate analyses with 42% protein and 12% lipid. Proximate analyses and amino acid composition of the test diets are presented in Table 4.

TABLE 3.—Diet formulations for white seabass trial 2, evaluating replacement of fish meal (FM) with soy protein concentrate (SPC; g/100 g of diet, as-is basis).

Ingredient	FM40-SBM	FM30	FM20	FM15
Menhaden FM[a]	40.0	30.0	20.0	15.0
Soybean meal (SBM), solvent extracted[b]	24.6	24.6	24.6	24.6
SPC, ADM (63% P)[c]	0.0	10.3	20.5	25.6
Menhaden fish oil[a]	7.0	7.9	8.9	9.3
Corn starch[d]	7.85	6.65	4.65	3.55
Whole wheat	16.0	16.0	16.0	16.0
ASA trace mineral premix[e]	0.25	0.25	0.25	0.25
ASA vitamin premix without choline[f]	0.50	0.50	0.50	0.50
Choline chloride[d]	0.20	0.20	0.20	0.20
Stay C, 35%[g]	0.10	0.10	0.10	0.10
CaP, dibasic (MP Biomedicals)[d]	0.00	0.00	0.80	1.40
Lecithin (soy refined, MP Biomedicals)[d]	1.00	1.00	1.00	1.00
Corn gluten meal[h]	2.50	2.50	2.50	2.50
Total	100.00	100.00	100.00	100.00

[a] Omega Protein, Inc.
[b] De-hulled solvent-extracted SBM, Faithway Feed Co., Inc.
[c] Soycomil P, SPC, 63% protein (P), Archer Daniels Midland (ADM).
[d] MP Biomedicals, Inc.
[e] ASA Premix (g/100 g of premix): cobalt chloride, 0.004; cupric sulfate pentahydrate, 0.250; ferrous sulfate heptahydrate, 4.0; manganous sulfate anhydrous, 0.650; potassium iodide, 0.067; sodium selenite, 0.010; zinc sulfate heptahydrate, 13.193; α cellulose, 81.826.
[f] ASA Premix (g/kg of premix): thiamin HCl, 0.5; riboflavin, 8.0; pyridoxine HCl, 5.0; Ca-pantothenate, 20.0; niacin, 40.0; biotin, 0.040; folic acid, 1.80; cyanocobalamin, 0.002; vitamin A acetate (500,000 international units [IU]/g) 2.40; vitamin D3 (400,000 IU/g), 0.50; DL-α-tocopheryl acetate, 80.0; α cellulose, 834.258.
[g] Stay C (L-ascorbyl-2-polyphosphate, 35% active C), Roche Vitamins, Inc.
[h] Grain Processing Corporation.

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. The Student–Newman–Keuls multiple range test was used to distinguish significant differences between treatment means. All statistical analyses were conducted using the Statistical Analysis System for Windows (SAS Institute, Cary, North Carolina).

Results

Trial 1

During trial 1, water quality was maintained at the following levels: temperature at 18°C, dissolved oxygen near saturation (>7.0 mg/L), and pH at 7.8 (Table 5). Production parameters measured in the first growth trial included final weight, percent weight gain, survival, and FCR (Table 6). Final weights (17.2 g) were significantly greater in fish that were offered the fish-meal-based diet. Final weights of white seabass reared on diets formulated with SBM (14.1 g) or SPC (15.4 g) were intermediate and not significantly different from the final weight of fish offered the commercial feed (14.9 g). A similar response was found for weight gain transformed to percent weight

TABLE 4.—Proximate composition (g/100 g of diet, as-is basis), protein digestibility, and amino acid profile of white seabass diets in trial 2, evaluating replacement of fish meal (FM) with soy protein concentrate (SPC)[a].

Variable	FM40-SBM[c]	FM30	FM20	FM15
Proximate components				
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[b]	86.71	82.93	79.86	79.21
Amino acids				
Methionine	0.95	0.87	0.79	0.80
Cysteine	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

[a] Analyses conducted by New Jersey Feed Laboratory.
[b] Using 0.0002% pepsin solution.
[c] SBM = soybean meal.

TABLE 5.—Water quality parameters (average ± SD) in semi-closed recirculating systems for two white seabass growth trials (TAN = total ammonia nitrogen).

Variable	Trial 1	Trial 2
Temperature (°C)	18.0 ± 0.8	18.1 ± 0.5
Dissolved oxygen (mg/L)	7.7 ± 0.3	7.4 ± 0.2
pH	7.8 ± 0.1	7.8 ± 0.1
Salinity (‰)	34.4 ± 0.6	34.5 ± 0.7
TAN (mg/L)	0.05 ± 0.03	0.03 ± 0.03

gain. Feed conversion ratio of fish offered the fish meal diet (1.0) was significantly lower than the FCR of fish offered the other diets (1.2). Survival across all treatments was good (93–98%), with no significant difference due to dietary treatments.

Trial 2

Trial 2 was designed to evaluate the efficacy of substituting fish meal with increasing inclusion levels of soy protein from 24.6% to 50.2%. As in the first trial, water quality was maintained within acceptable limits for this species and fish were in good health (Table 5). Final weight, percent weight gain, survival, and FCR values are summarized in Table 7. Final weight and percent weight gain of fish decreased significantly as fish meal was replaced, with final weights ranging from 36.5 g (116.6% gain) to 27.1 g (69.0% gain). Feed conversion ratio increased from 1.5 to 2.4 as the level of fish meal was decreased in the diets. In general, each incremental reduction in fish meal level resulted in significant reductions in final weight and percent weight gain and a significant increase in FCR.

Discussion

The white seabass is a high-value species for which there is a ready supply of fingerlings and a great deal of potential as an offshore commercial culture species (Leet et al. 2001). This research project was designed to initiate development of a simple practical diet formulation that could be used for culturing white seabass. In the first trial, fish that were maintained on the fish-meal-based diet outperformed the fish that were maintained on the other diets in terms of final weight, percent weight gain, and FCR. However, the similarity in performance of fish reared on the commercial diet compared with fish reared on the fish meal and soy protein test diets suggests that these diets are reasonable but not optimal for white seabass. The commercial feed was formulated to contain 50% protein and 14% lipid, yet the fish performed as well on the 42% protein test diets. Further evaluation of protein requirements for white seabass is warranted because the species has a relatively slow growth rate.

In the second series of diets, SPC was used as a replacement for fish meal. Soy protein concentrate was used because it has a protein level similar to that of fish meal and because it has been processed to remove some of the anti-nutrients and allergens that are found in soybeans. Final weight and percent weight gain were negatively correlated and FCR was positively correlated with the inclusion level of SPC. The reduced performance of white seabass receiving these diets was

TABLE 6.—Response of white seabass to practical soy-based diets in trial 1 (see Table 1 for diet codes and descriptions; Wt = weight; FCR = feed conversion ratio). Within a column, values with different letters are significantly different ($P < 0.05$).

Diet	Initial wt (g)	Final wt (g)	Gain (%)	Survival (%)	FCR
FM	3.4	17.2 z	401.8 z	97	1.0 y
FM40-SPC	3.5	15.4 y	344.8 y	98	1.2 z
FM40-SBM	3.5	14.1 x	307.2 y	93	1.2 z
Commercial	3.4	14.9 yx	332.4 y	98	1.2 z
Pooled SE	0.112	0.342	11.662	2.041	0.018
P	0.9886	0.0002	0.0007	0.3096	<0.0001

TABLE 7.—Response of white seabass to diets with increasing levels of soy protein concentrate in trial 2 (see Table 3 for diet codes and descriptions; wt = weight; FCR = feed conversion ratio). Within a column, values with different letters are significantly different ($P < 0.05$).

Diet	Initial wt (g)	Final wt (g)	Gain (%)	Survival (%)	FCR
FM40-SBM	16.9	36.5 z	116.6 z	100	1.5 x
FM30	16.8	33.4 y	99.2 y	100	1.8 y
FM20	16.8	29.2 x	74.3 x	98	2.2 z
FM15	16.0	27.1 w	69.0 x	95	2.4 z
Pooled SE	0.211	0.619	4.242	2.795	0.077
P	0.0531	<0.0001	<0.0001	0.5519	<0.0001

most likely attributable to a nutrient imbalance, reduced digestibility, or reduced palatability of the feed.

Reduced performance of white seabass in this study may have been due to shifts in amino acid profiles of the diet or digestibility of the protein sources (Table 4). The tested diets were balanced in terms of protein, but the amino acid levels varied. As would be expected, the amino acid analyses of the diets showed a shift in essential amino acids. Lysine and methionine are the most common limiting amino acids in production diets (Lovell 1989). Dietary lysine levels ranged from 2.71 to 2.89 g/100 g of diet (6.88–7.21% of the protein); the differences most likely represent variation in analytical procedures rather than ingredient substitution. These values are greater than the typical requirements for a number of fish species (NRC 1993) and were not likely to be limiting. Methionine level ranged from 0.79 to 0.95 g/100 g of diet (2.00–2.37 g/100 g of protein), and the total sulfur amino acid (TSAA) level (methionine plus cysteine) ranged from 1.28 to 1.39 g/100 g of diet (3.25–3.47 g/100 g of protein). Methionine level in the diets decreased as fish meal was replaced. However, there were minimal differences in TSAA level among the diets. Given that the methionine level was greater than or equal to 60% of the TSAA level, a deficiency in methionine or TSAA is possible but unlikely.

The TSAA requirement for white seabass has not been determined. For a number of species, including the red drum (Moon and Gatlin 1991), hybrid bass (white bass *Morone chrysops* × striped bass *M. saxatilis*; Keembiyehetty and Gatlin 1993), Japanese flounder *Paralichthys olivaceus* (Alam et al. 2000), yellowtail *Seriola quinqueradiata* (Ruchimat et al. 1997), and orange-spotted grouper *Epinephelus coioides* (Luo et al. 2005), the reported requirements for methionine or TSAA are less than or equal to the levels used in this study. However, higher requirements have been reported for a number of other species, such as the cobia *Rachycentron canadum* (Zhou et al. 2006) and large yellow croaker *Pseudosciaena crocea* (Mai et al. 2006). Another possibility is that taurine levels are limiting because plants do not contain significant levels of taurine. Recent studies have indicated that taurine is conditionally required in some species, such as the rainbow trout *Oncorhynchus mykiss* (Gaylord et al. 2007), Japanese flounder (Kim et al. 2003, 2005, 2007), European seabass *Dicentrarchus labrax* (Martinez et al. 2004), red sea bream *Pagrus major* (Goto et al. 2001), yellowtail (Takagi et al. 2005), and cobia (Lunger et al. 2007).

The results of this study and the values reported in the literature suggest that the experimental diets we used were marginal or deficient in methionine, taurine, or both. Further evaluation is needed to determine whether these compounds are limiting to white seabass that receive plant-based diets. In addition to a nutrient imbalance, shifts in digestibility could also have played a role in reduced performance. In the present study, pepsin digestibility values of the feeds were reduced as fish meal was replaced. This could indicate that the digestibility of this batch of SPC was below that of the fish meal, which would explain the reduced performance. However, because fish meal contains considerable levels of soluble protein, a modified pepsin digestibility may have been a better analysis and leaves the result unclear but presents another possible explanation.

Similar poor responses to increasing levels of soy protein have been found in other species, especially those for which nutrient requirements were not well understood at the time of the testing. For example, early research with juvenile red drum (Reigh and Ellis 1992; Davis et al. 1995; McGoogan and Gatlin 1997) and red snapper *Lutjanus campechanus* (Davis et al. 2005) found poor responses with increasing levels of soy protein. However, once palatability and nutrient requirements (primarily amino acid requirements) were more precisely determined, substitution strategies produced better results.

Our results demonstrate that a simple practical feed formulation will support white seabass growth and survival similar to those achieved by the currently used commercial formulation. Based on current results with this species, practical diets can be formulated with moderate levels of plant-based ingredients, but high levels resulted in reduced growth for these feed formulations. With additional research, it is likely that the nutritional value of the soy protein-based diets could be improved. The reduction in growth is presumably due to amino acid deficiency or palatability problems that should be investigated in future work.

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