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Replacement of Menhaden Fish Meal Protein by Solvent Extracted Soybean Meal and Soy Protein Concentrate Supplemented with L-Methionine and L-Lysine in the Diet of Juvenile Red Porgy *Pagrus Pagrus*

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Abstract

Eight experimental diets were prepared to replace menhaden Fish Meal Protein (FMP, 59% crude protein) by solvent extracted Soy Bean Meal Protein (SBP, 47.5% crude protein) and Soy Protein Concentrate (SPC, 66% crude protein) for juvenile red porgy *Pagrus pagrus*. Five isonitrogenous (48%) and isolipidic (12%) diets were prepared replacing 0, 15, 30, 45 and 60% of FMP by SBP. In addition, three diets were prepared replacing 30, 45 and 60% of FMP by SPC. The control diet contained 66% menhaden fish meal, 0% SBP and 0% SPC. Crystalline L-methionine and L-lysine were added to the diets to simulate the calculated values of methionine and lysine found in the control diet. Fifteen fish were stocked in each of twenty-four 75-L tanks, and each test diet was fed to triplicate groups of fish (mean weight = 2.21 ± 0.02 g) for 56 days. Fish were fed twice per day (09:00 and 16:00 h) to apparent satiation. Compared to the control diet, percent body weight gain, feed intake, feed conversion ratios and protein efficiency ratios were not significantly (P> 0.05) different for fish fed from 15-45% SBP and 30-60% SPC, but were lower in fish fed 60% SBP. Broken line regression analysis, however, indicated an optimal substitution level of 34.9% for SBP. Survival of fish after the feeding trial ranged from 84 to 91% among treatments, with no significant differences. Apparent digestibility coefficients of protein in fish fed 60% SBP diet was significantly lower than control diet, but not the 60% SPC diet. Total essential amino acid and fatty acid composition of diets were similar in all test diets. Results indicated that the optimum level of FMP replacement by SBP and SPC were 34.5% and 45%, respectively, with supplemental methionine and lysine in the diet of juvenile red porgy.

Keywords: Red porgy; *Pagrus pagrus*; Fish meal replacement; Alternative proteins and Fish feed

Abbreviations

FMP: Fish Meal Protein; SBP: Soy Bean Meal Protein; SPC: Soy Protein Concentrate; UNCW: University of North Carolina Wilmington; CMS: Center for Marine Science; NOAA: National Oceanic and Atmospheric Administration; BWG: Body Weight Gain; SGR: Specific Growth Rate; FI: Feed Intake; FCR: Feed Conversion Ratio; PER: Protein Efficiency Ratio; EPA: Eicosapentaenoic Acid; DHA: Docosahexaenoic Acid; ARA: Arachidonic Acid; SFA: Saturated Fatty Acids; MUFA: Mono Unsaturated Fatty Acids; PUFA: Poly Unsaturated Fatty Acids; ADC: Apparent Digestibility Coefficient; ANF: Anti Nutrition Factor; CCFHR: Center for Coastal Fisheries Habitat Research

Introduction

The red porgy *Pagrus pagrus*, also known as sea bream, silver snapper, or pink snapper, is a valuable marine finfish in the family Sparidae, inhabiting the Mediterranean Sea, the eastern Atlantic (from British Isles to Senegal) and the western Atlantic (from North Carolina to Mexico and from Venezuela to Argentina) [1]. Red porgy is an important component of the snapper-grouper complex in the coastal Atlantic off the SE US (particularly NC and SC), red porgy populations have declined severely [2,3]. Due to declining natural populations, high market value, and suitability for intensive culture in tanks and in offshore cages, red porgy are considered a promising species for farming in the Mediterranean and Atlantic coastal areas [1,4-7]. Significant progress has been made in understanding the environmental and feeding requirement for larval culture of Atlantic red porgy [8,9] and production of post-metamorphicjuvenilesat University of North Carolina Wilmington (UNCW) [8]. Until recently, little or no experimental work has been conducted to elucidate the nutritional requirements of red porgy, or to develop cost-effective, environmentally-friendly diets for this species. Research is underway at UNCW to investigate the substitution limits of underutilized and locally available ingredients as alternative protein sources to fish meal that will help formulate a cost-effective and environmentally-friendly diet for commercial aquaculture of the red porgy.

Alternate plant protein sources to fish meal can lower the cost of aquaculture diets, reduce the amount of wild fish used as protein,

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and potentially reduce the nutrient levels in effluent waste [10,11]. However, for most species, there is a limit to how much fish meal can be replaced by alternative plant protein sources without negatively affecting the growth and feed efficiency [12]. Several authors have investigated the tolerance of fresh and saltwater fish for alternative protein sources. The maximum replacement levels of alternative plant protein sources for fish meal varies greatly depending on species [13]. Among alternative protein sources, soybean meal appears to be the most appropriate because of its ample supply, moderate price and favorable essential amino acid profile [14,15]. In addition to good amino acid profile, soybean meal has high protein content, very low carbohydrate and fiber, high digestibility compared to other plant protein sources [12,16]. Presently, soybean meal is the most important protein source in feeds for aquaculture species and as a partial or complete replacement for fish meal. Soybean meal is used not only because of its high protein content but also due to its worldwide availability.

The amino acid profile of soy protein is generally superior to other plant proteins; although compared to menhaden meal protein, it is lower in lysine and Methionine [16]. Although soy protein has a relatively balanced amino acid profile for fish, it is low in some essential amino acids especially methionine and lysine [14]. Thus, more attention has been focused on the beneficial effect of essential amino acid supplementation in soy-based diets on growth performance for several fish species [17-19]. Soybean meal has produced varying results in diets for many marine fishes, including salmonids *Oncorhynchus mykiss* [20]; giltheadsea bream *Sparus aurata* [21]; Japanese yellow tail *Seriola quinqueradiata* [22]; Mediterranean yellow tail *Seriola dumerili* [10]; red snapper *Lutjanus argentimaculatus* [23]; Japanese flounder *Paralichthys olivaceus* [24]; red drum *Sciaenops ocellatus* [25]; cobia *Rachycentron canadum* [26]; Atlantic cod *Gadus morhua* [27]; black sea bass *Centropristis striata [19]*; southern flounder *Paralichthys lethostigma* [18]; rose snapper *Lutjanus guttatus* [28] and yellow tail kingfish *Seriola lalandi* [29].

Soybean meal contains several Antinutritional Factors (ANF) that may affect the digestion or absorption of nutrients [12]. Thus, a few other soy protein sources are used in replacement of fish meal (especially for diets with a high protein content) since appropriate processing can eliminate or deactivate several ANF [30]. Soy protein concentrate is produced through aqueous ethanol or methanol extraction of defatted soy flakes, which typically contains 65–70% crude protein [31]. This additional extraction removes or deactivates ANF soluble carbohydrates and fiber [14]. Further, the extraction by alcohol can eliminate bitter off-flavors [32]. Fish meal has been partly or totally been replaced by SPC without adverse effects on growth performance in Atlantic salmon *Salmo salar* [33]; rainbow trout *Oncorhynchus mykiss* [20,34]; turbot *Scophthalmus maximus* L. [35]; Senegale sole *Solea senegalensis* [36] and Atlantic cod *Gadus morhua* [37].

Despite the recent increase in red porgy aquaculture research in the Mediterranean and Asia [38], no published data was available on red porgy culture with diets replacing fish meal by soybean meal protein. The objectives of this study were to investigate the effects of replacement of menhaden fish meal protein (59% crude protein) by solvent extracted Soybean Meal Protein (SBP, 47.5% crude protein) and soy protein concentrate (SPC, 66% crude protein)supplemented with methionine and lysine on growth performance, feed utilization and body composition of juvenile red porgy.

Materials and Methods

Experimental animals

Red porgy juveniles were produced at the University Of North Carolina Wilmington (UNCW)-Aquaculture Facility (Wrightsville Beach) from eggs collected from natural spawning of captive brood stock held at the Center for Coastal Fisheries Habitat Research (CCFHR) (National Ocean Service, NOAA) Laboratory (Beaufort, NC). These fish were hatched and reared at UNCW Aquaculture Facility according to published protocols [8] (Morris et al. 2008). Early juveniles were raised in 2.61-m^3 in recirculating tanks until the feeding trial was conducted. Fish were fed a commercially prepared diet containing 50% protein and 15% lipid (Skretting, Vancouver, Canada) until the study commenced.

Experimental system

The experimental system consisted of twenty-four 75-L rectangular tanks supported by a recirculating aquaculture system located in an indoor climate-controlled laboratory. The recirculating aquaculture system included a Kaldness moving bed biofilter (Anox Kaldness Inc, Providence, Rhode Islands), a bead filter (Aquaculture Systems Technologies, LLC, New Orleans, Louisiana) to remove solids, a protein skimmer for removal of small particulate and dissolved materials and an ultra-violet sterilizer for disinfection. Temperature was controlled using a heat pump, and each tank was supplied with diffused air supplemented with pure oxygen when necessary. Dissolved oxygen, temperature, salinity and pH were measured using a multi-parameter probe (YSI 556 MPS, GEO Scientific Ltd., Vancouver, British Columbia). Total ammonia and nitrate were measured weekly using a portable data logging spectrometer (HACH DR/2010 SPEC, Loveland, CO, USA).

Experimental diets

Eight experimental diets were formulated to replace menhaden fish meal protein (59% crude protein) by solvent extracted soybean meal (47.5% crude protein) and soy protein concentrates (66% crude protein). Five isonitrogenous (48%) and isolipidic (12%) test diets (Table 1) were prepared replacing 0, 15, 30, 45 and 60% of menhaden Fish Meal Protein (FMP, 59% crude protein) by solvent extracted Soybean Meal Protein (SBP, 47.5% crude protein). The control diet (Diet 1) contained 66% menhaden fish meal, and all other nutrients in the diets were added according to the recent nutrients requirement information for marine fish [39,19]. Since menhaden fish meal (59 % protein) has a higher protein content than soybean meal (47.5 % protein), this was accomplished by reducing menhaden fish meal from control diet (Diet 1) by 0, 9.9 19.8, 29.7 and 39.6% and adding soybean meal to levels of 0, 12.2, 24.3, 36.5, and 48.0 in the diets, respectively (Table 1, Diets 1 to 5). In addition, three isonitrogenous (48%) and isolipidic (12%) test diets (Table 1, Diets 6 to 8) were prepared replacing 30, 45 and 60% of FMP by soybean protein concentrate (SPC, 66% crude protein). This was accomplished by reducing menhaden fish meal from control diet (diet 1) by 19.8, 29.7 and 39.6% and adding SPC to levels of 18.0, 27 and 36% in the diets, respectively (Table 1, Diets 6 to 8). To maintain isolipidic levels and to avoid a deficiency of highly unsaturated fatty acid profiles in all diets, menhaden fish oil content was increased as the fish meal level

Table 1: Compositions of diets (g 100 g^{-1}). Diets indicate % FMP replaced with SBP or SPC.

Diet No.	1	2	3	4	5	6	7	8
	0%	15%	30%	45%	60%	30%	45%	60%
		SBP	SBP	SBP	SPC	SPC	SPC	SPC
Soybean meal [a]	0	12.2	24.3	36.5	48	0	0	0
Soy protein conc. [b]	0	0	0	0	0	18	27	36
Menhaden meal [c]	66	56.1	46.2	36.3	26.4	46.2	36.3	26.4
Wheat starch	10	8	5.9	2	0	10	10	10
Wheat gluten[d]	10	10	10	10	10	10	10	10
Menhaden fish oil[e]	4	5	6	7	8	6	7	8
Soybean lecithin[f]	1	1	1	1	1	1	1	1
Vitamin premix [g]	2	2	2	2	2	2	2	2
Mineral premix [g]	2	2	2	2	2	2	2	2
Di-calcium phosphate	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Methionine	0	0.5	0.6	0.7	0.8	0.6	0.7	0.8
Lysine	0	0.5	0.6	0.7	0.8	0.6	0.7	0.8
Cellulose	4.5	2.23	0.9	1.29	0.5	3.13	2.84	2.56
Total	100	100	100	100	100	100	100	100
Analyzed crude protein and lipid composition (%)								
Crude protein	49.4	48.4	48.8	49.9	48.5	49	48.6	48.6
Digestible protein	43.3	43.7	43.9	44.6	40.7	43.9	43.6	42.6
Total lipid	14.7	13.9	14.3	14.1	13.8	13.9	13.3	13.7
Gross energy[h]	15	15	15	15	15	15	15	15
(Calculated kJ g^{-1} diet)								

[a] Southern States, Wallace, NC, USA (solvent extracted, crude protein 47.5%).
[b] Solae LLC, St. Louis, MO, USA (crude protein 66%)
[c] Omega protein, Houston, TX, USA (crude protein 59%, lipid 11%).
[d] VWR International, Radnor, PA, USA (crude protein 78%).
[e] Virginia Prime Silver, Omega Protein, Hammond, LA, USA.
[f] ADM, IL,USA. gSigma-Aldrich, St. Louis, MO. USA (crude protein 80%).
[g] Tomita Pharmaceutical Company, Kagoshima, Japan as Alam et al.[19].
[h] Calculated based on carbohydrates, proteins and lipids are 17.2, 23.6, and 39.5 kJ g-1, respectively [76].

decreased. Crystalline L-methionine and L-lysine were added to the diets to simulate the calculated values of methionine and lysine found in the control diet. Equal quantities of Kadai vitamin and mineral premix (Tomita Pharmaceutical Company, Kagoshima, Japan) for marine fish were used in the diets. Diets were prepared at UNCW-CMS using a feed mixer (Kitchen Aid Inc., St. Joseph, Michigan), meat grinder (Jacobi-Lewis Co. Wilmington, North Carolina) and a drying oven [18,19].

Feeding protocol

Fifteen fish were stocked in each of twenty-one 75-L tanks (360 fish total). Fish were acclimated to laboratory conditions and were fed the control diet (0% SBP) for one week, and then each test diet was fed to triplicate groups of fish (average weight = 2.21 ± 0.02 g) for 56 days. Fish were fed twice per day (9:00 and 16:00 h) to apparent satiation, and the amount of diet fed was recorded. Tanks were siphoned daily or as needed. A 10: 14 h light: dark photoperiod was maintained. Water quality was verified twice weekly. During the feeding period, water temperature ranged from 21.2 to 23.9 C and dissolved oxygen ranged from 6.07 to 7.41 mg/L. The ranges of other water quality parameters in the experimental tanks during the experimental periods were as follows: pH 7.69 to 8.3, salinity35.6 to 36.3 gL^{-1}, ammonia 0.25 to 0.34 mgL^{-1} and nitrite 0.07 to 0.13 mgL^{-1}. Fish from each tank were weighed in bulk every 4 weeks. After 56 days of feeding, five fish from each tank were sacrificed, freeze dried, and stored at -80 C for whole body proximate composition analysis.

Apparent digestibility coefficient (%) of protein

To determine the Apparent Digestibility Coefficient (ADC) of crude protein of fish, eight test diets were prepared by adding 0.5% chromic oxide to the diets as an inert marker. Each diet was reformulated to included 0.5% chromic oxide which was offset by a reduction of cellulose. All other ingredients and formulations were the same as the diets used in the growth study (Table 1). After 56 days of feeding, 15 fish from each treatment (5 from each triplicate tank) were stocked in one tank. Eight chromic oxide based diets were fed two times a day (08:00 and 16:00 h) to the respective tank, and feces were collected every morning at 08:00 h for 10 days. Before the second (15:00 h) feeding, all tanks were siphoned to remove any uneaten diets and feces. Uneaten diets (if any) were also removed from the tanks after 30 min. of feeding. Feces were stored at freezer at -32°C to analyze apparent digestibility of protein. Chromic oxide content was determined using a spectrophotometer (Thermo Fisher Scientific, Two Rivers, Wisconsin, USA) through a modified Furukawa & Tsukahara (1966) method [40].

The ADC of protein was calculated using the formula:

$$\text{ADC of protein (\%)} = 100 \text{ X } [1-(F_{protein} / D_{protein} \text{X } D_{chromic} / F_{chromic})]$$

Where *F* is the percent of proteinor chromic oxide in the fecal matter and *D* is the percent of proteinor chromic oxide in the diet.

Proximate composition

Proximate composition of all experimental diets and body tissues

Table 2: Analyzed amino acid composition (g 100 g^{-1} dry diet) of test diets. Values are means (*N*=2). Diet indicates percentage of FMP replaced with SBP and SPC.

Amino Acids	0%	15%	30%	45%	60%	30%	45%	60%	Red Sea
		SBP	SBP	SBP	SBP	SPC	SPC	SPC	Bream[a]
Threonine	1.51	1.7	1.43	1.49	1.82	1.48	1.46	1.56	0.86
Valine	1.75	1.97	1.68	1.71	2.08	1.72	1.67	1.82	1.2
Methionine	1.04	1.55	1.3	1.34	1.58	1.33	1.3	1.45	1.05[2]
Iso-leucine	1.44	1.64	1.42	1.49	1.86	1.46	1.45	1.62	1.06
Leucine	2.80	3.16	2.71	2.85	3.49	2.79	2.77	3.07	2.02
Phenylalanine	1.62	1.94	1.71	1.84	2.35	1.75	1.78	2.01	1.96[3]
Histidine	0.95	1.09	0.93	0.96	1.18	0.95	0.93	1.03	0.67
Lysine	2.66	3.26	2.88	2.92	3.73	2.98	2.9	3.19	2.11
Arginine	2.31	2.65	2.31	2.46	3.1	2.35	2.37	2.65	1.68
Hydroxyproline	0.59	0.58	0.36	0.33	0.31	0.41	0.33	0.22	
Aspertic acid	3.23	3.78	3.3	3.55	4.49	3.38	3.43	3.88	
Serine	1.55	1.81	1.55	1.72	2.17	1.61	1.7	1.91	
Glutamic acid	6.74	7.77	6.71	7.5	9.33	6.99	7.33	8.26	
Proline	2.36	2.52	2.18	2.25	2.84	2.27	2.23	2.39	
Glycine	2.57	2.67	2.11	2	2.27	2.13	1.98	1.96	
Alanine	2.18	2.33	1.87	1.84	2.11	1.91	1.81	1.86	
Tyrosine	1.22	1.42	1.26	1.37	1.69	1.3	1.28	1.42	

[a]Essential amino acid requirements (g 100g $^{-1}$dry diet) as per Forster & Ogata (1998) considering 48% crude protein in diets.
[2]Methionine + Cystine
[3]Phenylalanine + Tyrosine

for all three experiments were analyzed at UNCW-CMS. Crude protein was determined by the Kjeldahl method with a Labconco Kjeltec System (Rapid Digestor, Distilling Unit-Rapid Still II and Titration Unit, Labconco Corporation, Kansas city, MO, USA) using boric acid to trap ammonia. Crude lipid (Soxhlet byether extraction), ash (BARNSTAD Thermolyne Muffle Furnace, IA, USA) and moisture (Fisher Scientific Isotemp Oven, Pittsburgh, PA, USA) contents in the diets were analyzed by standard methods [41]. Moisture contents in whole bodies were determined by drying the fish in a freeze dryer (Labcanco Freeze Dryer, Kansas city, MO, USA).

Amino acid analysis of diets

Total amino acid composition of the diets was conducted by AAA Service Laboratory (Damascus, OR, USA). Nor-leucine was used as internal standard. After hydrolysis, samples were analyzed using post-column derivatization on Hitachi L8900 analyzers (Hitachi High Technologies America, Inc. USA).

Fatty acid analysis

Fatty acid compositions of the diets were determined by first extracting total lipids in chloroform: methanol [42]. One ml of a 0.001 g mL^{-1} solution of C19:0 fatty acid was added to each sample as an internal standard. Lipid fatty acids were converted to their methyl esters (FAMEs) for GC analysis by refluxing the concentrated lipid sample in 1.0 mL of 0.5 M NaOH/MeOH for 30 min., followed by addition of 1.5 mL of boron trifluoride-methanol (BF_3) and refluxing for an additional 30 minutes. The FAMEs were extracted into hexane, concentrated and re-dissolved in 1 mL of chloroform. GC analysis was performed on a HP-6890 Gas Chromatograph using a 25 meter x 0.25 µm HP-5 capillary column with FID detection (Department of Chemistry and Biochemistry, UNCW). Helium was used as the carrier gas. The column temperature profile was: 195 °C, hold for 8 min, ramp to 270 at 15 °C$min^{-1}$and hold at 270 °C for 2 min. FAME peaks were integrated using the HP Chemstation soft ware package and individual FAMEs were identified by comparison of retention times to standards: GLC-84 (Nu Chek Prep U.S.A) as well as individual standards of stearidonic, eicosapentaenoic and arachidonic acid methyl esters (Sigma-Aldrich, MO, U.S.A). FAMEs from all samples were quantified using their peak areas compared to the peak area of the C19:0 internal standards.

Statistical analysis

All data were subjected to statistical verification by using one-way Analysis Of Variance (ANOVA) (JMP, version 7.0, SAS Institute, Cary, North Carolina). Significant differences between means were evaluated by Tukey–Kramer tests [43]. Probabilities of $P < 0.05$ were considered significant. Broken-line regression analysis [44] was also used to determine the optimum dietary fish meal protein replacement by SBP. Regression analysis was performed using the software package JMP, version 7.0.

Results

Growth performance

Table 4 shows the growth performance of fish fed the test diets

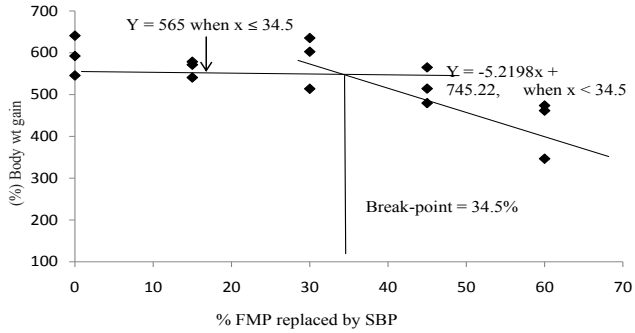

Figure 1: Relationship between (%) body weight gain of red porgy juvenile and optimum % FMP replaced by SBP as described by the broken line regression model.The optimum value determined based on body weight gain for FMP replaced by SBP of juvenile red porgy was estimated to be 34.5%.

Table 3: Fatty acid composition of diets (mg g^{-1} dry diets).Values are means (*N*=2). Diet indicates percentage of FMP replaced with SBP and SPC.

	0%	15%	30%	45%	60%	30%	45%	60%
Fatty acids		**SBP**	**SBP**	**SBP**	**SBP**	**SPC**	**SPC**	**SPC**
14:00	5.23	5.05	5.26	4.68	4.65	4.69	4.76	4.88
16:00	15.55	14.88	15.4	12.5	12.38	13.15	13.4	12.98
16:1n-7	7.89	7.72	8.11	6.88	6.99	7.2	7.61	7.79
18:00	3.4	3.29	3.36	2.72	2.67	2.8	2.93	2.82
18:1n-9	7.44	7.72	8.45	7	7.22	6.94	7.5	7.59
18:1n-11	2.58	2.55	2.61	2.02	2.04	2.3	2.4	2.31
18:2n-6	5.93	6.84	8.31	7.6	8.27	5.56	6.07	6.22
18:4n-3	2.44	2.5	2.58	2.14	2.21	2.27	2.52	2.57
20:00	0.21	0.2	0.21	0.18	0.18	0.18	0.18	0.18
20:1n-9	0.92	0.95	0.99	0.82	0.84	0.88	0.96	0.97
20:2n-6	0.25	0.26	0.27	0.24	0.25	0.24	0.27	0.27
20:4n-6 (ARA)	0.8	0.71	0.67	0.45	0.44	0.62	0.68	0.62
20:5n-3 (EPA)	9.19	9.39	9.84	7.77	7.98	8.74	9.38	9.47
22:5n-3	1.83	1.88	2.04	1.46	1.5	1.71	1.87	2.1
22:6n-3 (DHA)	10.7	10.8	10.9	8.03	8.06	9.68	10.2	9.99
Σ SFA	24.4	23.4	24.2	20.1	19.9	20.8	21.3	20.9
Σ MUFA	18.8	18.9	20.2	16.7	17.1	17.3	18.5	18.7
Σ n-3 PUFA	24.2	24.6	25.3	19.4	19.8	22.4	23.9	24.1
Σ n-6 PUFA	6.98	7.81	9.25	8.29	8.96	6.42	7.02	7.11
n-3/n-6 PUFA	3.46	3.14	2.74	2.34	2.2	3.49	3.42	3.39
DHA/EPA	1.17	1.15	1.1	1.03	1.01	1.11	1.09	1.05

Table 4: Percent body weight gain, Specific Growth Rate (SGR), feed intake, Feed Conversion Ratio (FCR), percent survival of juvenile red porgy fed different diets for 56 days. Diet indicates percentage of FMP replaced with SBP and SPC. Values are means ± SEM (*N*=3). Means with different letters in the same column differ significantly (*P*< 0.05).

Diets	(%) BWG	SGR	FI	FCR	PER	SR
0%	593 ± 28a	2.47 ± 0.02a	0.22 ± 0.01ab	0.97 ± 0.02bc	2.09 ± 0.02ab	91 ± 2.0
15%SBP	564 ±12a	2.41 ± 0.02a	0.22 ± 0.01ab	1.01 ± 0.03abc	2.02 ± 0.02abc	89 ± 5.8
30%SBP	584 ± 36a	2.41 ± 0.04a	0.24 ± 0.02a	1.03 ± 0.03abc	1.97 ± 0.05abc	87 ± 3.7
45%SBP	520 ± 25ab	2.31 ± 0.05ab	0.21 ± 0.01abc	1.01 ± 0.02abc	2.02 ± 0.05abc	85 ± 2.3
60%SBP	427 ± 40c	2.08 ± 0.11c	0.18 ± 0.01c	1.16 ± 0.05a	1.86 ± 0.11c	84 ± 4.3
30%SPC	598 ± 37a	2.42 ±0.07a	0.24 ± 0.03a	1.01 ± 0.03abc	2.02 ± 0.07abc	87 ± 3.7
45%SPC	577 ± 26a	2.42 ± 0.05a	0.21 ± 0.03abc	0.94 ± 0.04c	2.18 ± 0.05a	91 ± 5.8
60%SPC	451 ± 26bc	2.17 ± 0.08bc	0.19 ± 0.02bc	1.10 ± 0.03a	1.93 ± 0.08bc	84 ± 4.3

BWG: {(Final weight-Initial fish wet)/Initial weight} x 100.
SGR: (%d^{-1}): [ln (mean final weight) - ln (mean initial weight) /56d] x 100.
FI: Feed Intake (g/fish/d),
FCR: Feed Conversion Ratio (feed intake (g) / wet weight gain (g),
PER: wet weight gain (g) / protein intake (g).
SR: survival (%).

after 56 days of the feeding trial. No significant differences (*P*< 0.05) were observed in Body Weight Gain (BWG) among the fish fed 0 to 45% SBP (520-593%) and 30 to 45% SPC (451-598%) diets (Table 4). However, compared to fish fed 0% SBP (593%), BWG decreased significantly for the fish fed 60% SBP (427%) and 60% SPC (451%). BWG for fish fed 60% SPC (451%) was also significantly lower than in fish fed 30-45% SPC (598-577%), but not significantly different from fish fed 45%SBP (520%). The optimum FMP replacement by SBP in the diet of juvenile red porgy based on BWG was found to be 34.5% by broken-line regression analysis (Figure 1).

Survival of fish after the feeding trial was 84 to 91% with no significant differences among the groups (Table 4). Specific Growth Rate (SGR) followed a similar trend to BWG, with significantly lower values at the 60% FMP substitution level for both SBP (2.08% d^{-1}) and SPC (2.17%d^{-1}) than in the high fish meal control diet (2.47% d^{-1}) and with no significant differences among SBP and SPC diets at the lower substitution levels (Table 4).

Feed intake(FI, g/fish/d)was significantly lower for fish fed the 60% SBP diet (0.18) than for fish fed 0 to 30% SPB diets (0.22-0.24). However, feed intake for the fish fed 15 to 45% SBP (0.21 – 0.24) and 30 to 45% SPC (0.21 – 0.24) diets were not significantly different from those fed the 0% control diet (0.22). The lowest Feed Conversion Ratio (FCR) (0.94) was found for fish fed the 45% SPC diet, but was not significantly different from fish fed 0 to 45% SBP (0.97 – 1.03) and the 30% SPC diet (1.01). However, FCR was significantly higher for the 60% SPC diet (1.10) and the 60% SBP diet (1.16) than in the 0% control diet (0.97). Protein Efficiency Ratio (PER) was significantly lower for the fish fed 60% SBP diet (1.86) than for fish fed the 0% diet (2.09). However, PERs among the fish fed 0 to 45% SBP (1.97 – 2.09) and 30-45% SPC (2.02-2.18) were not significantly different (Table 6).

Apparent digestibility coefficients (%) of protein

The Apparent Digestibility Coefficients (ADC) (%) of protein in

Table 5: Effects of replacement of FMP (0-60%) by SBP and SPC on whole body proximate composition (% dry basis) of red porgy). Diet indicates percentage of FMP replaced with SBP and SPC. Values are means ± SEM (*N* = 3). Means with different letters in the same column differ significantly (*P*< 0.05).

Diet	Protein	Lipid	Ash
0%	61.5 ± 0.34	18.0 ± 0.21b	18.0 ± 0.11ab
15% SBP	62.9 ± 0.53	17.0 ± 0.39c	18.5 ± 0.22a
30% SBP	61.5 ± 0.96	18.8 ± 0.21a	18.6 ± 0.28a
45% SBP	61.5 ± 0.74	18.0 ± 0.23b	18.3 ± 0.18ab
60% SBP	60.8 ± 0.20	16.1 ± 0.05d	18.6 ± 0.14a
30% SPC	62.2 ± 0.36	18.7 ± 0.30ab	17.9 ± 0.03ab
45% SPC	61.3 ± 0.24	17.9 ± 0.27b	17.6 ± 0.13b
60% SPC	61.5 ± 0.16	17.2 ± 0.21c	17.6 ± 0.26b

Table 6: Apparent Digestibility Coefficient (%) (ADC) of protein in fish fed the test diets. Diet indicates percentage of FMP replaced with SBP and SPC. Values are means ± SEM (*N* = 3). Means with different letters in the same column differ significantly (*P*< 0.05).

Diets	ADC of Protein (%)
0%	87.7 ± 0.59b
15% SBP	90.3 ± 0.49a
30% SBP	90.0 ± 0.29a
45% SBP	89.4 ± 0.53a
60% SBP	83.9 ± 0.40c
30% SPC	89.5 ± 0.69a
45% SPC	89.7 ± 0.56a
60% SPC	87.8 ± 0.31b

fish fed 15, 30 and 45% SBP (89.4-90.3%) were significantly higher than in fish fed the 0% control and 60% SBP diets (83.9-87.7%) (Table 6). The ADC of protein in fish fed 30 and 45% SPC (89.5-89.7%) were also significantly higher than the control and 60% SPC (Table 6).

Whole body proximate composition

Whole body crude protein content was similar (61.3-62.9%) among fish in all diet treatments, with no significant differences (Table 5). Whole body lipid content was also similar among diet treatments, but was significantly lower in fish the fed 60% SPB diet (16.1%) than in fish fed the other test diets (17.2 – 18.8%). The whole body lipid in fish fed 45% SBP (18.0%) and 45% SPC (17.9%) were not significantly different from fish fed the 0% control diet (18.0%). Whole body ash content in fish fed15, 30 and 60% SBP diets were significantly higher than the fish fed 45 and 60% SPC diets (17.6%) (Table 5).

Amino acid composition of diets

Methionine and lysine level in all diets were ranged from 1.04 to 1.58%and2.66 to 3.73%of dry diet, respectively (Table 2). Ranges of other essential amino acids were: valine (1.67-2.08%), iso-leucine (1.42 – 1.64%), leucine (2.77 – 3.49%), phenyl alanine (1.62 – 2.35%), histidine (0.93 – 1.18%) and arginine (2.31 – 3.1%) (Table 2).

Fatty acid composition of diets

Total Saturated Fatty Acids (SFA) ranged from 19.9 – 24.4 mg g^{-1} diet among experimental diets (Table 3). The 45-60% SBP diets contained higher in linoleic acid (18:2n-6) (6.07 – 8.31 mg g^{-1}) than the 0% SBP diet (5.93 mg g^{-1}) and the 30% SPC diet (5.56 mg g^{1}). The concentration of n-3 Poly Unsaturated Fatty Acids (PUFA) ranged from 19.4-25.3 mg g^{-1} among treatments and was slightly lower in 45 and 60% (19.4-19.8 mgg^{-1}) SBP diets than in other diets (Table 3). Arachidonic acid (20:4n-6) (0.44-0.45), Eicosapentaenoic Acid (EPA, 20:5n-3) (7.77-7.98mg g^{-1}) and Docosahexaenoic Acid (DHA, 22:6n-3) (8.03-8.06mg g^{-1}) concentrations for the 45 and 60% SBP diets were lower than in the 0% SBP diet (0.80, 9.19 and 10.7mg g^{-1}, respectively). DHA/EPA ratios ranged from 1.01 – 1.15, with no big differences among diets (Table 3).

Discussion

No statistically significant differences were found in % body weight gain among fish fed the 0, 15, 30 and 45% SBP diets (Table 4), whereas body weight gain was significantly lower for fish fed a 60% SBP diet than in those fed the 0%SBP control diet. However, a broken line regression analysis of percent body weight gain showed a more conservative optimum replacement level of FMP by SBP of 34.5% (Figure 1). Varying levels of SBP have been incorporated successfully (i.e., without reduction of growth performance) into the diets of other marine and freshwater species in replacement of FMP. In the present study, the optimum replacement level of FMP with SBP (34.9%) in red porgy diets was lower than the values reported for other carnivorous marine finfish species, such as Japanese flounder (45%) [24], cobia (50%) [26], Atlantic cod (50%) [45]; herbivorous freshwater fish, such as carp *Cyprinus carpio* (100%) [46], and Nile tilapia *Oreochromis niloticus* (100%) [47]. The optimum replacement level of FMP with SBP for red porgy in the present study is higher than reported for higher than reported for the yellowtail (20%) [22].

A comparison of percent weight gain data by ANOVA suggested that the maximum replacement of FMP by SPC in the red porgy diet was not more than 45% (Table 4). This FMP replacement level (45%) by SPC is higher than the value reported for juvenile black sea bream (40%) [48], turbot (25%) [35], and Gilthead Sea bream (30%) [17].

No significant differences were found in feed intake, FCR and PER among the groups fed 0 to 45% SBP and SPC diets, but significantly lower feed intake was observed in the fish fed the 60% SBP and SPC diets (Table 4) compared to the other test diets, indicating reduced palatability of diets replacing 60%FMP by SBP or SPC. This is similar to what was reported in yellow perch fed diets replacing 63.5% of the FMP by SBP [49]. Lower feed intake of red porgy fed the 60% SBP and SPC diets is a primary reason for poor growth at high replacement levels. In addition, FCR for red porgy fed the 60% SBP and SPC diets was significantly higher than in the 0% SBP control diet. Gilthead sea bream fed diets with 60% or 75% FMP replaced by SBP also showed higher FCR compared to fish fed the 0% SBP control diet containing only FMP [50]. PER in the 60% SBP diet was significantly lower than the control diet (Table 4). In Mediterranean yellowtail [10] and gilthead sea bream [50], PER declined at 40 and 45% replacement of FMP by SBP, respectively. It has been suggested that the poor growth performance and low feed utilization of fish fed high substitution levels of soybean protein for fish meal could be due to lower digestibility of nitrogen and energy, the presence of non-digestible oligosaccharides, mineral deficiencies, amino acid deficiencies and anti-nutritional factors [14]. Results of the present study suggest that SBP in the 60% FMP replacement diets was not as well digested and assimilated by red porgy as in diets containing higher levels of protein originating from soybean meal. In Europeanseabass, replacement of FMP by soy protein at levels above 50% negatively affected feed intake, feed efficiency, specific growth rate and protein retention ratio [35]. Protein intake is directly proportional to the feed intake, which in turn affects growth rate [51]. Low feed intake driven by a poor feed

palatability appeared to have been a major factor restricting higher inputs of SBP and SPC in diet formulation of snapper [52], similar to the findings in the present study for red porgy juveniles.

In this study, survival remained high (84-91%) throughout the study, with no significant differences. This is similar to what was reported for black sea bass [19], southern flounder [18], Atlantic cod [27] and rainbow trout [53], which showed no significant differences in survival when fed diets containing high levels of soybean meal. Kasper et al. [49] however, found a significant decline in the survival of yellow perch when fed diets with 92% and 100% of the FMP replaced by SBP.

The ability of red porgy to grow on diets with 45% substitution levels of SBP and SPC for FMP indicates a superior ability to digest SBP compared to other marine finfish such as yellowtail [22]. Juvenile red porgy fed 15-45% SBP and 30-45% SPC diets had significantly higher Apparent Digestibility Coefficient (ADC) of protein than red porgy fed control, 60% SBP and 60% SPC (Table 7). In general, the higher AADC for the15, 30 and 45% SBP and 30-45% SPC diets suggests that SBP and SPC with fish meal more digestible than only fish meal protein and thus well assimilated by red porgy. In several finfish species including red sea bream, gilthead sea bream and Japanese flounder it has been suggested that the high growth performance and high feed utilization of fish fed high substitution levels of soybean meal for fishmeal could be due to higher digestibility of nitrogen and energy [24,50]. On the other hand, the results of the present study showed that red porgy fed 60% SBP diets had significantly lower ADC of protein than the diets with lower SBP levels. Diets with high plant protein can reduce digestibility of nutrients in fish [54]. Reduced ADC of protein was reported for Atlantic cod when solvent-extracted soybean meal was included in diets [27,55]. Different soybean products, such as soy protein concentrate, extracted and toasted (defatted) soybean meal, full-fat soybean meals, or low oligosaccharides soybean meal, have produced different growth performance in fish because of the different anti-nutritional factors, amino acid availability, leaching of supplemented amino acids, anti-nutrient compounds, and/or poorly digestible carbohydrates and soluble fiber content present in these products [56,57]. In contrast to SBP red porgy fed the 60% SPC diet showed no significant differences in ADC from the 0%SPC control diets. Many studies showed that there were no effects of incorporation of SPC on protein digestibility [35,58].

Supplementation of lysine and methionine to compensate for the deficiency of essential amino acids is beneficial in improving amino acid balance and palatability in high soybean protein based diets [39,59,60]. Methionine and lysine level (g 100 g^{-1} dry diet) in all diets in the present study with red porgy were similar to or higher than the methionine and lysine requirement levels reported for the congeneric red sea bream *Pagrus major* (Table 2) [61]. All other essential amino acids were more or less similar to or higher than the requirement values reported for red sea bream [61]. Similar growth and feed intake among the 0, 15, 30 and 45% SBP and 0, 30 and 45% SPC diets indicated that supplemental methionine and lysine improved feed utilization, and growth performance to the level observed in fish fed the control diet that has only intact protein sources. Supplementation of methionine and lysine in soybean based diets improved growth performance of red sea bream, *Pagrus major* [62] and Japanese yellowtail [63]. The lysine and methionine requirements of red porgy are unknown at this time. In the present study, diets were formulated with supplemental methionine and lysine in diets to simulate the methionine and lysine level found in the control diet. The analyzed values for total amino acids of the different diets (Table 2) appear to have been adequate for good growth and survival of juvenile red sea bream [39,61,64,65]. The amino acid availability of soybean meal for red porgy was not investigated. However, lower amino acid availabilities in the 60% SBP and SPC diets could affect the digestion, absorption and metabolism of the nutrients as observed for other species [54,58,66]. Nevertheless, the amino acid bioavailability from crystalline or ingredient-bound essential amino acid sources could be different [67] and may help explain the poor growth results achieved in red porgy fed a high level (60%) of SBP and SPC. Some aquatic animals, such as carp *Cyprinus carpio*, channel catfish, Japanese prawn *Penaeusjaponicus* appear to utilize free amino acids less efficiently than protein-bound amino acids [26,68].

In the present study, whole body protein content was not affected by SBP or SPC replacement of FMP up to 60% (Table 5), similar to what was reported in yellow croaker *Pseudosciaena crocea* (Richardson) [15]; rainbow trout [53] and Indian major carprohu *Lebeo rohita* L [69]. Whole body lipid content, however, was significantly lower in fish fed the 60% SBP diets compared to the 0% SBP diet (Table 5). A decline in whole body lipid level may indicate increasing use of lipid for energy in fish fed diets high in SBP and SPC [56] and may be related to lower protein digestibility and utilization as a source of energy at high substitution levels of these soybean protein ingredients. However the apparent lipid digestibility coefficients of diets were not measured in this experiment which could have some relation between whole body lipid and lipid digestibility. Mangrove red snapper also had a lower whole body lipid level when 50% FMP was replaced by SBP in the diets [23] a level of SBP replacement that reduced growth in these fish.

Total n-3 PUFA levels (1.9 – 2.5%) of the diets were within the range (0.5% - 2.5%) typically required for juvenile marine fish [70]. As SBP and SPC contents were increased in the diets, fish oil was increased to compensate for the low lipid content of SBP and SPC, producing similar n-3 PUFA levels in all test diets (Table 3). Red drum requires 0.5% dry diet of n−3dietary PUFA [71]. Requirements for n−3HUFA in other marine species such as red sea bream, yellowtail *Seriola lalandi* and turbot range from 0.5 to 2.0% of dry diet [72,73].

In this study, the ratio of DHA to EPA in the diets (1.0-1.1) was within the range of 0.5 – 1.0 required by gilthead sea bream [70]. Although the DHA or EPA requirements for juvenile red porgy are not known, based on the requirements of other marine finfish listed above, it appears that sufficient DHA and EPA was provided in all diets. There is no information on replacement of fish oil by vegetable oil in red porgy diets. However, these values meet the requirement (1.0 and 0.5%of the diet for DHA and EPA, respectively) for juvenile red sea bream as estimated by Takeuchiet al [74]. While ARA requirements for red porgy are unknown, the ARA concentrations in the test diets used in the present study ranged from 0.44 to 0.80%dry weight, lower than what was reported to maximize survival in juvenile gilthead sea bream (1.8% dry weight) [70,75]. Additional studies are needed to determine maximum fish oil replacement by vegetable oil in high soybean based diets for red porgy.

In summary, results suggested that the maximum levels of FMP replacement with SBP and SPC in red porgy diets were34.5% and 45%, respectively, when menhaden fish meal and wheat gluten were used as protein source in the control diet.

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