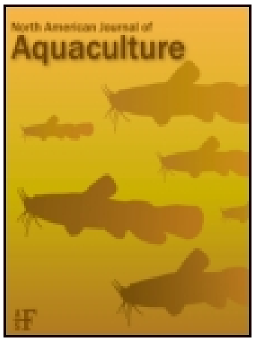

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ARTICLE

Replacement of Menhaden Fish Meal Protein by Solvent-Extracted Soybean Meal Protein in the Diet of Juvenile Black Sea Bass Supplemented with or without Squid Meal, Krill Meal, Methionine, and Lysine

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Abstract
Three experiments were conducted to determine the extent to which menhaden fish meal protein (FMP) can be replaced by solvent-extracted soybean meal protein (SBP) in the diet of juvenile black sea bass *Centropristis striata*. Diets were formulated replacing FMP by SBP at 0, 10, 20, 30, 40, 50, and 60% (experiment 1) and 0, 60, 70, 80, 90, and 100% (experiment 2), with supplementation with squid meal, krill meal, and attractants in both experiments. Experiment 3 was designed to replace FMP by SBP at 40, 50, 60, 70, and 80% without supplemental squid and krill meal and at 60% and 70% with supplemental methionine and lysine. Diets were fed twice daily to triplicate groups of fish ($N = 15$ per group) in 75-L tanks containing recirculating seawater. Fish were fed for 6, 10, and 8 weeks in experiments 1, 2, and 3, respectively. No significant differences in body weight gain, feed efficiency, and survival were observed among treatments in experiment 1. In experiment 2, no significant differences in percent weight gain were observed among fish fed diets replacing FMP at 0, 60, and 70%. In experiment 3, body weight gain was not significantly different for fish fed supplemental methionine and lysine in 70% SBP diets compared with fish fed 0% SBP diets. No significant differences were observed in whole-body n-3 polyunsaturated fatty acids among treatments in experiment 2. Broken-line regression of the specific growth rate data suggested that the maximum level of FMP replacement with SBP in black sea bass diets was 67.6–68.4% with 75 g/kg squid meal and 50 g/kg krill meal in the diet and 57.2–58.0% without squid and krill meal supplementation.

Alternate plant protein sources to replace fish meal in feeds can lower the cost of aquaculture diets, reduce the amount of wild fish used as protein, and potentially reduce the nutrient levels in effluent waste (Tomas et al. 2005; Trushenski et al. 2006). However, for most species there is a limit to how much fish meal can be replaced by alternative plant protein sources without negatively affecting fish growth and feed efficiency (Gatlin et al. 2007). Several authors have investigated the tolerance of freshwater and saltwater fishes for alternative protein sources. The maximum replacement levels of alternative plant protein sources for fish meal varies greatly depending on species (Lochmann and Kumaran 2006).

Soybean meal is considered to be one of the most suitable ingredients for replacing fish meal to produce cost-effective, nutritionally balanced diets in commercial fish feed. Compared with other plant protein sources, soybean meal has high protein

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content, very low carbohydrate and fiber, high digestibility, and good amino acid profile (Hardy 2006; Gatlin et al. 2007). Presently, soybean meal is the most important protein source in feed for aquaculture species and as partial or entire replacement of fish meal. Soybean meal is used not only because of its high protein content but also owing to its worldwide availability. The amino acid profile of soy protein is generally superior to other plant proteins; although compared with menhaden meal protein, it is lower in lysine and methionine (Hardy 2006). Soybean meal has produced varying results in diets for many marine fish (Gatlin et al. 2007), including rainbow trout *Oncorhynchus mykiss* (Kaushik et al. 1995), gilthead seabream *Sparus aurata* (Nengas et al. 1999), Japanese yellowtail *Seriola quinqueradiata* (also known as buri) (Shimeno et al. 1993), Mediterranean yellowtail, *S. dumerili* (Tomas et al. 2005), mangrove red snapper *Lutjanus argentimaculatus* (Catacutan and Pagador 2004), Japanese flounder *Paralichthys olivaceus* (also known as olive flounder) (Kikuchi 1999), red drum, *Sciaenops ocellatus* (McGoogan and Gatlin 1997), cobia *Rachycentron canadum* (Zhou et al. 2005), and Atlantic cod *Gadus morhua* (Hansen et al. 2007). Red snapper growth rate, feed efficiency, and mortality were not significantly affected when soybean meal was introduced at increments of 12% up to 48% (Catacutan and Pagador 2004). In juvenile cobia, 60% replacement of fish meal by soybean meal was successful (Zhou et al. 2005). However, use of soybean protein in marine fish diets is limited by its amino acid profile, which is deficient in the essential amino acids lysine and methionine (Gatlin et al. 2007). Reduced growth and feed utilization in Atlantic cod fed diets containing soybean meal protein compared with a diet based on fish meal was partly attributed to the lower lysine level in soybean meal (Hansen et al. 2007). Supplementation of methionine and lysine in soybean-based diets improved growth performance of red seabream *Pagrus major* (Takagi et al. 2001) and Japanese yellowtail (Watanabe et al. 2001).

Black sea bass *Centropristis striata* is a commercially important species found in waters along the Atlantic coast from the Gulf of Maine to Northern Florida, and a subspecies inhabits the eastern Gulf of Mexico. Their wide acceptance as an excellent food fish and their high market value has led to overharvesting of wild stocks in many areas. Black sea bass adapt well to captivity and have been raised successfully from eggs to marketable stages at the University of North Carolina Wilmington (Copeland et al. 2002; Watanabe et al. 2003). Black sea bass grow rapidly when fed artificial diets consisting largely of marine feedstuffs such as menhaden fish meal or natural diets such as live tilapia (Copeland et al. 2005). Previously, basic information on the nutrient requirements of black sea bass was investigated (Alam et al. 2008, 2009), but there is no published information available on fish meal replacement with alternative protein sources in black sea bass diets. The objective of this study was to investigate the effects of replacement of menhaden fish meal protein from 0% to 100% with solvent-extracted soybean meal protein supplemented with or without squid meal, krill meal, methionine and lysine on growth performance, feed utilization, and body composition of juvenile black sea bass.

METHODS

Experimental Fish

This experiment was conducted at the University of North Carolina Wilmington, Center for Marine Science (UNCW-CMS), Aquaculture Facility, Wrightsville Beach. Adult black sea bass broodstock held in photothermally controlled tanks were induced to spawn using luteinizing hormone releasing hormone analog (LHRHa) (Watanabe et al. 2003). These fish were hatched and reared according to published protocols (Copeland and Watanabe 2006, Watanabe et al., in press,). Early juveniles were raised in 150-L rectangular raceways and then in 2.61-m^3 recirculating tanks until the feeding trial was conducted. Fish were fed a commercially prepared diet containing 50% protein and 15% lipid (Skretting, Vancouver, British Columbia) 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 (Anox Kaldness, Providence, Rhode Island) biofilter, a bead filter (Aquaculture Systems Technologies, New Orleans, Louisiana) to remove solids, a protein skimmer for removal of small particulate and dissolved materials, and an ultraviolet sterilizer for disinfection. Temperature was controlled using a heat pump, and each tank was supplied with continuous diffused air supplemented with pure oxygen when necessary. Dissolved oxygen, temperature, salinity, and pH were measured with a YSI 556 MPS (Multi-Probe System, GEO Scientific, Vancouver). Ammonia and nitrate were measured weekly with a portable data logging spectrometer (HACH DR/2010 SPEC).

Experiment 1

Test diets.—Seven isonitrogenous and isolipidic test diets were prepared replacing 0, 10, 20, 30, 40, 50, and 60% of menhaden fish meal protein (FMP, 63.5% crude protein) with solvent-extracted soybean meal protein (SBP, 47.5% crude protein) (adding 0, 67, 134, 200, 267, 334, and 401 g/kg soybean meal) (Table 1). The control diet and all experimental diets also contained 75 g/kg squid meal and 50 g/kg krill meal. All other nutrients were added on the basis of the recent nutritional studies on black sea bass (Alam et al. 2008, 2009). To maintain isolipidic levels and to avoid deficiencies in highly unsaturated fatty acids (HUFAs) in all diets, menhaden fish oil content was increased as the fish meal level decreased. In addition, alanine, glycine, betaine, and taurine (each at 2.5 g/kg) were used as attractants in the diets. Energy levels of diets were calculated based on 23.6, 39.5, and 17.2 kJ/g for protein, lipid, and nitrogen-free extract, respectively (Blaxter

TABLE 1. Composition of diets (g/kg) for black sea bass in experiment 1. Diets indicate percentage of fish meal protein replaced with soybean meal protein (SBP).

	% SBP						
Diet	0%	10%	20%	30%	40%	50%	60%
Menhaden meal[a]	500	450	400	350	300	250	200
Soybean meal[b]	0	67	134	200	267	334	401
Squid meal[c]	75	75	75	75	75	75	75
Krill meal[d]	50	50	50	50	50	50	50
Menhaden fish oil[e]	40	40	45	50	55	60	60
Soybean lecithin[f]	10	10	10	10	10	10	10
Wheat gluten[g]	50	50	50	50	50	50	50
Wheat starch	50	50	50	50	50	50	50
Vitamin mix[h]	40	40	40	40	40	40	40
Mineral mix[h]	40	40	40	40	40	40	40
Attractants[i]	10	10	10	10	10	10	10
Cellulose	135	118	97	75	53	31	14
Total	1,000	1,000	1,000	1,000	1,000	1,000	1,000
Analyzed proximate composition (g/kg)							
Protein	442	441	445	439	441	441	443
Lipid	128	126	121	124	130	132	125
Ash	127	105	103	100	98	97	95
Gross energy[j] (calculated kJ/g diet)	16.3	16.5	16.8	17.1	17.7	18.2	18.2

[a] Omega Protein, Houston, Texas (crude protein, 63.5%; lipid, 12.5%).
[b] Southern States, Wallace, North Carolina (solvent-extracted crude protein, 47.5%).
[c] Scoular Company, Minnesota (crude protein, 78%; lipid, 11%).
[d] Aqion, Vincent, Ohio (crude protein, 60%; lipid, 10%).
[e] Virginia Prime Silver, Omega Protein, Hammond, Louisiana.
[f] ADM, Illinois.
[g] Sigma-Aldrich, St. Louis, Missouri (crude protein, 80%).
[h] As in Alam et al. (2008).
[i] Alanine, glycine, taurine, betaine (each 2.5 g/kg diet), Sigma-Aldrich.
[j] Based on values for carbohydrates, proteins, and lipids as 17.2, 23.6, and 39.5 kJ/g, respectively (Blaxter 1989).

1989). Diets were prepared at UNCW-CMS by using a Kitchen Aid mixer, meat grinder (Jacobi Lewis, North Carolina) and a drying oven (Yamato Mechanical Convection Oven Model DKM 600, Santa Clara, California) (Alam et al. 2008, 2009). Proximate composition of the test diets is presented in Table 1.

Feeding trial.—In experiment 1, 15 fish were stocked in each of twenty-one 75-L tanks. Each test diet was fed to triplicate groups of fish (initial weight was 5.1 ± 0.15 g [mean ± SE]). Fish were fed twice per day (0900 and 1600 hours) as much as they could consume during a 20-min period, and the amount of diet consumed was recorded. Tanks were siphoned daily or as needed to remove waste and uneaten feed. Each tank was covered with a glass lid to prevent fish from jumping out. A 14 h light : 10 h dark photoperiod (photo intensity, 500–600 lx) was maintained. Water quality was checked twice weekly. Water temperature was 21.2 ± 0.7°C during the experimental period and dissolved oxygen (7.0 ± 0.3 mg/L) was maintained near saturation. Mean ± SE values for other water quality variables were: pH, 7.5 ± 0.05; salinity, 32.4 ± 0.3 g/L; ammonia, 0.25 ± 0.01 mg/L; and nitrate, 0.10 ± 0.01 mg/L. Fish were weighed every 2 weeks. Growth was monitored for a period of 6 weeks. A pooled sample of 10 fish from each tank were sacrificed at the end of the feeding trial, freeze-dried, and stored at −85°C for whole-body proximate composition analysis.

Experiment 2

Test diets.—Six isonitrogenous and isoenergetic test diets were prepared by replacing 0, 60, 70, 80, 90, and 100% FMP with SBP (adding 0, 401, 468, 535, 602, and 669 g/kg soybean meal, respectively) (Table 2). Varying levels of wheat starch and menhaden fish oil were used in this feeding trial to maintain isoenergetic levels in all diets. The control diet and all experimental diets also contained 75 g/kg squid meal and 50 g/kg krill meal. To maintain isolipidic levels and to avoid deficiencies in HUFAs in all diets, menhaden fish oil content was increased as the fish meal level decreased. Diet preparation and sources of all ingredients were the same as in experiment 1. Vitamin and mineral mixture was slightly lower in experiment 2 to increase the fish meal substitution level up to 100% with isonitrogenous diets (for proximate composition, total amino acid content, and fatty acid composition of the diets see Tables 2, 4 and 6, respectively).

TABLE 2. Composition of diets (g/kg) for black sea bass in experiment 2. Diet indicates the percentage of fish meal protein replaced with soybean meal protein (SBP). The sources of all ingredients are as in Table 1.

	% SBP					
Diet	0%	60%	70%	80%	90%	100%
Menhaden meal	500	200	150	100	50	00
Soybean meal	00	401	468	535	602	669
Squid meal	75	75	75	75	75	75
Krill meal	50	50	50	50	50	50
Menhaden fish oil	30	60	65	70	75	76
Soybean lecithin	10	10	10	10	10	10
Wheat gluten	50	50	50	50	50	50
Wheat starch	150	80	62	40	18	00
Vitamin mix	30	30	30	30	30	30
Mineral mix	30	30	30	30	30	30
Attractants	10	10	10	10	10	10
Cellulose	65	4	00	00	00	00
Total	1,000	1,000	1,000	1,000	1,000	1,000
Analyzed proximate composition (g/kg diet)						
Crude protein	442	449	446	435	437	433
Crude lipid	126	121	119	113	117	118
Ash	123	97	90	86	81	76
Gross energy (calculated kJ/g diet)	17.9	18.6	18.6	18.3	18.5	18.6

TABLE 3. Composition of diets (g/kg) for black sea bass in experiment 3 (without squid and krill meal). Diet indicates the percentage of fish meal protein replaced with soybean meal protein (SBP). The sources of all ingredients are as in Table 1; M = methionine, L = lysine, and CMC = carboxymethyl cellulose.

	% SBP							
Diet	0%	40%	50%	60%	70%	80%	60% M + L	70% M + L
Menhaden meal	650	387	323	258	194	129	258	194
Soybean meal	00	345	431	517	603	689	517	603
Menhaden fish oil	20	53	60	65	71	71	65	71
Soybean lecithin	10	10	10	10	10	10	10	10
Wheat gluten	45	45	45	45	45	45	45	45
Wheat starch	110	40	40	30	7	1	30	7.1
Vitamin mix	30	30	30	30	30	30	30	30
Mineral mix	25	25	25	25	25	25	25	25
L-Methionine	0	0	0	0	0	0	7.2	7.8
L-Lysine	0	0	0	0	0	0	5.4	6.1
Cellulose	110	65	36	20	15	0	6.5	0
CMC	0	0	0	0	0	0	0.9	1
Total	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000
Analyzed proximate composition (g/kg diet)								
Crude protein	445	446	446	439	447	437	444	441
Crude lipid	112	111	115	117	116	115	116	114
Ash	120	110	105	96	91	86	81	79
Gross energy (calculated kJ/g diet)	16.4	16.9	17.3	17.5	17.5	17.7	17.7	17.8

TABLE 4. Analyzed amino acid composition (g/100 g dry diet) of test diets in experiment 2 and in whole bodies of juvenile black sea bass. Values are means ($N = 2$). Diet indicates the percentage of fish meal protein replaced with soybean meal protein.

			Diet					
Amino acid	Juvenile black sea bass (% of protein)	Calculated value in the 45% protein diet	0%	60%	70%	80%	90%	100%
Essential amino acids								
Methionine (MET)	3.25	1.46	0.85	0.74	0.70	0.61	0.62	0.58
Lysine (LYS)	7.12	3.20	2.51	2.41	2.36	2.22	2.25	2.27
Threonine (THR)	4.00	1.80	1.21	1.42	1.44	1.36	1.40	1.39
Isoleucine (ILE)	3.24	1.46	1.48	1.60	1.65	1.58	1.63	1.69
Leucine (LEU)	6.26	2.82	2.54	2.75	2.81	2.68	2.76	2.89
Valine (VAL)	3.76	1.69	1.67	1.63	1.70	1.62	1.67	1.72
Histadine (HIS)	1.88	0.85	0.86	0.84	0.88	0.84	0.87	0.89
Phenylalanine (PHE)	3.35	1.51	1.47	1.75	1.84	1.77	1.84	1.94
Arginine (ARG)	6.07	2.73	2.15	2.46	2.59	2.44	2.55	2.66
Nonessential amino acids								
Tyrosine (TYR)	2.59	1.17	1.05	1.20	1.28	1.21	1.28	1.32
Aspartic acid (ASP)	9.05	4.07	3.10	3.69	3.83	3.67	3.85	3.98
Serine (SER)	4.18	1.88	1.30	1.47	1.55	1.49	1.56	1.63
Glutamic acid (GLU)	12.17	5.48	5.14	6.43	6.80	6.56	6.83	7.13
Proline (PRO)	1.80	0.81	1.81	1.86	1.98	1.90	1.94	1.97
Glycine (GLY)	7.54	3.39	2.49	1.81	1.86	1.67	1.68	1.77
Alanine (ALA)	6.40	2.88	2.08	1.85	1.84	1.70	1.72	1.71

TABLE 5. Analyzed amino acid composition (g/100 g dry diet) of test diets in experiment 3. Values are means ($N = 2$). Diet indicates the percentage of fish meal protein replaced with soybean meal protein. For amino acid abbreviations, see Table 4.

	Diet							
Amino acid	0%	40%	50%	60%	70%	80%	60% M + L	70% M + L
Essential amino acids								
MET	0.70	0.69	0.59	0.52	0.51	0.46	1.05	1.02
LYS	2.64	1.98	1.98	2.24	2.33	2.21	2.42	2.38
THR	1.42	1.14	1.10	1.27	1.42	1.36	1.23	1.19
ILE	1.44	1.22	1.20	1.37	1.60	1.57	1.39	1.37
LEU	2.58	2.18	2.12	2.42	2.81	2.72	2.43	2.39
HIS	0.93	0.75	0.72	0.84	0.95	0.91	0.82	0.80
PHE	1.50	1.40	1.39	1.52	1.90	1.89	1.63	1.63
VAL	1.69	1.37	1.32	1.54	1.74	1.67	1.50	1.46
ARG	2.18	1.91	1.86	2.14	2.54	2.40	2.17	2.15
Nonessential amino acids								
TYR	1.02	0.95	0.91	1.04	1.24	1.13	1.06	1.06
ASP	3.16	2.88	2.85	3.14	3.85	3.81	3.37	3.35
SER	1.36	1.19	1.16	1.27	1.63	1.52	1.36	1.36
GLU	5.25	5.10	5.02	5.31	6.87	6.72	5.93	5.88
PRO	1.99	1.88	1.78	2.00	1.98	2.16	1.97	1.91
GLY	2.70	1.73	1.53	2.05	1.78	1.59	1.53	1.38
ALA	2.17	1.52	1.39	1.74	1.69	1.54	1.45	1.35

TABLE 6. Fatty acid composition (mg/g dry sample) of test diets in experiment 2. Values are means ± SEs ($N = 3$). Means followed by common letters are not significantly different ($P > 0.05$). Diet indicates the percentage of fish meal protein replaced with soybean meal protein; SFAs = saturated fatty acids, PUFAs = polyunsaturated fatty acids.

	Diet					
Fatty acid	0%	60%	70%	80%	90%	100%
14:0	9.08 ± 0.32 z	10.9 ± 0.39 y	9.04 ± 0.51 z	9.63 ± 0.28 yz	8.83 ± 0.28 z	10.9 ± 0.43 y
16:0	19.0 ± 0.29 y	19.2 ± 0.55 y	17.4 ± 0.60 yz	17.8 ± 0.33 yz	15.9 ± 0.59 z	18.8 ± 0.73 y
16:1	11.9 ± 0.25 z	14.6 ± 0.42 xy	12.7 ± 0.54 yz	13.5 ± 0.28 xyz	12.2 ± 0.40 z	15.6 ± 0.65 x
16:3	1.48 ± 0.03 x	1.55 ± 0.06 x	1.21 ± 0.07 yz	1.20 ± 0.06 yz	1.05 ± 0.01 z	1.33 ± 0.07 xy
16:4	1.30 ± 0.06 z	1.74 ± 0.06 y	1.36 ± 0.06 z	1.42 ± 0.04 z	1.32 ± 0.02 z	1.75 ± 0.07 y
18:0	3.57 ± 0.01 z	3.88 ± 0.25 z	3.36 ± 0.07 z	3.04 ± 0.35 z	3.05 ± 0.09 z	3.09 ± 0.52 z
18:1(n-11)	3.34 ± 0.04 yz	3.55 ± 0.17 yz	3.34 ± 0.14 yz	3.59 ± 0.10 yz	3.20 ± 0.14 z	3.96 ± 0.21 y
18:1(n-9)	9.12 ± 0.08 z	10.6 ± 0.24 y	10.2 ± 0.29 yz	10.5 ± 0.11 y	9.71 ± 0.39 yz	12.1 ± 0.46 x
18:2(n-6)	7.10 ± 0.08 z	11.5 ± 0.12 y	12.4 ± 0.30 y	12.8 ± 0.14 y	12.6 ± 0.44 y	15.3 ± 0.37 x
18:4(n-3)	2.54 ± 0.09 z	3.26 ± 0.11 xy	2.72 ± 0.11 z	2.91 ± 0.03 yz	2.65 ± 0.10 z	3.54 ± 0.18 x
20:1	1.08 ± 0.01 yz	1.27 ± 0.03 xy	1.20 ± 0.03 yz	1.25 ± 0.02 y	1.02 ± 0.09 z	1.48 ± 0.06 x
20:5(n-3)	14.4 ± 0.33 yz	15.7 ± 0.40 xy	14.4 ± 0.19 yz	14.8 ± 0.22 yz	13.2 ± 0.56 z	17.4 ± 0.77 x
22:5(n-3)	1.91 ± 0.02 yz	1.99 ± 0.05 xy	1.89 ± 0.05 yz	1.96 ± 0.01 xyz	1.66 ± 0.08 z	2.23 ± 0.12 x
22:6(n-3)	9.52 ± 0.13 yz	10.3 ± 0.19 xy	9.76 ± 0.26 yz	9.90 ± 0.09 yz	8.74 ± 0.38 z	11.7 ± 0.52 x
$\sum$SFAs	35.5 ± 4.13 z	33.8 ± 0.62 z	31.0 ± 0.75 z	30.2 ± 0.63 z	27.9 ± 0.61 z	32.6 ± 0.38 z
$\sum$MUFAs	25.4 ± 0.34z	29.9 ± 0.83 yz	27.5 ± 0.95 yz	28.9 ± 0.31 yz	26.2 ± 0.97 yz	33.2 ± 1.36 x
$\sum$n-3 PUFAs	29.9 ± 0.60 yz	32.8 ± 0.79 y	29.9 ± 0.66 yz	30.8 ± 0.39 yz	27.3 ± 1.13 z	36.2 ± 1.54 x
n-3/n-6 PUFAs	4.21 ± 0.03 w	2.84 ± 0.05 x	2.42 ± 0.01 y	2.41 ± 0.04 y	2.16 ± 0.02 z	2.37 ± 0.04 y
22:6(n-3)/ 20:5(n-3)	0.66 ± 0.01	0.65 ± 0.01	0.68 ± 0.01	0.67 ± 0.01	0.66 ± 0.00	0.67 ± 0.02

Feeding trial.—In experiment 2, six test diets (0, 60, 70, 80, 90, and 100% FMP replacement with SBP) were fed to triplicate groups of black sea bass (initial weight, 10.1 ± 0.74 g, 15 fish per tank) for 10 weeks. Dissolved oxygen (7.4 ± 0.1 mg/L), temperature (21.2°C ± 0.8°C), salinity (32.8 ± 0.2 g/L), water pH (7.8 ± 0.11), ammonia (0.30 ± 0.01 mg/L), and nitrate (0.12 ± 0.01 mg/L) were maintained at optimal levels (Copeland and Watanabe 2006). The feeding and sampling protocol was the same as in experiment 1. After 10 weeks, final fish biomass was measured, and 10 fish were sampled from each tank. The fish were freezedried and then stored at −80°C for biochemical analysis.

Experiment 3

Test diets.—Six isonitrogenous and isoenergetic test diets were prepared by replacing 0, 40, 50, 60, 70, and 80% FMP with SBP (adding 0, 345, 431, 517, 603, and 689 g/kg soybean meal, respectively) (Table 3) without supplemental squid and krill meal. Two other diets (60% SBP + M + L and 70% SBP + M + L) were prepared by replacing 60 and 70% FMP with SBP supplementing methionine (M) and lysine (L). L-Methionine and L-lysine were added to simulate the methionine and lysine levels found in 45% whole-body protein of juvenile black sea bass on the basis of reference protein concept (Alam et al. 2002, 2005; Wilson 2002; Table 4). L-Methionine and L-lysine were precoated to retard leaching losses from the diets (Alam et al. 2002). Diet preparation and sources of all ingredients were the same as in experiments 1 and 2 (for the proximate composition and total amino acid of the diets see Tables 3 and 5, respectively).

Feeding trial.—In experiment 3, eight test diets were fed to triplicate groups of black sea bass (initial weight, 5.3 ± 0.11 g, 15 fish per tank) for 6 weeks. Dissolved oxygen (7.2 ± 0.3 mg/L), temperature (23.2°C ± 0.5°C), salinity (33.2 ± 0.7 g/L), pH (7.8 ± 0.1), ammonia (0.20 ± 0.01 mg/L), and nitrate (0.15 ± 0.01 mg/L) were maintained at optimal levels (Copeland and Watanabe 2006). The feeding and sampling protocol was the same as in experiment 1. After 8 weeks, final fish biomass was measured, and 10 fish were sampled from each tank. The fish were freeze-dried and stored at −80°C for biochemical analysis.

Proximate Composition

Proximate composition of all experimental diets and body tissues for all three experiments were analyzed at UNCW-CMS and the New Jersey Feed Laboratory (Trenton, New Jersey). Crude protein was determined by the Kjeldahl method with a Labconco Kjeltec System (Rapid Digestor, Distilling

Unit-Rapid Still II and Titration Unit, Labconco, Kansas City, Missouri) by using boric acid to trap ammonia. Crude lipid (Soxhlet by ether extraction), ash (BARNSTAD Thermolyne Muffle Furnace, Dubuque, Iowa), and moisture (Fisher Scientific Isotemp Oven, Pittsburgh, Pennsylvania) contents in the diets were analyzed by standard methods (AOAC 2000). Moisture contents in whole bodies were determined by drying the fish in freeze dryer (Labconco).

Amino Acid Compositions of Diets

Total amino acid composition of the diets (experiments 2 and 3) (Tables 4 and 5) was conducted by AAA Service Laboratory (Damascus, Oregon). Samples were hydrolyzed in 6 N HCl at 110°C for 22 h. Nor-leucine was used as internal standard. After hydrolysis samples were analyzed by using postcolumn derivatization on Hitachi L8900 analyzers (Hitachi High Technologies America, Schaumburg, Illinois). Whole-body amino acid analysis of juvenile black sea bass (Table 4) from the initial stock (experiment 2) was analyzed at the New Jersey Feed Laboratory. Methionine level was lowest in the 100% SBP diet (0.58) and highest in the 0% SBP diet (0.85 g/100 g dry diet). Lysine content of the diets ranged from 2.22 to 2.51 g/100 g dry diet. As the SBP substitution level increased, the lysine level in the diets decreased (Table 4); however, some other essential amino acids such as arginine, isoleucine, leucine, and phenylalanine in the SBP diets (60–100%) were slightly higher than in the 0% SBP diet. Methionine levels in the 60% SBP + M + L and 70% SBP + M + L diets were higher than in the other treatments.

Fatty Acid Composition

Fatty acid composition of the diets and whole bodies of the fish (experiment 2) was determined by first extracting total lipids into chloroform–methanol (Folch et al. 1957). A 1 mL aliquot of a 0.001 g/mL solution of C19:0 fatty acid was added to each sample as an internal standard. Lipid fatty acids were converted to their fatty-acid methyl esters (FAMEs) for gas chromatography (GC) analysis by refluxing the concentrated lipid sample in 1.0 mL of 0.5 M NaOH in methanol for 30 min, followed by the addition of 1.5 mL of boron trifluoride–methanol (BF_3) and refluxing for an additional 30 min. The FAMEs were extracted into hexane, concentrated, and redissolved in 1 mL of chloroform. Analysis was performed on a HP-6890 gas chromatograph by using a 25-m × 0.25-μm HP-5 capillary column with a flame ionization detector (Department of Chemistry and Biochemistry, University of North Carolina Wilmington). 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, and hold at 270°C for 2 min. Peaks for FAMEs were integrated by using the HP Chemstation software package, and individual FAMEs were identified by comparing retention times to standards: GLC-84 (Nu-Chek Prep, Elysian, Minnesota) and individual standards of steariodonic, eicosapentaenoic, and arachidonic acid methyl esters (Sigma-Aldrich, St. Louis, Missouri). The FAMEs from all samples were quantified by comparing their peak areas with the peak areas of the C19:0 internal standards.

Total saturated fatty acids (SFAs) ranged from 27.9 to 35.5 mg/g diet with no significant ($P > 0.05$) differences among experimental diets (Table 6). Monounsaturated fatty acid (MUFA) concentration in the 100% SBP diet (33.2 mg/g diet) was significantly ($P < 0.05$) higher than in the 0% SBP diet (25.4 mg/g diet). Oleic acid (18:1[n-9])[1] concentration in the 100% SBP diet (12.1 mg/g diet) was significantly ($P < 0.05$) higher than in the other diets (9.1–10.6 mg/g diet). All SBP (60–100%) diets were significantly ($P < 0.05$) higher in linoleic acid (18:2[n-6]) (11.5–15.3 mg/g diet) than the 0% SBP diet (7.10 mg/g diet). The concentration of n-3 polyunsaturated fatty acids (PUFAs) ranged from 27.3 to 36.2 mg/g diet and was significantly higher in the 100% SBP diet than in other diets (Table 4). Eicosapentaenoic acid (EPA, 20:5[n-3]) and docosahexaenoic acid (DHA, 22:6[n-3]) concentrations were significantly ($P < 0.05$) higher for the 100% SBP diet (17.4 and 11.7 mg/g, respectively) than for the 0% SBP diet (14.4 and 9.52 mg/g, respectively). Ratios of DHA:EPA ranged from 0.65 to 0.68, with no significant differences among diets (Table 6).

Growth and Feed Efficiency Parameters

Growth and feed utilization were determined from the following formulas:

$$\text{Percent body weight gain} = [(\text{final weight} - \text{initial fish weight})/\text{initial weight}] \times 100$$

$$\text{Specific growth rate (\%/d)} = [\log_e (\text{mean final weight}) - \log_e (\text{mean initial weight})/42,\ 70,\ \text{or } 56\,\text{d}] \times 100$$

$$\text{Feed intake} = \text{percent of body weight per day (\% BW/d)}$$

$$\text{Feed conversion ratios} = \text{feed intake (g)}/\text{wet weight gain (g)}$$

$$\text{Protein efficiency ratio} = \text{weight gain (g)}/\text{protein intake (g)}$$

Statistical Analysis

All data were subjected to statistical verification by using one-way analysis of variance (ANOVA) (JMP, version 6.0, SAS Institute, Cary, North Carolina). Significant differences between means were evaluated by Tukey–Kramer tests (Kramer 1956). Probabilities of $P < 0.05$ were considered significant. Broken-line regression analysis (Zeitoun et al. 1976) was also used to determine the optimum dietary FMP replacement by SBP. Regression analysis was performed using the software package JMP, version 7.0.

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[1] In this notation, the number to the left of the colon is the number of carbon atoms in the compound, the number immediately to the right of the colon is the number of double bonds, and the number after the hyphen indicates the position of the first double bond from the methyl end.

RESULTS

Growth Performance and Feed Efficiency

Experiment 1.—The percent body weight gain (BWG), specific growth rate (SGR), feed intake, feed conversion ratio (FCR), (PER), and percent survival for black sea bass in experiment 1 after 42 d of feeding are presented in Table 7. No significant differences ($P > 0.05$) were observed in BWG (212–248%) among the fish fed diets replacing from 0% to 60% FMP with SBP. The SGRs (2.73–2.87%/d) also showed no significant differences among diets. Feed intake (3.20–3.39% BW/d), FCR (1.24–1.36), and survival (91–98%) were also not significantly different in fish among the diets. Protein efficiency ratios were not significantly different among the fish fed 0–60% SBP diets (1.49–1.70).

Experiment 2.—After 70 d of the feeding trial, percent BWG, SGR, feed intake, FCR, PER, and percent survival for fish in experiment 2 are presented in Table 7. The survival rate (87–100%) was not significantly different among the diets. Body weight gain of fish fed 60, 70, and 80% SBP (BWG range, 153–179%) was not significantly different from fish fed the 0% SBP diet (186%). However, weight gain of fish fed the 90% (BWG, 135%) and 100% SBP diets (BWG, 115%) was significantly lower than that of fish fed the 0% SBP diet (Table 7). The SGR was significantly lower in fish fed 100% SBP (1.10%/d) diets than in fish fed the 0% to 70% SBP diets (1.44–1.59%/d) (Table 7). The optimal FMP replacement for juvenile black sea bass based on SGR was calculated to be 68.4% by broken-line regression analysis (Figure 1).

Feed intake (1.74–2.22% BW/d) showed no significant differences among fish fed 0, 60, 70, and 80% SBP diets. The feed intake values for fish fed 90% and 100% SBP diets (1.74–1.76% BW/d) were significantly lower than for the 0% SBP (2.2% BW/d). The lowest FCR (1.27) was found for fish fed the 70% SBP diet, but it was not significantly different from FCRs in fish fed 0 (1.55), 60 (1.35), and 80% (1.63) SBP diets. However, FCRs were significantly higher in fish for the 90% (1.98) and 100% (2.18) SBP diets compared with the 70% SBP diet. The PERs were significantly lower for the fish fed more than 80% SBP diets (0.99–1.09) than for the fish fed 0% SBP diet (1.40).

Experiment 3.—After 56 d of the feeding trial, percent BWG, SGR, feed intake, FCR, PER, and survival rate for black sea bass in experiment 3 are presented in Table 7. Survival

TABLE 7. Percent body weight gain (% BWG), specific growth rate (SGR;%/d), feed intake (% body weight/d), feed conversion ratio (FCR), protein efficiency ratio (PER), and percent survival (%*S*) of juvenile black sea bass fed different diets for 42 d in experiment 1 (initial weight, 5.1 g), 70 d in experiment 2 (initial weight, 10.1 g), and 56 d in experiment 3 (initial weight, 5.3 g). Diet indicates the percentage of fish meal protein replaced with soybean meal protein (SBP). Values are means ± SEs ($N = 3$). Means with different letters in the same column differ significantly ($P < 0.05$).

Diet%	BWG	SGR	Feed intake	FCR	PER	%*S*
			Experiment 1			
0%	212 ± 11.1	2.80 ± 0.02	3.39 ± 0.08	1.29 ± 0.08	1.58 ± 0.11	93
10%	235 ± 37.9	2.85 ± 0.27	3.29 ± 0.12	1.33 ± 0.12	1.64 ± 0.07	91
20%	229 ± 6.3	2.83 ± 0.04	3.20 ± 0.16	1.33 ± 0.05	1.49 ± 0.41	91
30%	236 ± 24.4	2.87 ± 0.16	3.22 ± 0.09	1.29 ± 0.11	1.68 ± 0.12	98
40%	228 ± 34.3	2.79 ± 0.26	3.31 ± 0.01	1.36 ± 0.14	1.70 ± 0.01	96
50%	223 ± 6.3	2.79 ± 0.04	3.34 ± 0.09	1.33 ± 0.04	1.65 ± 0.17	96
60%	248 ± 16.0	2.73 ± 0.27	3.25 ± 0.11	1.24 ± 0.05	1.61 ± 0.07	93
			Experiment 2			
0%	186 ± 9.8 x	1.49 ± 0.04 xy	2.22 ± 0.15 y	1.55 ± 0.16 yz	1.40 ± 0.14 y	87 z
60%	179 ± 10.2 xy	1.59 ± 0.07 x	1.95 ± 0.09 yz	1.35 ± 0.04 yz	1.54 ± 0.05 y	95 z
70%	168 ± 10.8 xy	1.44 ± 0.08 xy	1.88 ± 0.10 yz	1.27 ± 0.04 z	1.68 ± 0.06 y	100 z
80%	153 ± 0.5 xyz	1.36 ± 0.02 xyz	1.77 ± 0.09 yz	1.63 ± 0.23 xyz	1.38 ± 0.21 yz	91 z
90%	135 ± 5.9 yz	1.23 ± 0.03 yz	1.76 ± 0.06 z	1.98 ± 0.15 x	1.09 ± 0.07 z	93 z
100%	115 ± 5.7 z	1.10 ± 0.03 z	1.74 ± 0.03 z	2.18 ± 0.10 x	0.99 ± 0.04 z	88 z
			Experiment 3			
0%	238 ± 14.2 y	2.17 ± 0.07 y	3.58 ± 0.05 z	1.83 ± 0.08 yz	1.24 ± 0.05 y	98 z
40%	239 ± 1.9 y	2.18 ± 0.01 y	4.25 ± 0.40 z	1.88 ± 0.01 yz	1.21 ± 0.01 yz	90 z
50%	236 ± 19.3 y	2.16 ± 0.11 y	3.56 ± 0.03 z	1.82 ± 0.12 z	1.26 ± 0.08 y	96
60%	230 ± 14.5 y	2.13 ± 0.08 y	3.69 ± 0.08 z	1.97 ± 0.07 yz	1.16 ± 0.04 yz	100 z
70%	181 ± 12.1 yz	1.85 ± 0.08 yz	3.73 ± 0.18 z	2.16 ± 0.20 yz	1.07 ± 0.09 yz 93 z	
80%	148 ± 12.4 z	1.62 ± 0.09 z	3.72 ± 0.03 z	2.38 ± 0.11 y	0.96 ± 0.04 z	93 z
60% + M + L	227 ± 11 y	2.11 ± 0.06 y	3.67 ± 0.03 z	1.88 ± 0.04 yz	1.21 ± 0.03 yz	95 z
70% + M + L	223 ± 9.2 y	2.09 ± 0.05 y	3.70 ± 0.05 z	1.88 ± 0.06 yz	1.21 ± 0.03 yz	93 z

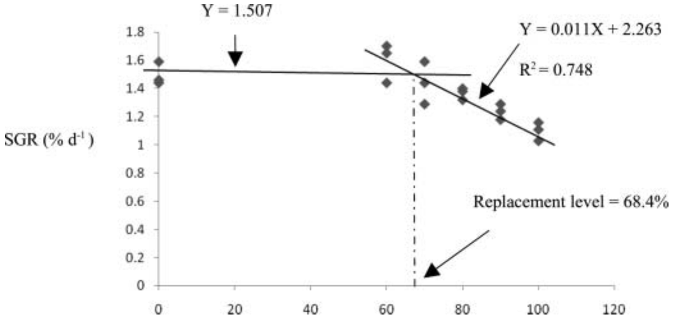

FIGURE 1. Relationship between specific growth rate (SGR,%/d) of juvenile black sea bass in experiment 2 and percentage of fish meal protein (FMP) replaced with soybean meal protein (SBP) with supplemental squid meal, krill meal, and attractants as described by the broken-line regression model.

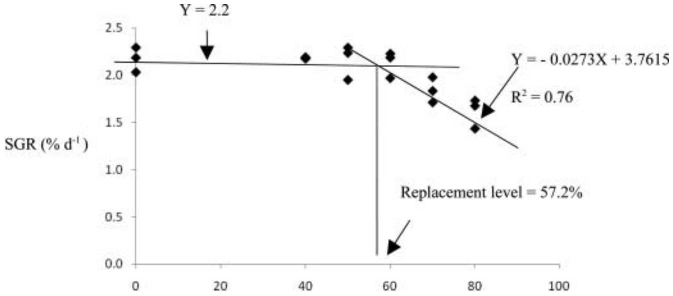

FIGURE 2. Relationship between specific growth rate (SGR,%/d) of juvenile black sea bass in experiment 3 and percentage of fish meal protein (FMP) replaced with soybean meal protein (SBP) without supplemental squid meal, krill meal, and attractants as described by the broken-line regression model.

(90–100%) was not significantly different among diets. Body weight gain (181–239%) of fish fed 40, 50, 60 and 70% SBP was not significantly different from fish fed the 0% SBP diet (BWG, 238%). However, body weight gain of fish fed the 80% (BWG, 148%) was significantly lower than that for fish fed the 0% SBP diet (Table 7). Without supplemental squid meal, krill meal, and methionine and lysine, the optimum FMP replacement for juvenile black sea bass based on BWG was 57.2%, as determined by broken-line regression analysis (Figure 2). In addition, body weight gain of fish fed 60% SBP + M + L (BWG, 227%) and 70% SBP + M + L (BWG, 223%) diets were not significantly different from that of fish fed the 0% SBP diet (BWG, 238%). Specific growth rate was significantly lower in fish fed the 80% SBP diet (1.62%/d) than in fish fed the 0% to 60% SBP diets (2.13–2.17%/d) (Table 7). The SGRs for the fish fed 60% SBP + M + L (2.11%/d) and 70% SBP + M + L (2.09%/d) were not significantly different from the SGR for fish fed the 0% SBP diet (2.17%/d). Feed intake showed no significant differences among fish fed test diets (3.56–4.25% BW/d). Although the lowest FCR (1.82) was found in fish fed the 50% SBP diet, it was not significantly different from that in fish fed the other diets (1.83–2.16) except for the 80% SBP diet (2.38). The PER was significantly lower for the fish fed the 80% SBP diet (0.96) than for fish fed the 0% SBP (1.24). The PERs for fish fed 60% SBP + M + L (1.21) and 70% SBP + M + L (1.21) diets were not significantly different compared with those for the fish fed the 0% SBP diet (1.24).

Whole-Body Proximate Composition

Experiment 1.—There were no significant differences in the whole-body moisture (666–679 g/kg), crude protein (165–175 g/kg), and lipid (92–102 g/kg) contents for black sea bass fed the 0% to 60% SBP diets (Table 8). Whole-body ash content of black sea bass fed 60% SBP diet (41.0 g/kg) was significantly lower than in fish fed 0% SBP diet (47.7 g/kg) (Table 8).

Experiment 2.—Fish whole body moisture content ranged from 668 to 691 g/kg among the treatments and there were no significant differences (Table 8). Crude protein content in fish fed the 0% SBP diet (177 g/kg) was significantly higher than in fish fed the other diets (160–170 g/kg). Crude lipid level tended to decrease in the whole body as soybean meal level increased in the diets and was significantly higher in the 0% SBP (130 g/kg) than in 60% to 100% SBP diets (109–121 g/kg). Whole-body ash content of the fish fed the 100% SBP diet (37.7 g/kg) was significantly lower than in fish fed the 0% SBP diet (45.7 g/kg) (Table 8); differences among the groups fed 60, 70, 80, 90, and 100% SBP diets (37.7–42.8 g/kg^1) were not significant.

Experiment 3.—Fish whole-body moisture content ranged from 656 to 668 gkg but there was no significant difference among the diets (Table 8). Crude protein content in fish fed the 0% SBP diet (151 g/kg) was not significantly different from fish fed the 40% to 70%SBP diets (148–155 g/kg). Crude lipid level was significantly lower in the 80% SBP diet (107 g/kg) than in the 40% and 60% SBP diets (both 117 g/kg). Whole-body ash content of the fish fed the 80% SBP diet (41 g/kg) was significantly lower than in fish fed 60% SBP (44 g/kg) (Table 8).

Fatty Acid Composition of the Whole Body (Experiment 2)

Saturated fatty acid concentration in fish fed the 100% SBP diet (88.6 mg/g) was significantly lower than in fish fed the 0% SBP diet (105.6 mg/g) (Table 9). Palmitic acid (16:0) concentration in fish fed the 90% SBP (57.6 mg/g) and 100% SBP (54.4 mg/g) diets were significantly lower than in fish fed the 0% SBP diet (68.2 mg/g). Oleic acid (18:1[n-9]) concentrations in the whole bodies were not significantly different among fish fed the 0, 60, 70, 80,and 90% SBP diets (50.4–56.7 mg/g). Fish fed 60% to 100% SBP diets (23.6–27.3 mg/g) were significantly higher in whole-body linoleic acid (18:2[n-6]) than in fish fed the 0% SBP diet (13.0 mg/g). Fish fed the 60, 80, and 90% SBP diets were significantly higher in EPA (20:5[n-3]) (37.0, 35.6, and 30.8 mg/g, respectively) than in fish fed the 0% SBP diet (23.7 mg/g). The DHA (22:6n-3) content in fish fed the 60 (28.9 mg/g), 80 (29.2 mg/g), and 90% diets (27.3 mg/g) was significantly higher than in fish fed the 0% SBP diet (21.8 mg/g). Fish fed the 60% SBP diet had a significantly lower DHA:EPA ratio (0.78) than fish fed the 0% SBP diet (0.93), but no significant ($P > 0.05$) differences were observed among 60% to 100% SBP

TABLE 8. Effects of replacement of fish meal protein (FMP; 0–100%) with soybean meal protein (SBP) on whole-body proximate composition (g/kg; wet weight basis) of black sea bass. Diet indicates the percentage of FMP replaced with SBP. Values are means ± SEs ($N = 3$). Means followed by different letters in the same column differ significantly ($P < 0.05$).

Diet	Moisture	Protein	Lipid	Ash
Experiment 1				
0%	666 ± 7.3 z	175 ± 2.1 z	96 ± 1.5 z	47.7 ± 1.7 y
10%	671 ± 5.5 z	173 ± 1.7 z	102 ± 0.5 z	44.8 ± 1.0 yz
20%	667 ± 3.0 z	174 ± 3.4 z	92 ± 2.0 z	42.9 ± 0.2 yz
30%	671 ± 4.7 z	170 ± 2.7 z	99 ± 4.7 z	41.7 ± 1.1 yz
40%	679 ± 3.6 z	165 ± 1.7 z	96 ± 2.2 z	44.3 ± 1.8 yz
50%	679 ± 2.7 z	166 ± 2.0 z	92 ± 1.9 z	44.5 ± 1.7 yz
60%	679 ± 2.0 z	167 ± 1.2 z	94 ± 2.0 z	41.0 ± 0.1 z
Experiment 2				
0%	668 ± 1.9 z	177 ± 0.7 w	130 ± 1.6 x	45.7 ± 1.2 y
60%	668 ± 5.1 z	166 ± 0.6 xy	121 ± 3.4 y	42.2 ± 1.8 yz
70%	669 ± 5.6 z	170 ± 1.0 xy	115 ± 0.4 yz	42.8 ± 0.8 yz
80%	675 ± 3.4 z	166 ± 0.8 xy	109 ± 1.4 z	42.6 ± 0.9 yz
90%	686 ± 1.4 z	162± 1.2 yz	112 ± 1.6 yz	41.3 ± 0.5 yz
100%	691 ± 2.8 z	160 ± 0.9 z	109 ± 0.7 z	37.7 ± 1.6 z
Experiment 3				
0%	668 ± 1.2 z	151 ± 1.2 yz	113 ± 1.2 xy	43 ± 1.0 yz
40%	667 ± 4.2 z	148 ± 1.1 yz	117 ± 1.2 x	42 ± 1.0 yz
50%	664 ± 2.6 z	155 ± 2.9 y	112 ± 3.5 xyz	43 ± 1.1 yz
60%	658 ± 1.5 z	151 ± 2.5 yz	117 ± 1.9 x	44 ± 1.0 y
70%	656 ± 1.4 z	149 ± 1.3 yz	110 ± 1.2 yz	44 ± 1.2 yz
80%	664 ± 2.6 z	145 ± 2.0 z	107 ± 1.0 y	41 ± 1.0 z
60% M + L	668 ± 1.05 z	148 ± 1.3 yz	111 ± 1.0 yz	41 ± 1.0 yz
70% M +L	658 ± 4.3 z	152 ± 1.8 yz	112 ± 1.0 xyz	41 ± 1.0 yz

diets (0.78–0.89). The n-3 PUFAs in fish fed the 60 (80.9 mg/g), 80 (79.0 mg/g), and 90% SBP (71.4 mg/g) diets were significantly ($P < 0.05$) higher than in fish fed the 0% SBP diet (56.3 mg/g). Fish fed all SBP diets had significantly lower n-3 : n-6 ratios (2.62–3.43) than did fish fed the 0% SBP diet (4.34).

DISCUSSION

The overall growth of the fish was satisfactory in experiment 1 (initial weight, 5.1 g; final weights, 15.8–17.5 g) for 42 d, experiment 2 (initial weight, 10.1 g; final weights, 22.8–28.5 g) for 70 d, and experiment 3 (initial weight, 5.3 g; final weights, 13.3–18.5 g) for 56 d, and was comparable with results of our previous study on black sea bass of the same size fed formulated diets (Alam et al. 2008) as well as with other marine fishes such as European sea bass *Dicentrarchus labrax* (Peres and Oliva-Teles 1999). The higher weight gain and SGR in experiment 1 was attributed to the smaller initial sizes of fish used in that experiment compared with experiment 2. The slightly higher growth and SGR found in experiment 1 compared with experiment 3 could be due to different parentage (cohorts) of fish, the different times experiments were conducted, presence of squid meal, krill meal, and attractants in the diets of experiments 1 and 2, and duration of the studies. In experiment 1, no significant differences were found in growth performance among the groups fed 0% to 60% SBP diets (Table 7), indicating that the FMP replacement with SBP could be more than 60% for juvenile black sea bass. In experiment 2, fish fed the 90% and 100% SBP diets showed significantly lower growth than did fish fed the 0, 60, and 70% SBP diets (Table 7), but growth was not significantly different from that in fish fed the 80% SBP diet. This suggested that the optimum level of FMP replacement with SBP was 70% with 75 g/kg squid meal and 50 g/kg krill meal. This finding is very similar to the results of broken-line regression analysis, which showed the optimum FMP replacement for juvenile black sea bass based on SGR was 68.4% (Figure 1) with squid and krill meal supplementation. Without supplemental squid and krill meal, the optimum FPM replacement level was 57.2% (Figure 2), but supplementing methionine and lysine in 60% and 70% SBP diets (experiment 3) improved body weight gain to a level not significantly different from the 0% SBP diets. When the results of all three experiments were combined SGR was compared as a percentage of the respective controls, break points for FMP replacement occurred at 67.6% and 58.0% for experiments 1 and 2, respectively (Figure 3), which is very similar to the values obtained from Figure 1 (68.4%) and Figure 2 (57.2%). Varying levels of SBP have been incorporated

TABLE 9. Fatty acid composition (mg/g dry sample) of whole bodies of juvenile black sea bass in experiment 2 after 70 d of feeding. Values are means ± SEs ($N = 3$). Means followed by common letters are not significantly different ($P > 0.05$). Diet indicates the percentage of fish meal protein replaced with soybean meal protein; SFAs = saturated fatty acids, MUFAs = monounsaturated fatty acids, and PUFAs = polyunsaturated fatty acids.

	Diet					
Fatty acid	0%	60%	70%	80%	90%	100%
14:0	18.2 ± 0.35 yz	22.5 ± 0.90 x	16.9 ± 0.25 z	20.2 ± 0.32 xy	16.7 ± 0.61 z	15.6 ± 1.06 z
16:0	68.2 ± 1.29 x	68.6 ± 1.45 x	63.2 ± 0.93 xyz	65.2 ± 0.70 xy	57.6 ± 1.61 yz	54.4 ± 3.65 z
16:1	31.2 ± 0.58 yz	38.3 ± 1.97 x	31.6 ± 0.57 yz	36.8 ± 0.39 xy	29.5 ± 2.03 z	29.4 ± 2.03 z
18:0	13.5 ± 0.48 z	13.4 ± 0.08 z	13.7 ± 0.25 z	13.6 ± 0.26 z	12.6 ± 0.22 z	12.1 ± 0.76 z
18:1(n-11)	10.3 ± 0.17 z	11.8 ± 0.24 yz	11.2 ± 0.24 yz	12.2 ± 0.14 y	11.5 ± 0.34 yz	11.2 ± 0.76 yz
18:1(n-9)	52.5 ± 0.51yz	56.7 ± 1.01y	55.5 ± 1.09 yz	55.7 ± 0.82 y	50.4 ± 1.47 yz	47.8 ± 3.26 z
18:2(n-6)	13.0 ± 0.30 z	24.2 ± 0.61 y	24.0 ± 0.60 y	27.3 ± 0.27 y	23.6 ± 0.83 y	25.8 ± 1.82 y
18:4(n-3)	4.9 ± 0.23 z	7.94 ± 0.36 x	6.07 ± 0.42 yz	7.17 ± 0.02 xy	6.52 ± 0.34 xyz	6.14 ± 0.49 yz
20:0	5.65 ± 0.17 z	5.94 ± 0.08 z	6.01 ± 0.16 z	6.31 ± 0.18 yz	7.15 ± 0.09 y	6.48 ± 0.42 yz
20:1	3.41 ± 0.19 z	3.93 ± 0.19 z	3.52 ± 0.14 z	3.82 ± 0.09 z	3.64 ± 0.06 z	3.48 ± 0.10 z
20:5(n-3)	23.7 ± 1.99z	37.0 ± 1.23 x	29.6 ± 0.61 yz	35.6 ± 0.61 xy	30.8 ± 1.13 xy	29.6 ± 2.1 yz
22:1	3.35 ± 0.03 z	3.61 ± 0.08 yz	3.71 ± 0.07 yz	3.88 ± 0.21 yz	4.90 ± 0.08 x	4.28 ± 0.28 xy
22:5(n-3)	5.88 ± 0.09 z	7.07 ± 0.14 y	6.32 ± 0.13 yz	7.11 ± 0.08 y	6.70 ± 0.20 yz	6.19 ± 0.40 yz
22:6(n-3)	21.8 ± 2.3 z	28.9 ± 0.68 xy	24.9 ± 0.50 yz	29.2 ± 0.40 x	27.3 ± 0.83 xy	25.6 ± 1.6xyz
$\sum$SFAs	105.6 ± 2.3 xy	110.4 ± 2.34 x	99.9 ± 1.48 xyz	105.4 ± 0.92 xy	93.9 ± 2.48 yz	88.6 ± 5.86 z
$\sum$MUFAs	100.7 ± 1.2 yz	114.4 ± 3.41 y	105.4 ± 1.79 yz	112.4 ± 1.51 y	99.9 ± 2.29 yz	96.2 ± 6.44 z
$\sum$n-3 PUFAs	56.3 ± 2.31 z	80.9 ± 2.24 x	66.9 ± 1.63 yz	79.0 ± 0.67 xy	71.4 ± 2.37 xy	67.5 ± 4.52 yz
$\sum$n-6 PUFAs	12.9 ± 0.30 z	24.2 ± 0.61 y	23.9 ± 0.60 y	27.3 ± 0.27 y	23.6 ± 0.83 y	25.8 ± 1.82 y
n-3/n-6 PUFAs	4.34 ± 0.11 w	3.43 ± 0.01 x	2.79 ± 0.02 yz	2.90 ± 0.03 y	3.03 ± 0.01 y	2.62 ± 0.02 z
22:6(n-3)/20:5(n-3)	0.93 ± 0.07 y	0.78 ± 0.01 z	0.84 ± 0.01 zy	0.82 ± 0.02 zy	0.89 ± 0.01 zy	0.87 ± 0.01 zy

successfully into the diets of other marine and freshwater species to replace FMP. In the present study, with supplemental squid meal, krill meal, and attractants, the maximum replacement level of FMP with SBP (67.6–68.4%) was higher than reported for other marine finfish species such as Japanese flounder (45%, Kikuchi 1999); gilthead seabream (45%, Martinez-llorens et al. 2008); Japanese yellowtail (20%, Shimeno et al. 1993), cobia (50%, Zhou et al. 2005), and Atlantic cod (50%, Walker et al. 2010), but lower than that found for freshwater fishes such as common carp *Cyprinus carpio* (100%, Viola et al. 1982) and Nile tilapia *Oreochromis niloticus* (100%, Deyab et al. 2002).

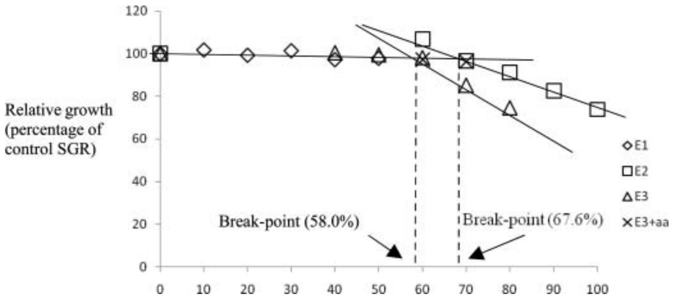

FIGURE 3. Relationship between relative growth (percentage of control specific growth rate [SGR]) of juvenile black sea bass and percentage of fish meal protein (FMP) replaced with soybean meal protein (SBP) with or without supplemental squid meal, krill meal, and attractants as described by the broken-line regression model. Abbreviations E1, E2, E3, and E3 + aa represent experiments 1, 2, and 3 and experiment 3 supplemented with amino acids, respectively. Data were calculated from SGR in each experiment as a percentage of the control value. The equations for the break point of 67.6% were $y = 100.114$ when $x \leq 67.6$ and $y = -0.7987x + 154.09$ when $x > 67.6$. The equations for the break-point of 58.0% were $y = 100.114$ when $x \leq 58.0$ and $y = -1.1751x + 168.28$ when $x > 58.0$.

The ability of black sea bass to grow on diets with high substitution levels of SBP for FMP indicates a superior ability to digest SBP compared with other marine finfishes such as yellowtail (Shimeno et al. 1993). This may indicate that black sea bass possess specific digestive enzymes, but further investigation is needed. Protein digestibility of the test feeds was not measured in the feeding trials; however, for several species it has been suggested that the high growth performance and high feed utilization of fish fed high substitution levels of soybean meal for fish meal is due to higher digestibility of nitrogen and energy (Storebakken et al. 2000; Hendricks 2002; Martinez-llorens et al. 2008).

In experiments 1 and 2 of the present study, growth performance of black sea bass was probably promoted by the addition of krill and squid meal as well as the addition of attractants (taurine, betaine, glycine, and alanine) to the diets to improve palatability. As a result, the optimum FMP replacement level was higher (68.4%, experiment 2) than in fish fed without supplemental attractants: methionine, lysine, squid meal, and krill meal (57.2%, experiment 3). Saitoh et al. (2003) found that 32% of extruded soybean meal could be added to the diet of Japanese flounder without compromising growth when the diet contained 6% squid powder and 4% krill meal but no supplemental amino acids. Reduced growth in juvenile black sea bass fed diets with 90% or 100% of the FMP replaced with SBP may be due to antinutritional factors in SBP and effects on intestinal pathology as reported in other species (Burrells et al. 1999). Heat treatment of SBP inactivates most but not all of the trypsin inhibitor (Tomas et al. 2005). Huismann and van der Poel (1989) demonstrated that the effects of antinutritional factors cannot be extrapolated from one species to another. Feed formulation and ingredient differences, changes in feed manufacturing technology, different environmental conditions, and differences in genetic stocks within each species all combine to make it impossible to prescribe absolute usage guidelines for soybean meals in aquaculture feeds. 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 fish growth performance because of the different quantities of antinutrient compounds or slowly digestible carbohydrates and soluble fiber content, or both, present in these products (Refstie et al. 1997).

In experiment 1, no significant differences were found in feed intake, FCR, and PER among the groups fed 0% to 60% SBP diets (Table 7), but in experiment 2, a significant decline in feed intake was observed in the groups fed 90% and 100% SBP (Table 7), indicating reduced palatability of diets containing greater than 80% SBP. However, in experiment 3, feed intake did not differ between the 0% SBP and the 60% and 70% SBP diets supplemented with methionine and lysine, suggesting that methionine and lysine promoted growth of black sea bass. A significant decline in feed intake was observed in yellow perch when fed diets that had 63.5% of the FMP replaced with SBP (Kasper et al. 2007). The lower feed intake of black sea bass fed the 90% and 100% SBP diets was one of the reasons for poor growth at these high replacement levels. In addition, FCR for black sea bass fed diets containing more than 70% SBP was significantly higher than that for fish fed the 0% SBP diet. Gilthead seabream fed diets with 60% or 75% FMP replaced with SBP had higher FCRs compared with fish fed only the FMP diet (Martinez-Llorens et al. 2008). The PER increased with SBP replacement for fish meal to a maximum at 70% SBP, but decreased at higher replacement levels (Table 7). The PER declined when 40% and 45% of FMP was replaced by SBP in Mediterranean yellowtail (Tomas et al. 2005) and gilthead seabream (Martinez-Llorens et al. 2008). The poor growth performance and low feed utilization of fish fed high substitution levels of soybean meal for fish meal may be due to lower digestibility of nitrogen and energy, the presence of nondigestible oligosaccharides, mineral deficiencies, amino acid deficiencies, and antinutritional factors (Storebakken et al. 2000).

In this study, survival in all three experiments remained high (87–100%) throughout the study, with no significant differences apparent. This is similar to what was reported for Atlantic cod (Hansen et al. 2007) and rainbow trout (Bureau et al. 2000), which showed no significant differences in survival when fed diets containing high levels of soybean meal. Kasper et al. (2007), however, found a significant decline in the survival of yellow perch when fed diets with 92% and 100% of the FMP replaced with SBP.

In experiment 1, whole-body moisture and protein and lipid contents were not affected by SBP replacement of FMP up to 60%. In experiments 2 and 3, no significant differences were found in whole-fish body moisture among treatment groups (Table 8), which is similar to findings for yellow croaker *Pseudosciaena crocea* (Ai et al. 2006), rainbow trout (Bureau et al. 2000), and rohu *Labeo rohita* (Khan et al. 2003). Whole-body lipid content, however, was significantly lower in fish fed the 60% to 100% SBP diets in experiment 2 compared with the 0% SBP diet in experiment 3 (Table 8). A decline in whole-body lipid level may indicate increasing use of lipid for energy in fish fed diets high in SBP (Krogdahl et al. 2003). Mangrove red snapper also had a lower whole-body lipid level when 50% FMP was replaced with SBP in the diets (Catacutan and Pagador 2004).

In the SBP diets (experiment 2), lysine and methionine content appeared to be lower than in the FMP diet (Table 4), and as SBP increased in the diets, lysine and methionine levels decreased. Other essential amino acids in the test diets did not show large deficits compared with to the control diet (Table 4). Diets of juvenile cobia (Zhou et al. 2005), gibel carp *Carassius gibelio* (Jose et al. 2006), Mediterranean yellowtail (Tomas et al. 2005), and Atlantic cod (Hansen et al. 2007) with high inclusion of SBP were also deficient in methionine and lysine compared with the diets based on fish meal. The lysine and methionine requirements of black sea bass are unknown at this time. Lower methionine and lysine in SBP diets than 45% whole-body protein (Table 4) suggest that these amino acids were deficient. The amino acid availability of soybean meal for black sea bass was not investigated. Unbalanced dietary amino acids and lower amino acid availabilities in the test diets could affect the digestion, absorption, and metabolism of these nutrients (Rawles et al. 2009).

In experiment 3, two diets were formulated with supplemental methionine and lysine in 60% and 70% SBP diets to simulate the amino acid profile found in 45% whole-body protein of juvenile black sea bass. Whole-body amino acid profiles were used as the reference of amino acid requirements for several species (Wilson 2002; Alam et al. 2005). In the 60% SBP + M + L and 70% SBP + M + L diets (experiment 3), methionine contents were higher than in the 0% SBP diet (Table 5), and lysine contents were similar to the 0% SBP diets. Similar growth and feed intake among these diets indicated that supplemental methionine and lysine improved feed utilization and growth performance to the level observed in control fish. However, higher substitution of FMP by SBP could be possible with methionine and lysine supplementation in diets of black sea bass, but this remains for future investigation. Red drum grew successfully with diets in which 90% and 95% of FMP was replaced with SBP, but also with additions of amino acids (McGoogan and Gatlin 1997).

In general, the fatty acid composition of the fish whole body (experiment 2) reflected the composition of their diets (Table 6 and 9). Linoleic acid (18:2[n-6]) was higher in the SBP diets and also higher in the whole body of fish fed those diets. Studies on yellow perch (Kasper et al. 2007), pikeperch *Sander lucioperca* (Schultz et al. 2005), and Japanese flounder (Lee et al. 2000) also found a higher level of linoleic acid in fish fed diets containing SBP or soybean oil. The total n-3 PUFA level (comprising 2.7–3.6% of the diets) was higher in fish fed the 60% to 100% SBP diets compared with the 0% SBP diet (Table 6) and exceeded the typical minimum requirements (0.8– 2.5%) for juvenile marine fish (Sargent et al. 2002). As SBP content was increased in the diets, fish oil was increased to compensate for the low lipid content of SBP, leading to higher n-3 PUFA levels in the SBP diets (Table 6). There is no information on replacement of fish oil by vegetable oil in black sea bass diets. The main objective of this study was to determine the influence of fish meal protein replacement (not fish oil) on growth performance. Fish meal also contains oil, and fish oil was therefore increased as fish meal was reduced in the diets. To estimate wet weight equivalents of forage fish used in the different diets, global average processing yields of wet fish to fish meal and fish oil of 22.5% and 5%, respectively, were used (Tacon and Metian 2008). In this study, the wet weight equivalent of forage fish used in the 70% SBP diets (15% fish meal and 6.5% fish oil) was 31% lower than in the control diet (50% fish meal and 3% fish oil, experiment 2). To further improve the efficiency of forage fish transformation, additional studies are needed to determine maximum fish oil replacement by vegetable oil in high soybean based diets for black sea bass.

Eicosapentaenoic acid (20:5[n-3]) and docosahexaenoic acid (22:6[n-3]) concentrations were the same or higher in whole bodies of fish fed all the SBP diets compared with fish fed the control diet (Table 9), and this was related to the EPA and DHA levels in the diets. Levels of DHA (0.8–1.1%) and EPA (1.3–1.7%) in the diets were within the range required in other marine finfish species, such as red seabream (0.5–1.0% and 1.0% of the dry diet, respectively) (Sargent et al. 2002). In this study, the ratio of DHA to EPA in the diets (0.64–0.68) was within the range of 0.5–1.0 required by gilthead seabream (Sargent et al. 2002). Although the DHA or EPA requirements for juvenile black sea bass are not known, based on the requirements of other marine finfishes listed above, it appears that sufficient DHA and EPA was provided in all diets.

In summary, our results suggested that the maximum level of FMP replacement with SBP in black sea bass diets was 67.6–68.4% with 75 g/kg squid meal, 50 g/kg krill meal, and 10 g/kg attractants (alanine, glycine, taurine, and betaine) in the diet and 57.2–58.0% without squid meal, krill meal, and attractants supplementation. However, supplemental methionine and lysine in diets having 60% and 70% FMP replaced with SBP (without squid meal, krill meal, and attractants) improved growth performance compared with the control diet, but replacement levels higher than 70% were not tested and warrant further investigation. All three feeding trials proved that black sea bass juveniles are able to utilize high levels of soybean meal in their diet without reducing growth performance. Addition of squid, krill meal, and attractants or supplementation with methionine and lysine increased FMP replacement with SBP by about 10%.

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