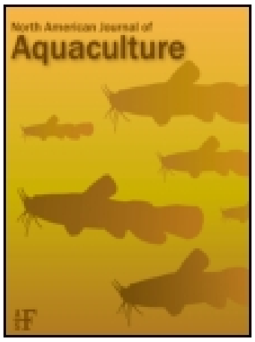

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ARTICLE

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Abstract
Two feeding experiments were conducted to study the effects of different levels of solvent-extracted soybean meal on the growth and body composition of juvenile southern flounder *Paralichthys lethostigma*. In experiment 1, seven diets were formulated, replacing menhaden fish meal protein (FMP) with soybean meal protein (SBP) at 0, 10, 20, 30, 40, 50, and 60% without supplementing amino acids. In experiment 2, eight diets were formulated, replacing FMP with SBP at 0, 10, 20, 30, 40, 50, 60, and 70% and supplementing with L-methionine and L-lysine. In the test diets, L-methionine and L-lysine were supplemented to approximate the methionine and lysine level found in the 45% whole-body protein of southern flounder. All diets were formulated to have the same crude protein (45%) and lipid levels (12%), and all diets contained 9% squid meal and 5% krill meal to improve palatability. Each of the test diets was fed two times a day to triplicate groups of flounder (average weight = 0.61 g for experiment 1 and 1.63 g for experiment 2). Experiments 1 and 2 were conducted for 42 and 60 d, respectively. Fish were held in 75-L recirculating seawater tanks at 15 fish per tank. In both experiments, no significant differences were observed in body weight gain (WG), specific growth rate, feed intake, feed conversion and protein efficiency ratios, and the whole-body proximate composition among fish fed diets replacing 0–40% of FMP with SBP. When compared with fish fed 0% SBP, WG decreased significantly for the fish fed more than 40% SBP. Broken-line regression showed that the optimum levels of FMP replacement with SBP in the diet of southern flounder without and with supplemental methionine and lysine were 35.1% and 38.9%, respectively, when diets contained 9% squid meal and 5% krill meal.

The southern flounder *Paralichthys lethostigma* (family Bothidae) is a flatfish found in coastal waters from North Carolina through the South Atlantic states to Texas, except for South Florida (Wenner et al. 1990). Their euryhaline character, tolerance of a wide range of temperatures, and status as a highly desirable food and recreational species make southern flounder an ideal candidate for aquaculture. Declining commercial and recreational landings has also stimulated interest in culturing southern flounder for stock enhancement. The methodology for spawning and larval rearing of this species is well documented (Watanabe et al. 2001b, 2006; Daniels and Watanabe 2003); however, development of sustainable feeds for production of market size fish from juvenile stage is critical for commercial production of this ongoing species.

Because of declining production and increasing price, fish meal is one of the most expensive macro-ingredients used in

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high percentages in aquaculture feeds (Hardy 2006). Alternate protein sources can reduce the amount of wild fish used as protein, lower the cost of aquaculture diets, and potentially reduce the nutrient levels in effluent waste (Trushenski et al. 2006). However, for most finfish species, there is a limit to how much fish meal can be replaced by alternative plant protein sources without negatively affecting growth and feed efficiency (Gatlin et al. 2007). The maximum replacement levels of alternative plant protein sources for fish meal vary greatly, depending on species (Webster et al. 1999; Lochmann and Kumaran 2006; Lim et al. 2008; Jirsa et al. 2010).

Among the plant protein sources considered, soybean has been used preferentially for replacement of fish meal because of its high protein content (Hardy 2006; Gatlin et al. 2007) and global availability. Soybean meal has been used successfully to partially replace fish meal in many carnivorous marine fish species, including gilthead sea bream *Sparus aurata* (Nengas et al. 1999); Japanese yellowtail *Seriola quinqueradiata* (Shimeno et al. 1993); Mediterranean yellowtail *S. dumerili* (Tomas et al. 2005); red snapper *Lutjanus argentimaculatus* (Catacutan and Pagador 2004); Japanese flounder *Paralichthys olivaceus* (Kikuchi 1999); red drum *Sciaenops ocellatus* (McGoogan and Gatlin 1997); cobia *Rachycentron canadum* (Zhou et al. 2005); Atlantic cod *Gadus morhua* (Hansen et al. 2007), and white sea bass *Atractoscion nobilis* (Jirsa et al. 2010). These studies have shown that maximum levels of fish meal replaced by soybean meal vary considerably among marine species.

However, the 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 fish meal–based diet were partly attributed to the lower lysine level in soybean meal diets (Hansen et al. 2007). Supplementation of methionine and lysine in the diets improved growth performance of red sea bream *Pagrus major* (Takagi et al. 2001) and yellowtail (Watanabe et al. 2001a). No information has been published on fish meal replacement with alternative protein sources in southern flounder diets. The objectives of this study were to investigate the effects of replacing menhaden fish meal protein from 0% to 70% with solvent-extracted soybean meal protein, with or without supplemental L-methionine and L-lysine, on growth performance, feed utilization, and body composition of juvenile southern flounder.

METHODS

Experimental fish and system.—Adult southern flounder broodstock held in photothermally controlled tanks were induced to spawn by using luteinizing hormone releasing–hormone analog (Watanabe et al. 2006) at the University of North Carolina Wilmington–Center for Marine Science (UNCW–CMS), Aquaculture Facility (Wrightsville Beach, North Carolina). Embryos were hatched and larvae reared according to published protocols (Daniels and Watanabe 2003). Early juveniles were raised in 150-L rectangular raceways and later in recirculating tanks until the feeding experiments were conducted. Fish were fed a commercially prepared diet containing 50% protein and 15% lipid (Skretting, Vancouver, British Columbia) until the studies commenced.

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, Inc., Providence, Rhode Island) biofilter, 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 ultraviolet sterilizer for disinfection. Temperature was controlled with 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).

Experiment 1 test diets.—Seven isonitrogenous (45%) and isolipidic (12%) test diets (Table 1) were prepared in which 0, 10, 20, 30, 40, 50, and 60% of menhaden fish meal protein (FMP; 63.5% crude protein) was replaced by solvent-extracted soybean meal protein (SBP; 47.5% crude protein). This was accomplished by reducing menhaden fish meal by 0, 5, 10, 15, 20, 25, and 30% and adding soybean meal to levels of 0, 6.7, 13.4, 20.0, 26.7, 33.4, and 40.1% in the diet, respectively (Table 1). The control diet contained 50% menhaden fish meal, and all experimental diets contained 9% squid meal and 5% krill meal to improve palatability. To maintain isolipidic levels and to avoid deficiency of highly unsaturated fatty acid profiles in all diets, menhaden fish oil content was increased as the proportion of fish meal in the diet decreased. Equal quantities of Kadai vitamin and mineral premix (Kohkin Chemical Industries, Inc., Kagoshima, Japan) for marine fish were used in the diets. Energy levels of diets were calculated based on 23.6, 39.6, and 17.2 kJ/g for protein, lipid, and nitrogen-free extract, respectively (Blaxter 1989). Diets were prepared at UNCW–CMS using a feed mixer (Kitchen Aid, Inc., St. Joseph, Michigan), a meat grinder (Jacobi-Lewis Co., Wilmington, North Carolina), and a drying oven (Yamato Scientific Co., Ltd., Japan; Alam et al. 2009). Proximate composition of the test diets is shown in Table 1.

Experiment 1 experimental conditions.—Fifteen fish were stocked in each of twenty-one 75-L tanks. After the fish were acclimated to laboratory conditions, they were fed the control diet (0% SBP) for 1 week and then each test diet was fed to triplicate groups of fish (weight = 0.61 ± 0.03 g [mean ± SD]) for 42 d. Fish were fed twice per day (at 0900 and 1600 hours) to apparent satiation, and the amount of diet fed was recorded daily. Tanks were siphoned daily or as needed. To prevent fish

TABLE 1. Composition of diets in experiment 1, by percentage of fish meal protein replaced with soybean meal protein.

Diet item or component	0%	10%	20%	30%	40%	50%	60%
Components (g/100 g)							
Menhaden meal[a]	50.0	45.0	40.0	35.0	30.0	25.0	20.0
Soybean meal[b]	0.0	6.7	13.4	20.0	26.7	33.4	40.1
Squid meal[c]	9.0	9.0	9.0	9.0	9.0	9.0	9.0
Krill meal[d]	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Menhaden fish oil[e]	3.5	4.0	4.5	5.0	5.5	6.0	6.5
Soybean lecithin[f]	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Wheat gluten[g]	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Wheat starch	14.5	12.3	10.1	8.0	7.8	7.6	7.4
Vitamin mix[h]	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Mineral mix[h]	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Cellulose	6.0	6.0	6.0	6.0	4.0	2.0	0.0
Total	100	100	100	100	100	100	100
Proximate composition (%)							
Protein	45.2	45.3	44.3	45.5	45.4	44.4	45.3
Lipid	12.8	12.6	12.1	12.4	12.0	12.2	12.5
Ash	13.0	12.6	12.1	11.4	11.2	10.9	10.3
Gross energy[i]	18.3	18.1	18.0	18.0	18.1	18.3	18.9

[a]International Proteins Corp., Minneapolis, Minnesota (protein, 63.5%; lipid, 12.5%).
[b]Southern States, Wallace, North Carolina (protein, 47.5%).
[c]The Scoular Co., Minneapolis, Minnesota (protein, 78%; lipid, 11%).
[d]Aqion, Vincent, Ohio (protein, 60%; lipid, 10%).
[e]Virginia Prime Silver; Omega Protein, Hammond, Louisiana.
[f]ADM, Decatur, Illinois.
[g]Sigma-Aldrich, St. Louis, Missouri (protein, 80%).
[h]As in Alam et al. (2009).
[i]Calculated (kJ/g diet) based on carbohydrates, proteins, and lipids at 17.2, 23.6, and 39.5 kJ/g, respectively (Blaxter 1989).

from jumping out, all the tanks were covered with glass lids. A photoperiod of 10 h light : 14 h dark was maintained. Water quality was verified twice weekly. During the feeding period, water temperature ranged from 22.5°C to 23.7°C and dissolved oxygen ranged from 7.1 to 7.7 mg/L. The ranges of other water quality parameters in the experimental tanks during the experimental periods were as follows: pH 7.3–7.8, salinity 33.2–33.8 g/L, ammonia 0.21–0.26 mg/L, and nitrite 0.06–0.11 mg/L. Fish from each tank were weighed in bulk every 2 weeks. After 42 d of feeding, 10 fish from each tank were killed, freeze-dried, and stored at −80°C for whole-body proximate composition analysis.

Experiment 2 test diets.—In experiment 2, eight isonitrogenous (45%) and isolipidic (12%) test diets were prepared, replacing 0, 10, 20, 30, 40, 50, 60, and 70% of FMP with solvent-extracted SBP (Table 2). Total amino acid profiles of juvenile southern flounder whole bodies were analyzed (Table 3) before preparing the diets. Test diets were supplemented with crystalline L-methionine and L-lysine to simulate the levels of dietary methionine and lysine found in 45% whole-body protein of southern flounder from a reference protein concept (Wilson 2002; Alam et al. 2005; Tables 2, 3). Carboxymethyl cellulose was added to coat the crystalline amino acids to retard leaching losses into water (Alam et al. 2002, 2005). All other ingredients were added as in experiment 1. Diet preparation and sources of all other ingredients were the same as in experiment 1. The proximate composition and total amino acid composition of the diets are shown in Tables 2 and 3, respectively.

Experiment 2 experimental conditions.—In experiment 2, eight test diets were fed to triplicate groups (15 fish per tank) of juvenile southern flounder (1.63 ± 0.14 g) for 60 d. The ranges of water quality parameters in the experimental tanks during the experimental periods were as follows: dissolved oxygen from 7.1 to 7.8 mg/L, temperature from 22.2°C to 23.5°C, salinity from 33.4 to 33.9 g/L, pH from 7.5 to 7.8, ammonia from 0.23 to 0.26 mg/L, and nitrite from 0.04 to 0.11 mg/L. Feeding and sampling protocols were the same as described in experiment 1. Fish were weighed every 2 weeks. After 60 d of feeding, final fish weight was measured, and 10 fish were sampled from each tank, killed, freeze-dried, and stored at −80°C for proximate analysis as in experiment 1.

Proximate composition.—The proximate composition of diets and whole-fish bodies for both experiments were determined at UNCW–CMS. Crude protein was determined by the Kjeldahl method (Kjeltec System; Labconco Corp., Kansas City, Missouri), using boric acid to trap ammonia. Crude lipid (Soxhlet by ether extraction), ash (BARNSTAD Thermolyne Muffle Furnace, Iowa), and moisture (Isotemp Oven; Fisher Scientific

TABLE 2. Composition of diets in experiment 2, by percentage of fish meal protein replaced with soybean meal protein. In this experiment, diets were supplemented with L-methionine and L-lysine.

Diet item or component	0%	10%	20%	30%	40%	50%	60%	70%
Component (g/100 g)								
Menhaden meal	50.0	45.0	40.0	35.0	30.0	25.0	20.0	15.0
Soybean meal	0.0	6.7	13.4	20.0	26.7	33.4	40.1	46.8
Squid meal	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0
Krill meal	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Menhaden fish oil	3.5	4.0	4.5	5.0	5.5	6.0	6.5	6.5
Soybean lecithin	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Wheat gluten	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Wheat starch	14.5	12.3	10.1	8.0	7.8	7.6	5.6	3.7
Vitamin mix	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Mineral mix	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
L-Methionine[b]	0.17	0.21	0.25	0.29	0.33	0.37	0.41	0.45
L-Lysine[b]	0.48	0.53	0.59	0.64	0.69	0.75	0.80	0.85
CMC[c]	0.05	0.06	0.06	0.07	0.07	0.08	0.09	0.10
Cellulose	5.3	5.2	5.1	5.0	2.91	0.80	0.5	0.6
Total	100	100	100	100	100	100	100	100
Proximate composition (%)								
Protein	45.1	45.3	44.6	44.7	45.8	45.7	45.9	46.1
Lipid	12.5	12.8	12.4	12.7	12.4	12.2	12.7	12.3
Ash	13.1	12.8	12.2	11.4	11.3	11.1	10.6	9.6

[a]Sources of all ingredients are as in Table 1.
[b]To simulate 45% southern flounder whole-body protein (see Table 3).
[c]Carboxymethyl cellulose.

Co., Pittsburgh, Pennsylvania) contents of diets were analyzed by standard methods (AOAC International 2000); the moisture contents of whole bodies were determined after freeze-drying (Labconco Freeze Dryer).

Amino acid compositions of diets and juvenile southern flounder.—The total amino acid composition of the diets (experiment 2) was determined 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. The whole-body amino acid content of juvenile southern flounder was analyzed (New Jersey Feed Laboratory, Trenton, New Jersey) before formulating and preparing the test diets. Amino acid composition (% dry samples) of the diets and of whole-body southern flounder (% protein) is summarized in Table 3.

Statistical analysis.—All data were subjected to statistical analysis using one-way analysis of variance (ANOVA; software package JMP, version 7.0; SAS Institute, Inc., Cary, North Carolina). Significant differences between means were evaluated by the Tukey–Kramer test (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 fish meal protein replacement. Regression analysis was analyzed with JMP, version 7.0. All percentages data were arcsine-transformed before analysis.

RESULTS

Growth Performance and Feed Efficiency

Table 4 shows the final percent body weight gain (WG), specific growth rate (SGR), feed intake (FI), feed conversion ratio (FCR), protein efficiency ratio (PER), and percent survival (SR) for experiments 1 and 2. In experiment 1, no significant differences ($P < 0.05$) were observed in WG (646–767%) and SGR (4.78–5.14) among the fish fed 0–40% SBP diets. Compared with WG in fish fed 0% SBP (767), WG increased at a significantly slower rate for the fish fed more than 40% SBP (428–521). Feed intake (3.10) for fish fed 60% SBP diets was significantly lower than the 0% SBP diet (3.63); however, FI (3.12–3.63) and FCR (0.77–0.95) were not significantly different among fish fed 0–50% SBP diets. Protein efficiency ratios were significantly lower for the 50% and 60% SBP diets (0.64–0.90) than for the 0% and 10% SBP diets (1.35–1.5). However, there were no significant differences among the groups fed 0–40% SBP diets (1.09–1.50). The lowest SR (67%), which was found for fish fed the 60% SBP diet, was significantly lower than that for fish fed the 0% SBP diet (93%). The SR was not significantly different among the fish fed 0–50% SBP diets (80–93%). The results (experiment 1) showed that FMP can be replaced by 40% SBP without supplementing methionine and lysine when diets contained 9% squid and 5% krill meal. However, broken-line regression analysis showed a growth optimum at 35.1%

TABLE 3. Analyzed amino acid composition (g/100 g dry diet) of experiment 2 diets and juvenile southern flounder whole bodies. Values are means ($N = 2$). The percentages are the percentages of fish meal protein replaced with soybean meal protein.

			Test Diets							
Amino acid[16]	Juvenile southern flounder (% of protein)	Calculated value in 45% protein diet	0%	10%	20%	30%	40%	50%	60%	70%
Essential										
Methionine	2.67	1.20	1.09	0.97	0.96	0.92	0.96	0.89	0.95	0.98
Lysine	7.91	3.56	2.62	2.39	2.44	2.32	2.52	2.39	2.58	2.69
Threonine	4.22	1.90	1.39	1.23	1.24	1.18	1.28	1.22	1.32	1.35
Isoleucine	2.99	1.35	1.44	1.26	1.30	1.24	1.37	1.31	1.43	1.55
Leucine	5.50	2.48	2.59	2.26	2.32	2.23	2.43	2.33	2.54	2.67
Valine	3.74	1.68	1.64	1.42	1.45	1.38	1.49	1.42	1.55	1.65
Histidine	1.97	0.88	0.86	0.78	0.80	0.76	0.84	0.80	0.90	0.89
Phenylalanine	1.33	0.60	1.57	1.39	1.46	1.42	1.57	1.54	1.70	1.78
Arginine	6.15	2.77	2.19	1.95	2.02	1.96	2.15	2.08	2.28	2.38
Nonessential										
Tyrosine	2.57	1.16	1.18	0.98	1.03	1.02	1.14	1.09	1.22	1.25
Hydroxylysine	0.42	0.19	0.26	0.16	0.11	0.15	0.10	0.09	0.05	0.05
Aspartic acid	9.49	4.27	3.09	2.77	2.88	2.81	3.08	2.99	3.30	3.52
Serine	4.67	2.10	1.24	1.16	1.21	1.16	1.30	1.28	1.40	1.41
Glutamic acid	13.79	6.21	6.04	5.19	5.35	5.28	5.74	5.65	6.16	6.46
Proline	4.67	2.10	1.53	1.54	1.53	1.49	1.59	1.55	1.66	1.55
Glycine	6.68	3.01	2.32	1.99	1.89	1.81	1.79	1.66	1.75	1.72
Alanine	6.60	2.97	1.98	1.70	1.66	1.57	1.63	1.52	1.60	1.61
Hydroxyproline	1.66	0.75	0.26	0.27	0.23	0.19	0.21	0.20	0.17	0.12

(Figure 1) replacement of FMP by SBP without methionine and lysine supplementation.

In experiment 2, the SR was 84–93% among dietary treatments, and there were no significant differences. Percent body WG was not significantly different among fish fed the 0–40% SBP diets (747–873); however, increasing FMP replacement by SBP from 40% to 70%, significantly decreased WG (589–267) from that of fish fed the 0% SBP diet (873). Specific growth rate was significantly lower in fish fed the 50, 60, and 70% SBP diets (2.14–3.12) than in fish fed 0–40% SBP diets (3.56–3.81; Table 4). Feed intake (g/fish for 60 d) showed no significant differences among fish fed the 0–50% SBP diets (11.1–13.7), but the FI for fish fed 70% SBP diet (7.8) was significantly lower. The lowest FCR was found for fish fed the 0% SBP diet (0.91), but this was not significantly different from fish fed the 10, 20, 30, and 40% SBP diets (0.93–1.17). However, FCRs were significantly higher for the 50, 60, and 70% SBP diets (1.40–1.79) compared with the 0% and 10% SBP diet (0.91–0.93). Protein efficiency ratios were significantly lower for the fish fed 40, 50, 60, and 70% SBP diets (1.25–1.88) than for the fish fed 0% SBP diet (2.53); however, PER was not significantly different for the fish fed 0–30% SBP diets (1.93–2.53). No abnormalities in morphology or behavior were observed throughout both experiments.

In experiment 2, the results showed that FMP can be replaced up to 40% by SBP without adverse effects on the WG and SGR of southern flounder with supplemented methionine and lysine when diets contained 9% squid meal and 5% krill meal. However, based on a broken-line regression analysis between WG and the percent replacement of FMP, the optimum levels of FMP replacement by SBP was 38.9% (Figure 2) when diets were supplemented with methionine and lysine.

Whole-Body Proximate Composition

In experiment 1, there were no significant differences in the whole-body moisture contents among fish fed 0–40% SBP diets (78.2–78.4%); however, moisture content in fish fed the 60% SBP diet (79.1%) was significantly higher than that in fish fed the 0% SBP diet (78.2%; Table 5). Percent carcass crude protein content in fish fed 60% SBP diet (14.4%) was significantly lower than in fish fed the 0% SBP diet (15.2%), while there were no differences among the fish fed 0–50% SBP diets (14.6–15.2%).

TABLE 4. Body weight gain (WG), specific growth rate (SGR), feed intake (FI), feed conversion ratio (FCR), protein efficiency ratio (PER), and percent survival (SR) of juvenile southern flounder fed different diets in experiments 1 and 2. Values are means ± SEs ($N = 3$); within columns, means with different letters differ significantly ($P < 0.05$). The initial average weight of the fish was 0.61 g in experiment 1 and 1.63 g in experiment 2.

Diet	WG[a]	SGR[b]	FI[c]	FCR[d]	PER[e]	SR[f]
Experiment 1						
0%	767 ± 22.2 x	5.14 ± 0.06 y	3.63 ± 0.06 y	0.77 ± 0.01 z	1.50 ± 0.05 x	93 y
10%	717 ± 20.9 yx	4.99 ± 0.06 y	3.52 ± 0.10 zy	0.79 ± 0.01 z	1.35 ± 0.06 x	88 z,y
20%	671 ± 31.2 yx	4.85 ± 0.09 y	3.41 ± 0.04 zy	0.85 ± 0.03 zy	1.12 ± 0.09 yx	82 z,y
30%	646 ± 11.0 yx	4.78 ± 0.03 y	3.37 ± 0.12 zy	0.87 ± 0.02 zy	1.12 ± 0.01 yx	84 z,y
40%	649 ± 20.4 yx	4.79 ± 0.06 y	3.32 ± 0.09 zy	0.85 ± 0.01 zy	1.09 ± 0.07 yx	82 z,y
50%	521 ± 72.2 zy	4.31 ± 0.29 zy	3.12 ± 0.183 zy	0.95 ± 0.05 zy	0.90 ± 0.06 zy	80 z,y
60%	428 ± 66.9 z	3.92 ± 0.31 z	3.10 ± 0.06 z	1.20 ± 0.21 y	0.64 ± 0.18 z	67 z
Experiment 2						
0%	873 ± 50.1 x	3.78 ± 0.08 w	13.2 ± 0.61 yx	0.91 ± 0.09 z	2.53 ± 0.23 w	87
10%	877 ± 45.2 x	3.80 ± 0.08 w	12.7 ± 0.35 yx	0.93 ± 0.08 z	2.39 ± 0.19 xw	84
20%	882 ± 23.2 x	3.81 ± 0.04 w	13.7 ± 0.92 x	0.97 ± 0.04 zy	2.36 ± 0.09 yxw	88
30%	805 ± 23.9 x	3.67 ± 0.04 w	13.7 ± 0.53 x	1.16 ± 0.04 zy	1.93 ± 0.06 yxw	84
40%	747 ± 30.6 yx	3.56 ± 0.06 xw	13.7 ± 0.48 x	1.17 ± 0.02 zy	1.88 ± 0.03 zyx	84
50%	589 ± 52.4 y	3.12 ± 0.068 y	11.1 ± 0.64 yx	1.40 ± 0.03 yx	1.74 ± 0.1 zy	93
60%	546 ± 49.0 y	3.10 ± 0.12 y	10.5 ± 0.41 zy	1.41 ± 0.04 yx	1.87 ± 0.04 zyx	84
70%	267 ± 43.6 z	2.14 ± 0.21 z	7.8 ± 0.40 z	1.79 ± 0.22 x	1.25 ± 0.13 z	91

[a](Final fish weight – initial fish weight)/initial fish weight] × 100.
[b]Experiment 1: $\{[\log_e(\text{mean final weight}) - \log_e(\text{mean initial weight})]/42\text{ d}\} \times 100$; experiment 2: $\{[\log_e(\text{mean final weight}) - \log_e(\text{mean initial weight})]/60\text{ d}\} \times 100$.
[c]Experiment 1: g/fish after 42 d; experiment 2: g/fish after 60 d.
[d]Feed intake (g) / wet weight gain (g).
[e]Weight gain (g) / protein intake (g).
[f]Survival rate (%).

Crude lipid content in fish fed 60% SBP diet (2.20) was significantly lower than in the fish fed 0% SBP diet (2.61%); however, no differences were found among fish fed 0–50% SBP diets (2.59–2.86%). Ash contents in the whole bodies were not significantly different among treatments (2.89–3.16%; Table 5).

In experiment 2, the whole-body moisture content in the fish fed the 70% SBP diet (76.6%) was significantly higher than that in fish fed the 0% SBP diet (75.7%; Table 5). Crude protein content in fish fed the 70% SBP diets (14.9%) was significantly lower than in fish fed the 0% SBP diets (16.6%). Despite no significant differences in the whole-body lipid contents among fish fed 0–60% SBP diets (2.97–3.39%), crude lipid content in fish fed 70% SBP (2.87%) was significantly lower than in fish fed 20% SBP (3.39%). Whole-body ash content was not significantly different among the treatments (3.10–3.24%).

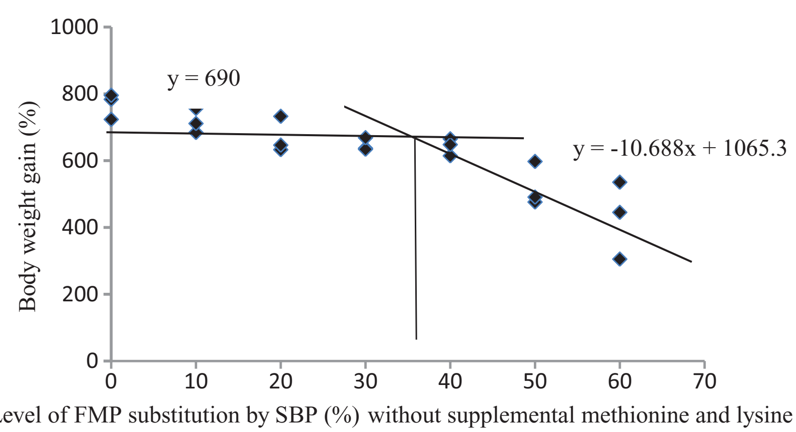

FIGURE 1. Relationship between percent body weight gain by juvenile southern flounder and the percentage of fish meal protein (FMP) replaced by soybean meal protein (SBP) without supplementation with methionine and lysine (experiment 1). Symbols represent replicate values at the different replacement levels. The optimum replacement level was 35.1%.

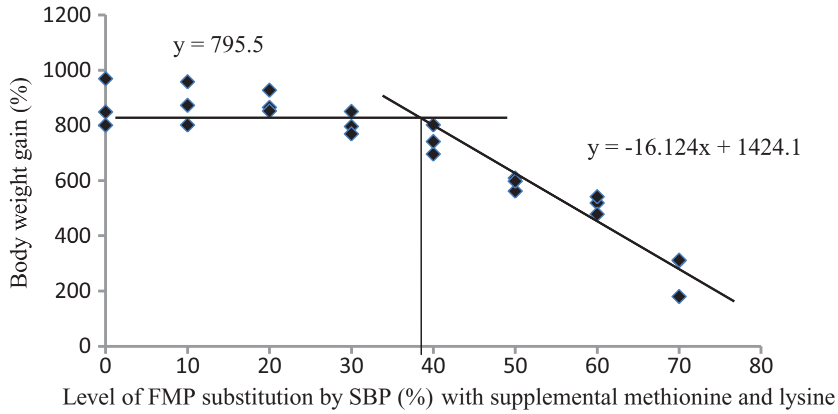

FIGURE 2. Relationship between percent body weight gain by juvenile southern flounder and the percentage of FMP replaced by SBP with supplementation with methionine and lysine (experiment 2). Plotted symbols represent replicate values at each replacement level. Optimum replacement level was 38.9%.

DISCUSSION

No significant differences were found in growth performance (WG and SGR) among the groups fed the 0–40% SBP diets in experiment 1 (Table 4), but increasing the replacement of FMP with SBP from 40% to 60% significantly lowered WG compared with 0% SBP replacement. Broken-line regression analysis indicated 35.1% as an optimum replacement level in juvenile southern flounder diets without supplementing methionine and lysine (Figure 1). These results indicated that optimum amount of SBP replacement of FMP for southern flounder diets was not more than 40% when diets contained 9% squid meal and 5% krill meal and no supplemental amino acids. Maximum replacement level of FMP with SBP without supplementing amino acids for southern flounder (40%) in this study was similar to what was found in cobia (40%; Zhou et al. 2005) and slightly lower than reported in the gilthead sea bream (45%; Martinez-Llorens et al. 2008).

When methionine and lysine were added to the diets in experiment 2, the growth performance of fish fed the 10–40% SBP diets was not significantly different from that of fish fed the 0% SBP diet (Table 4) and WG decreased significantly when SBP levels were increased from 40% to 70%. Hence, no increase in maximum FMP replacement levels was observed when methionine and lysine were supplemented to the diets (experiment 2). Broken-line regression analysis indicated 38.9% as an optimum replacement level in juvenile southern flounder diets when supplemented with methionine and lysine (Figure 2). This optimum replacement value (38.9%) obtained from

TABLE 5. Whole-body proximate composition (% wet-weight basis) of juvenile southern flounder fed different diets in experiments 1 and 2. See Table 4 for additional information.

Diet	Moisture	Protein	Lipid	Ash
		Experiment 1		
0%	78.2 ± 0.08 z	15.2 ± 0.10 y	2.61 ± 0.06 y	3.16 ± 0.04
10%	78.3 ± 0.09 zy	14.7 ± 0.16 zy	2.84 ± 0.05 y	2.98 ± 0.09
20%	78.5 ± 0.15 zy	15.1 ± 0.12 y	2.79 ± 0.03 y	2.89 ± 0.11
30%	78.4 ± 0.12 zy	14.9 ± 0.14 zy	2.86 ± 0.01 y	2.92 ± 0.05
40%	78.4 ± 0.13 zy	15.1 ± 0.10 y	2.71 ± 0.01 y	2.97 ± 0.05
50%	78.8 ± 0.07 yx	14.6 ± 0.09 zy	2.59 ± 0.06 y	2.89 ± 0.07
60%	79.1 ± 0.08 x	14.4 ± 0.09 z	2.20 ± 0.11 z	2.90 ± 0.01
		Experiment 2		
0%	75.7 ± 0.28 z	16.6 ± 0.25 y	3.21 ± 0.07 zy	3.10 ± 0.14
10%	76.1 ± 0.23 zy	16.3 ± 0.04 zy	3.14 ± 0.05 zy	3.10 ± 0.11
20%	75.8 ± 0.06 zy	16.1 ± 0.69 zy	3.39 ± 0.14 y	3.16 ± 0.02
30%	75.7 ± 0.15 y	16.6 ± 0.16 y	3.32 ± 0.09 zy	3.10 ± 0.15
40%	75.7 ± 0.09 y	16.1 ± 0.14 zy	3.32 ± 0.11 zy	3.13 ± 0.15
50%	76.5 ± 0.17 zy	16.0 ± 0.31 zy	3.01 ± 0.04 zy	3.10 ± 0.01
60%	76.5 ± 0.12 zy	15.8 ± 0.17 zy	2.97 ± 0.09 zy	3.17 ± 0.19
70%	76.6 ± 0.13 x	14.9 ± 0.34 z	2.87 ± 0.10 z	3.24 ± 0.02

experiment 2 by broken-line regression analysis is slightly higher than the value obtained from experiment 1 (35.1%). Therefore, the optimum level of FMP replacement with SBP was not more than 40%, even when supplementing methionine and lysine and when the diets contained 9% squid meal and 5% krill meal. In Japanese flounder, 40% FMP could be replaced by SBP, but only when amino acids were supplemented to the diets (Kikuchi et al. 1994; Kikuchi 1999; Kikuchi and Takeda 2001).

Species-specific responses to dietary SBP supplementation are well known (Refstie et al. 2000; Davis et al. 2005), and various levels of SBP have been incorporated successfully into the diets of marine and freshwater finfish species in replacement of FMP. Among carnivorous marine finfish species, these levels vary significantly. In cobia, for example, up to 40% of FMP can be replaced by defatted SBP in the diet without compromising fish growth and feed efficiency (Zhou et al. 2005), similar to results of the present study. However, in yellowtail, growth and feed efficiency were reduced when FMP replacement by SBP was greater than 20% (Shimeno et al. 1993). In contrast, juvenile red drum and Atlantic cod tolerated a replacement of FMP with SBP up to 50% (Reigh and Ellis 1992; Walker et al. 2010).

In the present study, krill and squid meal were added to the diets to improve palatability. 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. In many finfish species, the dietary value of soybean meal may be improved by supplementing essential amino acids to prevent lysine and methionine content deficiency of extracted soybean meal (Gallagher 1994; McGoogan and Gatlin 1997; Watanabe et al. 1998). In experiment 2, we attempted to remedy such a possible deficiency by balancing lysine and methionine in all diets, using crystalline amino acids. We also attempted to prevent the leaching losses of crystalline amino acid by precoating these materials with carboxymethyl cellulose (Alam et al. 2002). However, only slight improvement on FMP replacement level was observed. This could be due to the different sizes of the fish in the two studies; larger fish, which are often more amenable to reduced fish meal feeds, were used in experiment 2. Alternatively, the lysine and methionine content in the diets of experiment 2 were slightly lower than the calculated values and may have been insufficient. In addition, other growth-limiting factors (e.g., antinutrients) probably contributed to impaired growth at SBP substitution levels above 40%.

The lysine and methionine requirements of southern flounder are unknown at this time. Several studies have shown that whole-body amino acid patterns of an aquatic animal could reflect its dietary amino acid requirements (Alam et al. 2002, 2005; Wilson 2002). In the SBP diets, lysine and methionine contents were similar to those in the FMP diet (Table 3). The differences between the calculated (45% whole-body protein) and analyzed values of lysine and methionine in the diets may reflect slight differences in amino acids in the protein sources. Concentrations of other essential amino acids such as threonine and arginine in the test diets were slightly lower than in the 45% whole-body protein (Table 3). This indicates that although methionine and lysine were added in the feed formulations, deficiencies in other essential amino acids may have resulted in low growth performance and poor feed utilization by southern flounder fed the diets that contained high levels of soybean meal. Another possibility is that taurine levels were limiting in the diets because plants do not contain significant levels of taurine. Several studies have indicated that taurine is required in some species, such as channel catfish (Buentello and Gatlin 2002), Japanese flounder (Kim et al. 2003), yellowtail (Takagi et al. 2005), cobia (Lunger et al. 2007), and rainbow trout *Oncorhynchus mykiss* (Gaylord et al. 2007). Alternatively, perhaps supplemented crystalline amino acids were not efficiently utilized by the fish. The amino acid availability of soybean meal for southern flounder has not been reported. Unbalanced dietary amino acids, lower amino acid availabilities in the test diets, and inconsistent absorption between digested protein and crystalline amino acids could affect the digestion, absorption, and metabolism of these nutrients (Rawles et al. 2009).

Reduced growth in juvenile southern flounder fed diets with more than 40% SBP may also be related to antinutritional factors in SBP and the effects on intestinal pathology that limited dietary amino acid utilization (Davies and Morris 1997; Burrells et al. 1999). Trypsin inhibitor, a protein found in SBP, depresses growth of fish (Hendricks 2002). Heat-treatment of SBP inactivates most of the trypsin inhibitor but not all of it (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.

In experiment 1, no significant differences were found in FI, FCR, and PER among the groups fed the 0–40% SBP diets (Table 4). A significant decline in FI was observed in yellow perch *Perca flavescens* fed diets with 63.5% of the FMP replaced by SBP (Kasper et al. 2007). The lower FI of southern flounder fed the 60% (experiment 1) and 70% SBP diets (experiment 2) was one of the reasons for poor growth at these high replacement levels. Feed conversion ratios for southern flounder fed more than 50% SBP diets were significantly higher than for those fed the 0% SBP diets. Gilthead sea bream fed diets with 60% or 75% FMP replaced by SBP had higher FCR than did fish fed the FMP-only diet (Martinez-Llorens et al. 2008). For both experiments 1 and 2, the PER of fish fed the 60% SBP diets was significantly lower than in the fish fed 0% SBP diet (Table 4). Protein efficiency ratios declined when 40% and 45% of FMP was replaced by SBP in Mediterranean yellowtail (Tomas et al. 2005) and gilthead sea bream (Martinez-Llorens et al. 2008). Lower growth and nutritive efficiency was also reported in gilthead seabream fed FMP substitution levels of more than 40% (W. G. Kissil and L. Lupatsch, paper presented at the 10th International Symposium on Nutrition and Feeding of Fish, 2002).

Because the protein digestibility of the test feeds was not measured in the feeding trials, we could not evaluate how the effects of inclusion of soybean meal on growth and feed utilization of southern flounder were related to digestibility. However, it has been suggested that the poor growth performance and low feed utilization of fish fed high substitution levels of soybean meal for fish meal could be the result of lower digestibility of nitrogen and energy and the presence of nondigestible oligosaccharides (Storebakken et al. 2000; Hendricks 2002; Martinez-Llorens et al. 2008).

In both experiments 1 and 2, whole-body moisture, protein, and lipid contents were not affected by SBP replacement of FMP up to 40% (Table 5), similar to what has been reported in yellow croaker *Pseudosciaena crocea* (Ai et al. 2006), rainbow trout (Bureau et al. 2000), and Indian major carp *Labeo rohita* (Khan et al. 2003). In the present studies, southern flounder fed more than 50% and 60% FMP replaced by SBP in experiments 1 and 2 respectively, had lower whole-body protein and lipid content compared those fed the 0% SBP diet (Table 5). Similar findings were made in red drum (McGoogan and Gatlin 1997), discus *Symphysodon aequifasciata* (Chong et al. 2003), and Asian sea bass *Lates calcarifer* (Tantikitti et al. 2005), but not in Korean rockfish *Sebastes schiegeli* (Lim et al. 2004), when high fish meal was replaced by soybean meal. The lower body protein and lipid content of southern flounder fed more than 50% in the present study is presumably related to lower feed intake and hence lower protein and energy intake, and lower digestibility, causing reduced protein and energy retention.

In summary, broken-line regression showed that the optimum levels of FMP replacement with SBP in southern flounder without and with supplemental methionine and lysine were 35.1% and 38.9%, respectively. From a practical standpoint, however, statistical (ANOVA) comparisons of growth performance of fish fed diets replacing FMP with SBP indicated that the maximum substitution level was 40%, with no reduction in growth when diets also contained 9% squid meal and 5% krill meal.

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