REPLACEMENT OF FISH MEAL BY ALTERNATIVE PROTEIN SOURCES IN DIETS FOR JUVENILE BLACK SEA BASS

Katherine B. Sullivan

A Thesis Submitted to the
University of North Carolina Wilmington in Partial Fulfillment
of the Requirements for the Degree of
Masters of Science

Department of Biology and Marine Biology

University of North Carolina Wilmington

2008

Approved by

Advisory Committee

Pamela J. Seaton	Frederick S. Scharf
Md. Shah Alam	Wade O. Watanabe Chair

Accepted by

DN: cn=Robert D. Roer, o=UNCW,
ou=Dean of the Graduate School &
Research, email=roer@uncw.edu,
c=US
Date: 2009.05.05 21:52:55 -04'00'

Dean, Graduate School

TABLE OF CONTENTS

ABSTRACT

Fish meal is the protein source traditionally used in aquaculture diets, yet it is a limited resource and is expensive. Alternative protein sources 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. Soybean meal, poultry-by-product meal, and meat-and-bone meal have high protein contents and amino acid profiles similar to that of fish meal. They are readily available and less expensive than fish meal.

In a previous study, black sea bass fed diets with up to 60% of the fish meal replaced by soybean meal showed no significant differences in growth rates compared to those fed a control FM diet (Alam et al., unpublished data). Based on these results, five diets were formulated that replaced fish meal protein by soybean meal at 0, 60, 70, 80, 90 and 100%. In addition, a diet with 60% of the fish meal replaced by poultry by-product meal and a diet with 30% of the fishmeal replaced by meat-and-bone meal was formulated. These substitution levels were decided based upon published information for other marine finfish. All diets were formulated to have the same protein level (44% crude protein) and lipid level (10%). All diets contained the same amount of attractants (alanine, glycine, betaine, and taurine), vitamin and mineral premix, and wheat gluten as a binder. All diets contained 5% krill meal and 7.5% squid meal to enhance the palatability. Menhaden fish oil and soybean lecithin were used as lipid sources and cellulose was used as a filler. The diets were prepared at the UNCW. Triplicate groups of 15 juvenile black sea bass (average initial weight = 10 g) were fed to apparent satiation twice daily for 10 weeks. Fish were held in 75 liter rectangular tanks supported by a recirculating seawater system.

At the end of the experiment, survival was greater than 86% for all dietary treatments with no significant differences. No significant differences ($P<0.05$) in growth, liver tissue proximate composition, muscle tissue moisture, protein and ash, and whole boy moisture and protein were observed between fish fed the diets replacing 60% or 70% of the fish meal protein with soybean meal compared to fish fed the fish meal diet. No significant ($P< 0.05$) differences in growth, whole body proximate composition, digestive organ proximate composition, liver tissue proximate composition, and muscle tissue moisture, protein or ash were observed between fish fed the diet with 30% of the fish meal protein replaced by PBM compared to fish fed the fish meal diet. No significant $P<0.05$ differences in muscle tissue proximate composition, liver tissue proximate composition, digestive organ proximate composition and whole body moisture, lipid or ash were observed between fish fed the diet with 30% of the fish meal protein replaced by MBM compared to fish fed the fish meal diet.

Results indicated that fish meal protein maybe replaced by soybean meal (without amino acid supplementation) at levels up to 70% in the diets of juvenile black sea bass with, no dimution of fish performance. Fish meal replacement by meat-and-bone meal and poultry by-product meal at levels of at least 30% and 60%, respectively, were also successful, with higher substitution levels possible.

ACKNOWLEDGMENTS

I would like to thank my advisor, Dr. Wade Watanabe for his guidance and patience throughout this process. I appreciate his knowledge, kindness and willingness to be flexible with me. This experiment would not have happened without the leadership and guidance of Dr. Md. Shah Alam. Thank you for teaching me so much and helping me to learn and understand. For assistance in the fatty acid portion of this thesis, I would like to thank Dr. Pamela Seaton and Christopher Bentley. Thank you also to Dr. Frederick Scharf for his willingness to serve on my thesis committee.

For research funding, I would like to thank the National Ocean and Atmospheric Administration.

Thank you to the staff and students at the UNCW Aquaculture facility, especially: Dustin Allen, Patrick Carroll, Amanda Myers, Andy Ostrowski, Troy Rezek, James Wilde, Chris Woolridge, and Walker Wright-Moore.

DEDICATION

To my husband Dave and my son, Charlie who always make me smile.

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Overview

Black sea bass, *Centroprista striata*, is a commercially valuable marine finfish species. In 2006, the North Carolina commercial black sea bass landings totaled 790,988 lbs, valued at $1,740,469 (Division of Marine Fisheries, www.ncfisheries.net). North of Cape Hatteras, North Carolina, the stock is considered viable, but south of Cape Hatteras, the stock is considered overfished (Division of Marine Fisheries, www.ncfisheries.net). A carnivorous fish, black sea bass prefer hard-bottom areas such as reefs and shipwrecks (Musick & Mercer, 1977). In the wild, their diet consists of mostly fishes, amphipods and decapods (Sedberry, 1988). Protogynous hermaphrodites, black sea bass begin life as females but later mature into males (Vaughn et al., 1995). Wild-caught black sea bass have been raised successfully from subadult to full marketable stage at the University of North Carolina Wilmington aquaculture facility and have been shown to adapt well to captivity (Copeland et al., 2002, 2005; Cotton, 2002). Recently, hatchery-raised juvenile BSB have been successfully grown to marketable stages. Dietary protein and lipid levels of 44% and 15% were found to be ideal for optimum growth of black sea bass (Alam et al., 2008).

Fish meal (64% protein) is the protein source traditionally used in aquaculture diets, yet it is a limited and expensive resource (Tomas et al., 2005). Alternate protein sources 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. However, for most species, there is a limit to how much fish meal can be replaced by alternative protein sources without negatively affecting the fish (Catacutan & Pagador, 2004). Several studies have been done on fresh and saltwater fish to investigate their tolerance for alternative protein sources. The maximum replacement levels varied greatly depending on the species studied. This review will focus on soybean meal, meat and bone meal, and poultry by-product meal as potentially viable protein sources in black sea bass diets.

Fish meal substitution by soybean meal

Defatted soybean meal (SBM) is less expensive than fish meal ($376.5 per metric ton versus $930 per metric ton for fish meal (Feedstuffs 7/30/08 www.ams.usda.gov), is readily available and does not directly impact the ocean ecosystem. Nutrient content in effluent waste from aquaculture can negatively impact local waterways. SBM has significantly less phosphorous (6.5 g kg^{-1}) than fish meal (17-42 g kg^{-1}) (NRC 1993 in Cheng et al., 2003). SBM has a relatively high protein content (44% – 48%) (Cheng et al., 2003) and a fairly balanced amino acid profile (Zhou et al., 2005). However, soybean meal is deficient in methionine and lysine (Alam et al., 2005).

A number of studies have shown that varying levels of SBM can be incorporated successfully into the diets of other marine and freshwater species in replacement of fish meal. Studies on saltwater species had varying results. Mediterranean yellowtail, *Seriola dumerili*, were able to feed and grow successfully on diets with 20% and 30% SBM replacement for fishmeal (Tomas, 2005). Red snapper, *Lutjanus argentimaculatus*, growth rate, feed efficiency and mortality were not significantly affected when SBM was introduced at increments of 12% up to 48% (Catacutan & Pagador, 2004). However, fish fed the high replacement diets (36% and 48% SBM) had lipid deposits in the liver and low hematocrit levels, indicating a decline in fish health. It was concluded that 24% SBM (replacing 25% of the fish meal) could be successfully fed to red snapper, with higher substitution levels possible with phosphorous supplementation to reduce liver damage (Catacutan & Pagador, 2004). Zhou et al. (2005) fed diets containing 0 to 60% SBM in increments of 10% to juvenile cobia, *Rachycentron canadum*. In terms of growth rate, up to 40% SBM was successful, but lipid levels in the liver, blood cell composition, and feed conversion ratio were negatively affected as SBM content increased. A replacement level of 18.9% was recommended. Turbot, *Scophthalmus maximus*, raised on diets with up to 50% fish meal replaced by soybean concentrate showed no significant differences in growth or fish health from those raised on diets with 0% replacement (Day, 2000).

Studies on freshwater species have generally shown higher maximum replacement levels of SBM for fishmeal. However; some of the freshwater species studied, such as catfish and tilapia, are herbivorous. Khan et al. (2003) were able to successfully replace 100% of the fish meal with SBM in the diets of Rohu, *Labeo rohita*. Kasper et al. (2007) concluded that up to 47.6% of the fish meal could be replaced with SBM in the diets of yellow perch (*Perca flavescens*) without affecting the feed consumption, weight gain, feed efficiency or survival of the fish.

Several studies have investigated the effects of supplementation of SBM diets with various enzymes or amino acids on growth rates, feed efficiency and fish health. Phytase supplementation was shown to increase the utilization of dietary phosphorous in farm-raised fish (Van Weerd et al., 1999, Papatryphon & Soares, 2001, Masumoto et al., 2001). Phytase breaks down the indigestible phytic acid in grains and oilseeds to release digestible phosphorus and calcium, increasing amino acid digestibility. This reduces the amount of phosphorous excreted in the feces and the amount of nutrient pollution generated. Methionine hydroxy analogue (MHA) supplementation increases the methionine amino acid content in SBM diets to a level comparable to that found in fish meal-only diets. In rainbow trout, *Oncorhynchus mykiss*, weight gain, feed conversion, and retention of crude protein and phosphorus improved with 1.65 g MHA kg^{-1} supplementation (Cheng et al., 2003). Alam et al. (2005) found that juvenile kuruma shrimp, *Marsupenaeus japonicus*, had a significantly higher growth rate when fed SBM diets supplemented with methionine and lysine than when fed unsupplemented SBM diets. Gallagher (1994) replaced up to 75% of the FM protein with SBM supplemented with methionine in the diets of hybrid striped bass, *Morone saxatilis x M. chrysops.* No significant differences in weight gain, feed efficiency, protein efficiency ratio, or body composition were observed.

Fish meal substitution by poultry by-product meal

Poultry by-product meal (PBM) also has a high protein content (60%), an appropriate amino acid profile, and costs less than fish meal, $470 per metric ton versus $930 per metric ton for fish meal (Hammersmith Marketing Ltd, 3/09/08 and Feedstuffs 7/30/08 www.ams.usda.gov). Like soybean meal and meat and bone meal, the highest amount of dietary PBM incorporated successfully varied with species. Cuneate drum, *Nibea miichthioides*, fed successfully on a diet with 50% of the fish meal replaced by PBM (Wang, 2006). In red drum, *Sciaenops ocelatus,* Kureshy et al. (2000) were able to successfully raise juvenile fish on a diet with 66.7% of the fish meal replaced by PBM. This was the highest replacement level studied, so further investigation may show that even higher substitution levels are possible. Black sea turbot, *Psetta maeotica*, grew poorly when their diets exceded 25% replacement of fish meal by PBM (Yigit, 2006).

Diet experiments using PBM on freshwater fish were also successful. Muzinic et al. (2006) replaced 100% of the fish meal protein with turkey meal without any negative effects on the growth of sunshine bass (*Morone chrysops x Morone saxatilis*). Gibel carp, *Carassius* *auratus gibelio,* grew well on diets with 50% of the fish meal replaced by PBM protein (Yang, 2004).

Fish meal substitution by meat and bone meal

Meat and bone meal is less expensive than fish meal ($372 per metric ton versus $910 per metric ton for fish meal (Feedstuffs 7/30/08 www.ams.usda.gov), but has a higher protein level (51%) and superior amino acid profile than many plant proteins (Tan et al., 2005 in Zhang, 2006). Several studies on the replacement of fish meal with meat-and-bone meal have been done on marine finfish. Cuneate drum grew successfully with MBM replacing 30% of the fish meal (Wang, 2006), while Gilthead seabream, *Sparus aurata*, was able to utilize diets with 20% MBM substitution for fishmeal (Robaina, 1997). In yellow croaker, *Pseudosciaena crocea*, growth and fish health at 45% substitution of MBM for fish meal were not significantly different from the control (Ai, 2006). However, a study involving red drum, *Sciaenops ocelatus*, found a negative correlation between growth rate and MBM substitution (Kureshy, 2000). Possible reasons stated by the authors were a lower apparent digestibility coefficient (APD) or reduced feed intake due to poor palatability, although feed intake was not measured in their study.

Studies on MBM replacement in freshwater fish yielded similar results. In studies of gibel carp, diets with 20% of the fish meal replaced by MBM produced growth equivalent to the control diet (Zhang, 2006). Higher replacement levels resulted in reduced weight gain and feed efficiency. All diets containing MBM, including the 20% replacement diet, resulted in increased phosphorus and nitrogen in effluent waste compared to the diet containing only fish meal. The authors proposed that this was related to higher ash content and a lower digestibility of MBM compared to fish meal. Yang et al. (2004) also investigated the effects of MBM on gibel carp and they concluded that, without reducing growth rate, MBM could replace up to 50% of the fish meal (Yang, 2004). There were some differences in the methodologies that could possibly explain the discrepancy in substitution level success in these two studies. The Zhang (2006) experiment was a longer experiment, perhaps allowing for the negative effects on growth to become evident. Furthermore, the diet used by Zhang also contained SBM, which may have negatively impacted growth. Rainbow trout was able to utilize diets incorporating up to 24% MBM (Bureau, 2000), and Australian silver perch, *Bidyanus bidyanus*, grew successfully on a diet with 30% of the fish meal replaced by MBM (Stone, 2000). In the latter study, the MBM diets were supplemented with lysine, methione and threonine. The highest level investigated was 30%, so greater substitution may be possible.

Objectives

The objective of this study is to further the mariculture potential of black sea bass by investigating their capacity to grow on diets containing alternative protein sources to fish meal. Replacement of fish meal by soybean meal, meat-and-bone meal or poultry by-product meal can reduce the production costs, increase the sustainability, and lower the environmental impact of black sea bass aquaculture (Cheng et al., 2003, Tomas et al., 2005, Zhang et al., 2006). In a previous study on black sea bass, juveniles fed diets with up to 60% of the fish meal protein replaced by soybean meal showed no significant difference in growth rates compared to those fed a control diet with 100% fishmeal (Alam et al., unpublished data). This experiment extends these results by determining the maximum substitution limits of soybean meal for fish meal in juvenile black sea bass. In addition, a preliminary evaluation of meat-and-bone meal and poultry by-product meal as substitutions for FM is made.

Null hypotheses to be tested:

Replacement of fish meal by SBM, PBM or MBM will have no effect on the growth, feed utilization, fatty acid or amino acid composition of black sea bass juveniles.

METHODS

This experiment was conducted at the University of North Carolina Wilmington Center for Marine Science (UNCW - CMS) Aquaculture Facility at Wrightsville Beach, North Carolina from November 3, 2006 - January 26, 2007.

Experimental Animals

Juvenile black sea bass (*N* = 360, 10 g mean initial weight) were cultured from eggs spawned by captive broodstock held at the UNCW Aquaculture Facility in the summer of 2006. Fish were raised in a recirculating system and fed a commercially prepared diet containing 50% protein and 18% lipid (Skretting, Vancouver, Canada) until the study commenced.

Experimental System

The experimental system consisted of twenty-four 75-L rectangular tanks supported by a recirculating aquaculture system located in an indoor, climate-controlled laboratory. Water quality was maintained by a bubble wash bead filter, a foam fractionator, and UV sterilizer. Tanks were subjected to natural photoperiod conditions and ambient light levels from sunlight entering the laboratory windows.

Experimental Design

A sample of five fish from the experimental group was used to determine initial proximate and biochemical composition. These fish were weighed and stored in the deep freezer (-27.2°C) for later analysis. To begin the experiment, tanks were stocked at a density of fifteen fish per tank. The fish were allowed to acclimate for several days, and then they were weighed to determine the initial weight. Mean fish weight was 10.1 ± 0.7 g with no significant ($P > 0.05$) differences among treatments.

Eight diets (Table 1) were formulated and prepared at the UNCW - CMS Aquaculture Facility. Diets were formulated (Table 1) with 60%, 70%, 80%, 90% or 100% of the fish meal replaced by SBM. A diet with 0% replacement served as the control. In addition, diets with PBM replacing 60% of the fish meal protein and with MBM replacing 30% of the fish meal protein were formulated based on the replacement levels successfully used with other species (Kureshy, 2000; Stone, 2000; Yang, 2004; Ai, 2006; Wang, 2006;). The diets were formulated to have the same protein percentage (44.6 – 44.8) (Table 1). Energy (kJ/g) ranged from 14.1 – 15.6, carbohydrate percentage ranged from 14.1 – 15.6, and lipid percentage ranged from 10.5 to 14.5 (Table 1). Lipid content was provided partially by the protein sources and by soy lecithin and menhaden fish oil. Soy lecithin content was the same for all diets, but the fish oil content was increased as the soy protein level increased, due to the low fat content of soybean meal compared to fish meal (Table 1). All diets contained the same amount of vitamins, minerals, squid meal, krill meal, wheat gluten and attractants. The Kadai vitamin and mineral premix (University of Kagoshima, Kagoshima, Japan) ingredients is listed in Appendix A. Squid and krill meal provided additional protein and also served as attractants due to their odor. Wheat gluten was used as a binder. Amino acid attractants were also used to make the food more palatable to the fish.

The soybean meal was ground in a blender, and then sieved (355 μm) before being measured. The squid meal was also sieved (500 μm). All dry ingredients were measured into 4-L Ziploc bags and partially mixed by shaking the sealed bag. The dry ingredients were mixed in a KitchenAid (St. Joseph, Minnesota, USA) mixer for approximately 5 min. Meanwhile, menhaden fish oil and soy lecithin were measured into 50 mL glass beakers, and then heated on a hot plate to melt the soy lecithin. The wet ingredients were stirred, and then added slowly to the dry ingredients in the mixer. A spatula was used to ensure that all of the wet ingredients were transferred from the beaker. The wet and dry ingredients were mixed together for 10-15 min. Filtered water was added slowly, until the diet reached the desired moisture level. The amount of water added ranged between 420 – 520 mL. The diets with cellulose added as a filler required more water. The diets were mixed for another 15 min. to incorporate the water. Pellets were formed using a meat grinder, then dried in an oven preheated to 121 ºC. Once cool, the pellets were placed in labeled Ziploc bags and stored in a refrigerator. This procedure was repeated once to create a second batch of diets, this time with slightly larger pellets used to feed the larger fish. Diets were assigned to each tank at random, with three replicate tanks for each of the eight diets. The experiment was conducted for 10 weeks (70 d).

Feeding

The fish were fed to apparent satiation (until fish stopped feeding) twice daily during the week and once each day on the weekends. Fish were considered satiated when the food began to accumulate on the bottom of the tank after approximately 10 min of gradual hand feeding. To monitor the amount of feed administered, each tank had its own labeled container. Food was weighed before being added to the container. Apparent feed intake was calculated.

Environmental Conditions

Water quality data was measured twice weekly. Dissolved oxygen, temperature, salinity and pH were measured using a YSI meter. Ammonia and nitrate were measured using a spectrometer. Dissolved oxygen was maintained at 7.4 ± 0.1 mg/L. Temperature was maintained at 19.2 ºC ± 0.3 °C. Salinity was maintained at 31.8 ± 0.2 g/L. Water pH was maintained at 7.7 – 7.9. Ammonia levels were 0.30 ± 0.01 mg/L and nitrite levels were 0.12 ± 0.01 mg/L. Tanks were subjected to ambient light levels (520 ± 170 lux at midday). Tanks were siphoned daily or as needed.

Growth and Survival

Mortalities were recorded as they occurred and the fish were lot weighed every two weeks. After ten weeks, the final fish biomass was measured and 10 fish were sampled from each tank for biochemical analysis. Five of the fish were used for whole body proximate analysis. The other 5 fish were dissected for their muscle, liver and digestive organs.

Growth was analyzed by calculating the final body weight, percent weight gain [((final wet weight – initial wet weight)/initial wet weight) x 100%], specific growth rate [(ln(mean final weight) – ln(mean initial weight))/70 d) x 100], feed intake (g day^{-1} $fish^{-1}$), feed conversion ratio (total feed intake (g) / wet weight gain(g)), and protein efficiency ratio (wet weight gain (g) / total protein intake in dry basic (g)).

Chemical Analysis

Proximate composition (moisture, ash, lipid and protein content) of the whole body, liver, muscle, and digestive organs was analyzed.

Moisture content was calculated using the formula below. Samples were weighed before and after freeze-drying.

$$\text{Moisture} = \frac{\text{Initial mass} - \text{Final mass}}{\text{Initial mass}} \times 100\%$$

To determine the ash content, samples were weighed before and after being placed in a muffle furnace for approximately 6 h or until powdery white. The samples were cooled, and then placed in a desiccator for further cooling to room temperature before the final mass was taken. The formula below was used to calculate the ash content.

$$\text{Ash} = \frac{\text{Initial mass} - \text{Final mass}}{\text{Initial mass}} \times 100\%$$

To determine the crude lipid content, the Soxhlet method (AOAC, 1990) was used to extract the lipid from the samples. Approximately 1 g of sample was placed in the cellulose thimble and extracted using 150 mL of acetone solvent. The system was heated in a water bath for approximately 10 h after which time the solvent was evaporated using a rotary evaporator. The flasks were then placed in a drying over for 1 h to remove water. After cooling, the flasks were weighed and the lipid content calculated using the following formula.

$$\text{Lipid} = \frac{\text{Final Flask Mass} - \text{Initial Flask Mass}}{\text{Initial Sample Mass}} \times 100\ \%$$

Crude protein analysis was conducted by New Jersey Feed Labs, Trenton, New Jersey. Amino acid composition of the fish whole body was conducted by AAA Service Laboratory, Damascus, Oregon.

Fatty acid composition of the fish whole body was analyzed using a modified Folch et al. (1957) method. A 1:1 v/v chloroform:methanol solution was used to extract the lipid. Samples were weighed, then transferred to tubes. One ml of a 0.001g/mL solution of C19:0 fatty acid was added to each sample as an internal standard. Samples were then homogenized for 5 min. After 2 min of sonication, samples were vacuum filtered through a 50 mL fine porosity Buchner filter into a round bottom flask. The sample was then transferred by triple rinse of 1:1 chloroform:methanol to a separatory funnel. Distilled water was added until two layers were formed. The bottom layer (containing the lipids and chloroform) was transferred to a pre-weighed 25 mL round bottom flask and the chloroform was evaporated. The lipid content was then calculated after reweighing.

Next, the lipids were converted to methyl esters (FAMEs). The lipids were transferred to a conical vial by triple rinse of 20% methanol in dichloromethane. The solvent was evaporated with nitrogen gas. One ml of 0.5M NaOH in MeOH was added to the vial. It was then heated for 30 min at 80 ºC. Next, 1.5 mL of Boron Trifluoride was added and the vial was heated for an additional 30 min. One mL of saturated NaCl and 1 mL of hexanes were added. The vial was capped and shaken. Once the layers separated, the top layer containing the FAMEs was removed and passed through 32 μm silica in a Pasteur pipette into a glass vial. This process was repeated with 1 mL of hexanes and with 1 mL of 20% ether hexane. Finally, the pipette was rinsed with 1 mL of 50% ether hexane. The solvent was evaporated with nitrogen gas and the samples were transferred to a GC vial with 900 – 1200 μl of 100% chloroform. Nitrogen gas was added before capping the vials and storing them in a refrigerator.

The GC-FID was used to identify and quantify the FAMEs.

Diet evaluations:

Protein, lipid, ash and moisture content were analyzed as described above, except for the moisture content. Moisture content was determined using the oven, instead of the freeze drier. Samples were weighed before and after drying in the oven for 2 h and then moisture content was found using the same formula.

Amino acid composition and fatty acid composition were determined using the methods described above.

Statistical Analysis

Analyses were performed using the SAS 9.1 statistical software (SAS Institute Inc., Cary, North Carolina). Treatment means were compared using one-way ANOVA. The assumptions of the ANOVA were verified by Bartlett's Test for Homogeneity of Variance. Significant differences between the means were further analyzed using the Tukey-Kramer test (1956). Probabilities of $P < 0.05$ were considered significant.

Table 1 – Diet Formula for diets with different protein sources (fishmeal, FM; soybean meal, SBM; poultry by-product meal, PBM; meat-and-bone meal, MBM) All values are in g per 100g diet unless otherwise noted.

Fishmeal protein replacement	**FM**	**60% SBM**	**70% SBM**	**80% SBM**	**90% SBM**	**100% SBM**	**60% PBM**	**30% MBM**
Soybean meal[1]	0	40.1	46.8	53.5	60.2	66.9	0	0
Menhaden meal[2]	50	20	15	10	5	0	20	35
Poultry by-product meal[3]	0	0	0	0	0	0	28	0
Meat and bone meal[4]	0	0	0	0	0	0	0	19.1
Squid meal[5]	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5
Krill meal[6]	5	5	5	5	5	5	5	5
Wheat starch	15	8	6.2	4	1.8	0	15	15
Wheat gluten	5	5	5	5	5	5	5	5
Menhaden fish oil[7]	3	6	6.5	7	7.5	7.6	6	4.5
Soybean lecithin	1	1	1	1	1	1	1	1
Vitamin Premix[8]	3	3	3	3	3	3	3	3
Mineral Premix[8]	3	3	3	3	3	3	3	3
Attractants								
Taurine	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Alanine	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Glycine	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Betaine	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Cellulose	6.5	0.4	0	0	0	0	5.5	0.9
	100	100	100	100	100	100	100	100
Protein*	44.8	44.6	44.6	44.6	44.6	44.6	44.6	44.8
Lipid*	10.5	11.3	11.4	11.5	11.7	11.4	14.5	12.6
Carbohydrate*	15.9	20.9	21.1	20.9	20.7	20.9	15.9	15.9
Energy (kJ/g)*	14.1	15.2	15.3	15.3	15.3	15.2	15.6	14.9

[1] Southern States Cooperative, Inc., Farmville, NC, 47.5% protein
[2] Scoular Company, International Protein Corp., Minneapolis, MN, 64% protein
[3] Scoular Company, International Protein Corp., Minneapolis, MN, 68% protein
[4] Scoular Company, International Protein Corp., Minneapolis, MN, 50% protein
[5] Aquion, Vincent, OH, 80% protein
[6] Aquion, Vincent, OH, 56% protein
[7] Aquion, Vincent, OH
[8] Kadai, University of Kagoshima, Japan
* calculated value

RESULTS

Amino acid composition of diets

Amino acid composition of the diets and of final (d70) juvenile black sea bass whole body fed the FM diet is summarized in Table 2. Among the diets, lysine ranged from 6.4 – 7.7% of total amino acids (Table 2). As the SBM substitution level increased, the percent of lysine decreased. Lysine was lowest in the PBM diet (6.4%) and highest in the FM diet (7.7%). All diets contained less lysine than the final fish whole body (9.1% of the total amino acids).

Methionine content of the diets ranged from 1.7 – 2.8% of the total amino acids (Table 2). As the SBM substitution level increased, the percent of methionine decreased. Methionine content was similiar in the PBM (2.5%), MBM (2.6%) and the FM diet (2.8%). All diets contained less methionine than the fish whole body (3.1% of the total amino acids).

Other essential amino acids ranged in concentration from 2.1 – 8.2% of the total amino acids (Table 2) with no distinguishable trends or large deficits compared to the fish whole body. Non-essential amino acids ranged in concentration from 1.9 – 19.6% of the total amino acids (Table 2). Glutamic acid was the predominant non-essential amino acid, comprising 16.3 – 19.6% of the total amino acids. Aspartic acid was also found in relatively high concentrations, ranging from 8.8 – 11.2% of the total amino acids (Table 2).

Table 2 – Percent of total amino acids of diets (n = 1) with different protein sources (fishmeal, FM; soybean meal, SBM; poultry by-product meal, PBM; meat-and-bone meal, MBM) and of the final (d70) fish whole body fed Diet 1 – FM.

Amino Acid	Diet 1 – FM	Diet 2 – 60% SBM	Diet 3 – 70% SBM	Diet 4 – 80% SBM	Diet 5 – 90% SBM	Diet 6 –100% SBM	Diet 7 – 60% PBM	Diet 8 – 30% MBM	Fish Whole Body
Essential Amino Acids									
Lysine	7.7	7.3	6.8	6.8	6.6	6.5	6.4	7.1	9.1
Methionine	2.8	2.3	2.1	1.9	1.9	1.7	2.5	2.6	3.1
Threonine	3.8	4.2	4.0	4.1	4.1	3.8	4.1	4.0	3.9
Arginine	6.7	7.5	7.6	7.4	7.6	7.7	7.1	7.0	7.1
Serine	3.8	4.2	4.4	4.4	4.6	4.6	4.5	3.8	4.1
Histidine	2.5	2.5	2.5	2.6	2.5	2.5	2.1	2.2	2.3
Isoleucine	5.0	4.8	4.7	4.8	4.8	4.8	4.5	4.2	4.6
Leucine	8.0	8.1	7.9	8.0	8.0	8.2	7.7	7.4	7.7
Phenylalanine	4.7	4.9	5.1	5.1	5.2	5.3	4.6	4.4	4.5
Valine	5.4	4.9	4.8	4.9	4.8	4.8	5.0	4.8	5.2
Non-essential Amino Acids									
Aspartic acid	10.0	10.8	10.9	11.1	11.1	11.2	8.8	9.1	10.6
Hydroxyproline	0	0	0	0	0	0	1.9	2.3	0
Glutamic acid	17.3	18.7	19.2	19.4	19.4	19.6	17.0	16.3	14.9
Proline	5.3	5.3	5.8	5.8	5.6	5.5	6.7	6.4	4.7
Glycine	7.1	5.4	5.4	5.1	5.0	5.0	7.6	8.3	8.1
Alanine	6.5	5.4	5.2	5.1	5.0	4.8	6.1	6.7	6.7
Tyrosine	3.3	3.6	3.7	3.6	3.7	3.9	3.5	3.3	3.4

Fatty acid composition of diets

The fatty acid composition of the diets is summarized in Table 3. Total saturated fatty acids (SFA) ranged from 27.95 - 42.18 mg/g diet with no significant (P > 0.05) differences observed between the FM diet and the experimental diets (Table 3). Myristic acid (14:0) concentration ranged from 8.83 – 10.93 mg/g diet (Table 3). The 60% SBM (10.92 mg/g diet) and 100% SBM (10.93 mg/g diet) diets were significantly (P < 0.05) higher than the FM diet (9.08 mg/g diet). Palmitic acid (16:0) concentration ranged from 15.94 – 26.21 mg/g diet (Table 3). The 90% SBM diet (15.94 mg/g diet) was significantly (P < 0.05) lower in palmitic acid than the FM diet (19.04 mg/g diet). The 60% PBM (24.55 mg/g diet) and 30% MBM diet (26.21 mg/g diet) were significantly (P < 0.05) higher than the FM diet. No significant (P > 0.05) differences were observed between all other experimental diets and the FM diet (Table 3). Stearic acid (18:0) concentration ranged from 3.04 – 7.62 mg/g diet (Table 3). The 60% PBM (5.41mg/g diet) and 30% MBM (7.62 mg/g diet) were significantly (P < 0.05) higher than the FM diet (3.57 mg/g diet). No significant (P > 0.05) differences were observed between all other experimental diets and the FM diet (Table 3).

Monounsaturated fatty acid (MUFA) concentration ranged from 25.48 – 40.92 mg/g diet (Table 3). The 100% SBM (33.18 mg/g diet), 60% PBM (40.93 mg/g diet) and the 30% MBM (39.82 mg/g diet) diets were significantly (P < 0.05) higher than the FM diet (25.48 mg/g diet). No significant (P > 0.05) differences were observed between all other experimental diets and the FM diet (Table 3). Palmitoleic acid (16:1) concentration ranged from 11.94 – 15.35 mg/g diet (Table 3). The 60% SBM (14.57 mg/g diet), 100% SBM (15.62 mg/g diet), and 60% PBM diet (15.35 mg/g diet) diets were significantly (P < 0.05) higher than the FM diet (11.94 mg/g diet). No significant (P > 0.05) differences were observed between all other experimental diets and the FM diet (Table 3). Oleic acid (18:1n-9) concentration ranged from 9.12 – 20.84 mg/g diet

(Table 3, Fig. 1). The 100% SBM diet (10.57 mg/g diet), 60% PBM diet (20.84 mg/g diet) and the 30% MBM diet (20.05 mg/g diet) were significantly ($P < 0.05$) higher than the 100% FM diet (9.12 mg/g diet). No significant ($P > 0.05$) differences were observed between all other experimental diets and the FM diet (Table 3, Fig. 1).

Linoleic acid (18:2n-6) concentration ranged from 7.10 – 15.27 mg/g diet (Table 3, Fig. 2). All SBM diets (11.53 – 15.27 mg/g diet) were significantly ($P < 0.05$) higher in linoleic acid than the FM diet (7.10 mg/g diet). The 60% PBM diet (12.84 mg/g diet) was also significantly ($P > 0.05$) higher than the FM diet. No significant ($P > 0.05$) differences in linoleic acid concentration were observed between the FM diet and the 30% MBM diet (Table 3, Fig. 2).

The concentration of n-3 polyunsaturated fatty acids (PUFA) ranged from 27.30 – 36.15 mg/g diet (Table 3, Fig. 3). The 100% SBM diet (32.15 mg/g diet) was significantly ($P < 0.05$) higher than the FM diet (29.87 mg/g diet). No significant ($P > 0.05$) differences were observed between all other experimental diets and the FM diet (Table 3, Fig. 3). The ratios of n-3/n-6 PUFA ranged from 2.16 – 4.21 (Table 3). All experiment diets (2.16 – 3.95) were significantly ($P < 0.05$) lower than the FM diet (4.21) (Table 3). Eicosapentaenoic acid (EPA, 20:5n-3) concentration ranged from 13.20 – 17.40 mg/g diet (Table 3, Fig. 4). The 100% SBM diet (17.40 mg/g diet) was significantly ($P < 0.05$) higher than the FM diet (14.42 mg/g diet). No significant ($P > 0.05$) differences in were observed between all other experimental diets and the FM diet (Table 3, Fig. 4). Docohosahexaenoic acid (DHA, 22:6n-3) concentration ranged from 8.74 – 11.65 mg/g diet (Table 3, Fig. 5). The 100% SBM diet (11.65 mg/g diet) was significantly ($P < 0.05$) higher than the FM diet (9.53 mg/g). No significant ($P > 0.05$) differences in were observed between all other experimental diets and the FM diet (Table 3, Fig.

5). DHA/EPA ratios ranged from 0.64 – 0.68 with no significant differences observed between the treatment groups (Table 3, Fig. 6).

Table 3: Fatty acid composition in mg/g diet (X ± SEM, n = 3) of diets with different protein sources (fishmeal, FM; soybean meal, SBM; poultry by-product meal, PBM; meat-and-bone meal, MBM). Means with a common letter were not significantly different.

Fatty Acid	**1 - FM**	**2 - 60% SBM**	**3 - 70% SBM**	**4 - 80% SBM**	**5 - 90% SBM**	**6 - 100% SBM**	**7 - 60% PBM**	**8 - 30% MBM**
14:0	9.08 ± .32 b	10.92 ± .39 a	9.04 ± .51 b	9.63 ± .28 ab	8.83 ± .28 b	10.93 ± .43 a	9.61 ± .33 ab	9.81 ± .27 ab
16:0	19.04 ± .29 b	19.18 ± .55 b	17.43 ± .60 bc	17.79 ± .33 bc	15.94 ± .59 c	18.81 ± .73 b	24.55 ± .59 a	26.21 ± .37 a
16:1	11.94 ± .25 c	14.57 ± .42 ab	12.71 ± .54 bc	13.52 ± .28 abc	12.23 ± .40 c	15.62 ± .65 a	15.35 ± .59 a	13.92 ± .51 abc
16:3	1.48 ± .03 ab	1.55 ± .06 a	1.21 ± .07 ab	1.20 ± .06 ab	1.05 ± .01 ab	1.33 ± .07 ab	0.97 ± .28 b	1.46 ± .03 ab
16:4	1.30 ± .06 ab	1.74 ± .06 a	1.36 ± .06 ab	1.42 ± .04 ab	1.32 ± .02 ab	1.75 ± .07 a	1.11 ± .27 b	1.41 ± .04 ab
18:0	3.57 ± .01 c	3.88 ± .25 c	3.36 ± .07 c	3.04 ± .35 c	3.05 ± .09 c	3.09 ± .52 c	5.41 ± .17 b	7.62 ± .09 a
18:1n-11	3.34 ± .04 b	3.55 ± .17 ab	3.34 ± .14 b	3.59 ± .10 ab	3.20 ± .14 b	3.96 ± .21 ab	3.64 ± .50 ab	4.61 ± .05 a
18:1n-9	9.12 ± .08 c	10.57 ± .24 bc	10.21 ± .29 c	10.53 ± .11 bc	9.71 ± .39 c	12.12 ± .46 b	20.84 ± .54 a	20.05 ± .29 a
18:2n-6	7.10 ± .08 c	11.53 ± .12 b	12.40 ± .30 b	12.79 ± .14 b	12.63 ± .44 b	15.27 ± .37 a	12.84 ± .49 b	8.01 ± 0.23 c
18:4n-3	2.54 ± .09 c	3.26 ± .11 ab	2.72 ± .11 bc	2.91 ± .03 bc	2.65 ± .10 c	3.54 ± .18 a	2.64 ± .16 c	2.76 ± .06 bc
20:1	1.08 ± .01 b	1.27 ± .03 ab	1.20 ± .03 bc	1.25 ± .02 abc	1.02 ± .09 c	1.48 ± .06 a	1.11 ± .08 bc	1.24 ± .02 abc
20:5n-3	14.42 ± .33 b	15.70 ± .40 ab	14.42 ± .19 b	14.80 ± .22 ab	13.20 ± .56 b	17.40 ± .77 a	14.62 ± 1.06 ab	15.25 ± .55 ab
22:5n-3	1.91 ± .02 bc	1.99 ± .05 ab	1.89 ± .05 bc	1.96 ± .01 abc	1.66 ± .08 c	2.23 ± .12 a	1.84 ± .08 bc	2.01 ± .03 ab
22:6n-3	9.52 ± .13 bc	10.27 ± .19 ab	9.76 ± .26 bc	9.90 ± .09 bc	8.74 ± .38 c	11.65 ± .52 a	9.25 ± .41 bc	10.15 ± .16 bc
∑SFA	35.48 ± 4.13 ab	33.80 ± .62 ab	31.03 ± .75 b	30.17 ± .63 b	27.95 ± .61 b	32.57 ± .38 b	36.69 ± 2.87 ab	42.18 ± 1.39 a
∑MUFA	25.48 ± 0.34 c	29.95 ± 0.83 bc	27.47 ± 0.95 c	28.88 ± .31 bc	26.16 ± .97 c	33.18 ± 1.36 b	40.93 ± 1.59 a	39.82 ± .66 a
∑n-3 PUFA	29.87 ±.60 bc	32.77 ± .79 ab	29.99 ± 0.66 bc	30.77 ±.39 abc	27.30 ± 1.13 c	36.15 ± 1.54 a	29.32 ± 1.97 bc	31.62 ± .81 abc
n-3/n-6 PUFA	4.21 ± 0.03 a	2.84 ± .05 c	2.42 ± .01 d	2.41 ± .04 d	2.16 ± .02 e	2.37 ± .04 de	2.28 ± .08 de	3.95 ± .04 b
22:6n-3/20:5n-3	0.66 ± .007 a	0.65 ± .004 a	0.68 ± .010 a	0.67 ± .005 a	0.66 ± .002 a	0.67 ± .023 a	0.64 ± .021 a	0.67 ± 0.017 a
Total Fatty Acids	95.48	109.98	101.05	104.33	95.23	119.18	123.78	124.51

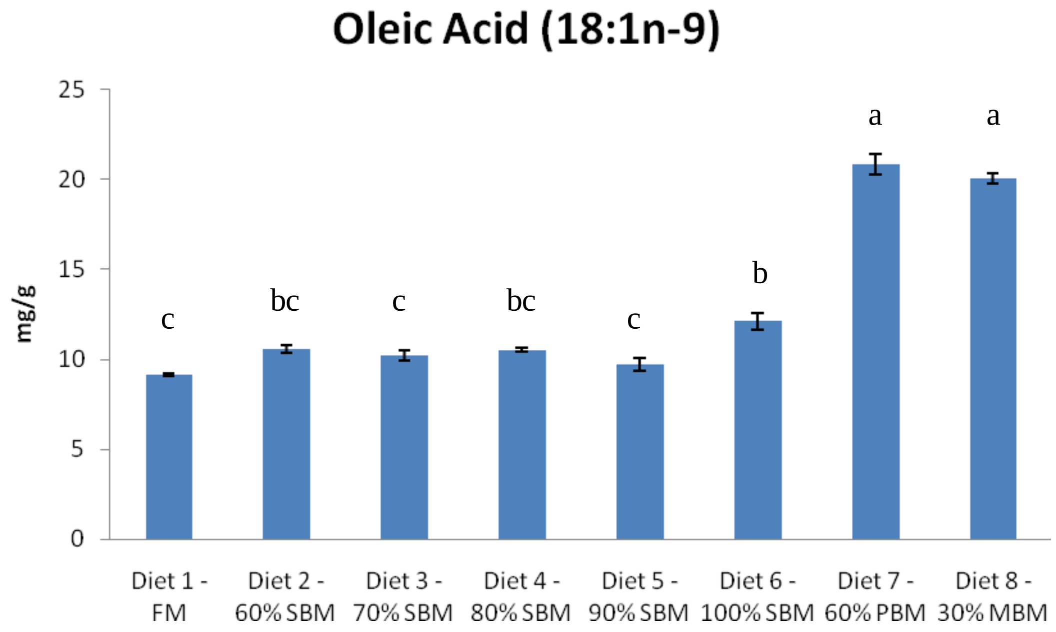

Figure 1. Oleic acid (18:1n-9) concentration ($\bar{X}$ ± SEM, n =3) of diets with different protein sources (fishmeal, FM; soybean meal, SBM; by-product meal, PBM; meat-and-bone meal, MBM). Means with a common letter were not different.

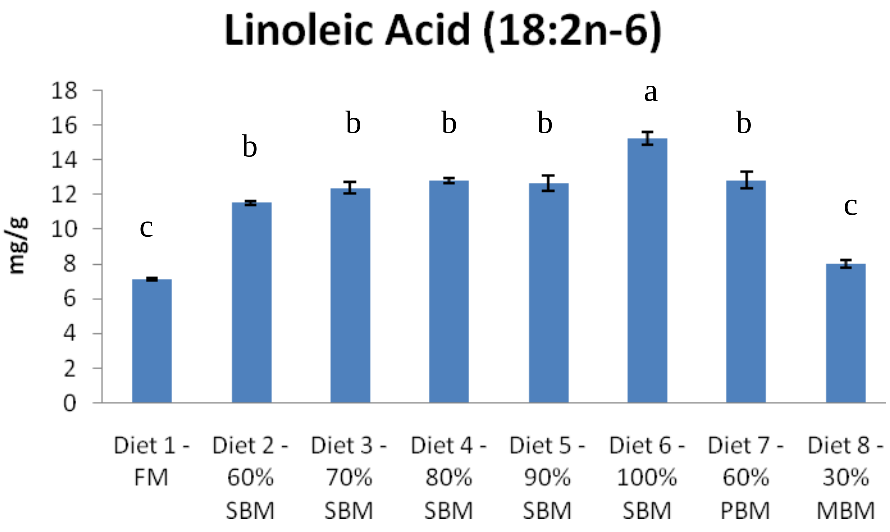

Figure 2. Linoleic acid (18:2n-6) concentration ($\bar{X}$ ± SEM, n =3) of diets with different protein sources (fishmeal, FM; soybean meal, SBM; poultry by-product meal, PBM; meat-and-bone meal, MBM). Means with a common letter were not significantly different.

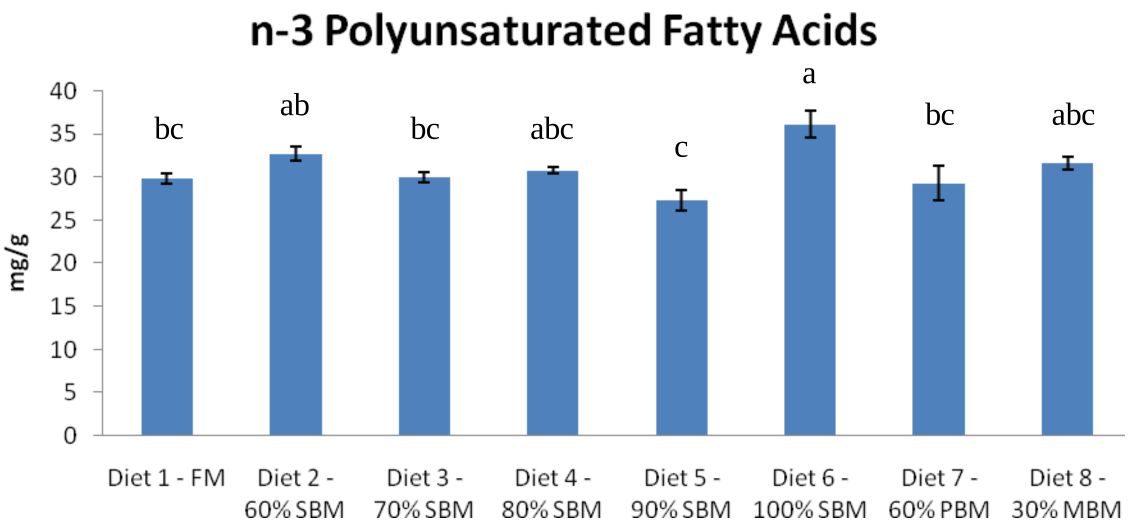

Figure 3. n-3 polyunsaturated fatty acids (PUFA) concentration ($\bar{X}$ ± SEM, n =3) of diets with different protein sources (fishmeal, FM; soybean meal, SBM; poultry by-product meal, PBM; meat-and-bone meal, MBM). Means with a common letter were not significantly different.

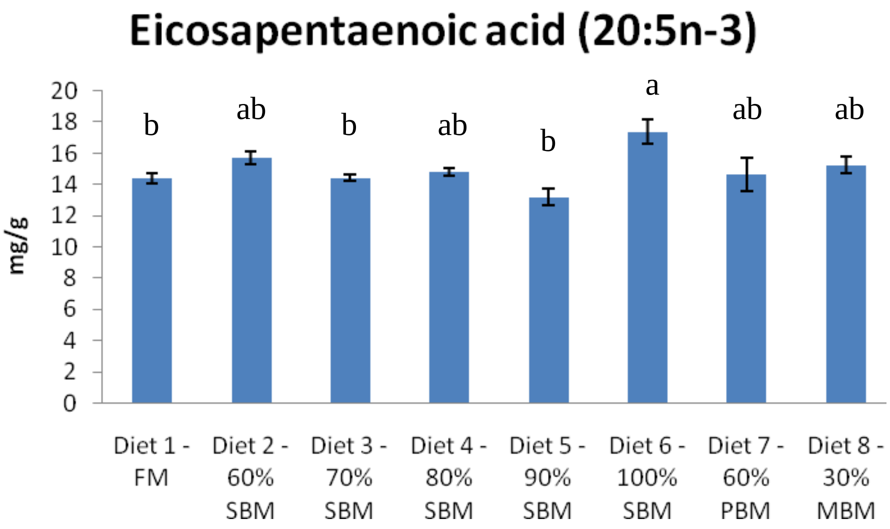

Figure 4. Eicosapentaenoic acid (EPA) (20:5n-3) concentration ($\bar{X}$ ± SEM, n =3) of diets with different protein sources (fishmeal, FM; soybean meal, SBM; poultry by-product meal, PBM; meat-and-bone meal, MBM). Means with a common letter were not significantly different.

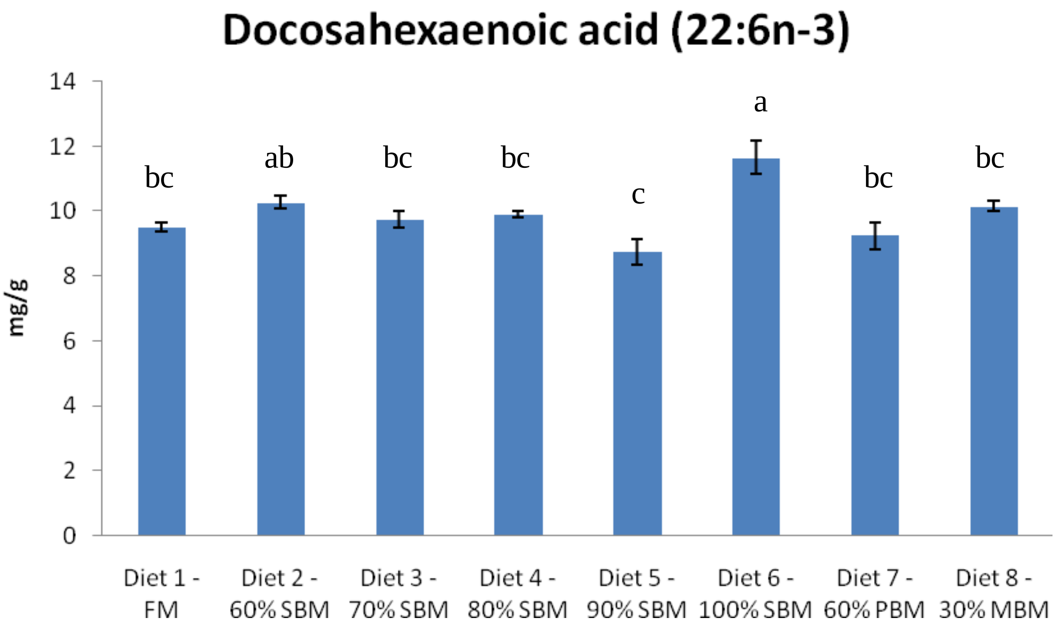

Figure 5. Docosahexaenoic acid (DHA) (22:6n-3) concentration ($\bar{X}$ ± SEM, n =3) of diets with different protein sources (fishmeal, FM; soybean meal, SBM; poultry by-product meal, PBM; meat-and-bone meal, MBM). Means with a common letter were not significantly different.

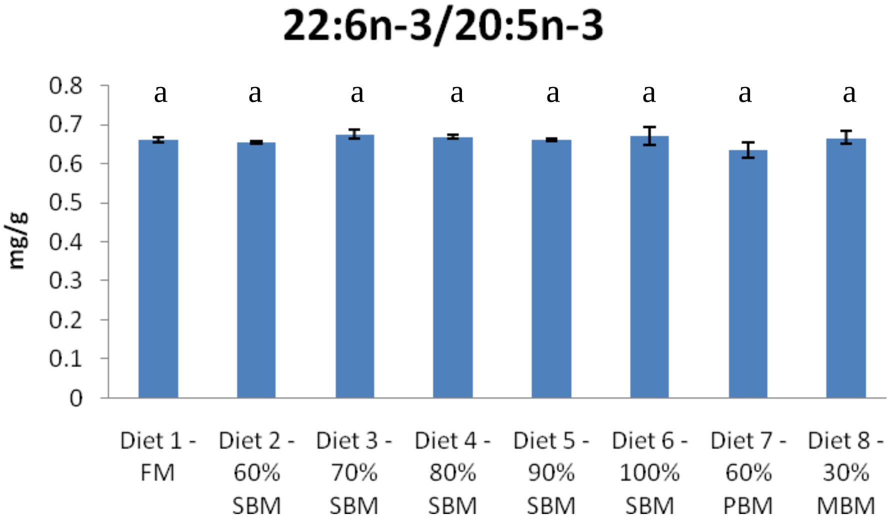

Figure 6. Ratio of 22:6n-3 (DHA) / 20:5n-3 (EPA) concentration ($\bar{X}$ ± SEM, n =3) of diets with different protein sources (fishmeal, FM; soybean meal, SBM; poultry by-product meal, PBM; meat-and-bone meal, MBM). Means with a common letter were not significantly different.

Fatty acid composition of fish

The fatty acid composition of the fish whole body is summarized in Table 4. Total saturated fatty acids (SFA) ranged from 88.61 – 110.42 mg/g (Table 4). Fish fed the 100% SBM diet (88.61 mg/g) were significantly lower in SFA concentration than fish fed the FM diet (105.60 mg/g). No significant ($P > 0.05$) differences were observed between fish fed all other experimental diets and fish fed the FM diet (Table 4). Myristic acid (14:0) concentration ranged from 15.58 – 22.45 mg/g (Table 4). Fish fed the 60% SBM (22.45 mg/g) diet were significantly ($P < 0.05$) higher than the fish fed the FM diet (18.21 mg/g). Fish fed the 70% SBM (16.87 mg/g), 90% SBM (16.67 mg/g), 100% SBM (15.58mg/g) and 30% MBM (17.34 mg/g) were significantly ($P < 0.05$) lower in myristic acid than fish fed the FM diet. No significant ($P > 0.05$) differences were observed between the fish fed the remaining experimental diets and fish fed the FM diet (Table 4). Palmitic acid (16:0) concentration ranged from 54.42 – 68.63 mg/g (Table 4). Fish fed the 90% SBM (57.58 mg/g) and 100% SBM (54.42) diets were significantly ($P < 0.05$) lower than fish fed the FM diet (68.21 mg/g). No significant ($P > 0.05$) differences were observed between fish fed all other experimental diets and the FM diet (Table 4). Stearic acid (18:0) concentration ranged from 12.13 – 15.86 mg/g (Table 4). Fish fed the 30% MBM diet (15.86 mg/g) were significantly ($P < 0.05$) higher in stearic acid than fish fed the FM diet (13.53 mg/g). No significant ($P > 0.05$) differences were observed between fish fed all other experimental diets and fish fed the FM diet (Table 4).

Total MUFA ranged from 96.23 – 118.46 mg/g (Table 4). Fish fed the 60% PBM diet (118.46 mg/g) were significantly ($P < 0.05$) higher in MUFA than fish fed the FM diet (100.71 mg/g). No significant ($P > 0.05$) differences were observed between fish fed all other experimental diets and fish fed the FM diet (Table 4). Palmitoleic acid (16:1) concentration ranged from 29.12 – 38.30 mg/g (Table 4). Fish fed the 60% SBM (38.30 mg/g) and 80% SBM (36.75 mg/g) diets were significantly higher in palmitoleic acid (16:1) concentration than fish fed the FM diet (31.17 mg/g). No significant ($P > 0.05$) differences were observed between fish fed all other experimental diets and fish fed the FM diet (Table 4). Oleic acid (18:1n-9) concentration ranged from 47.81 – 66.43 mg/g (Table 4, Fig. 7). No significant differences were observed between fish fed the SBM diets (47.81 -56.74 mg/g) and fish fed the FM diet (52.50 mg/g). Fish fed the 60% PBM (66.43) and 30% MBM (64.77) diets were significantly ($P < 0.05$) higher in oleic acid than fish fed the FM diet (Table 4, Fig. 7).

Linoleic acid (18:2n-6) concentration ranged from 12.83 – 27.25 mg/g (Table 4, Fig. 8). Fish fed all SBM diets and the 60% PBM diets (21.36 – 27.25 mg/g) were significantly ($P < 0.05$) higher in linoleic acid than fish fed the FM diet (12.96 mg/g). No significant differences were observed between fish fed the 30% MBM diet and fish fed the FM diet (Table 4, Fig. 8).

The concentration of n-3 PUFA ranged from 56.25 – 80.90 mg/g (Table 4, Fig. 9). Fish fed the 60% SBM (80.90 mg/g), 80% SBM (79.01 mg/g) and 90% SBM (71.36 mg/g) were significantly ($P < 0.05$) higher in n-3 PUFA than fish fed the FM diet (56.25 mg/g). No significant ($P > 0.05$) differences were observed between fish fed all other experimental diets and fish fed the FM diet (Table 4, Fig. 9). The ratio of n-3 / n-6 PUFA ranged from 2.62 – 4.87 (Table 4). Fish fed all SBM diets (2.62 – 3.34) and the 60% PBM diet (3.05) had significantly ($P < 0.05$) lower n-3 / n-6 ratios than fish fed the FM diet (4.34). Fish fed the 30% MBM diet (4.87) were significantly ($P < 0.05$) higher than fish fed the FM diet (Table 4). Eicosapentaenoic acid (20:5n-3) concentration ranged from 23.70 – 37.03 mg/g (Table 4, Fig. 10). Fish fed the 60% SBM (37.03 mg/g), 80% SBM (35.60 mg/g), and 90% SBM (30.82 mg/g) diets were significantly ($P < 0.05$) higher in EPA than fish fed the FM diet (23.70 mg/g). No significant (P

> 0.05) differences were observed between fish fed all other experimental diets and fish fed the FM diet (Table 4, Fig 10). Docosohexaenoic acid (22:6n-3) concentration ranged from 21.76 – 29.14 mg/g (Table 4, Fig. 11). Fish fed the 60% SBM (28.87 mg/g) and 80% SBM (29.14 mg/g) diets were significantly ($P < 0.05$) higher in DHA than fish fed the FM diet (21.76 mg/g). No significant ($P > 0.05$) differences were observed between fish fed all other experimental diets and fish fed the FM diet (Table 4, Fig. 11). DHA/EPA ratios ranged from 0.78 - 0.93 (Table 4, Fig. 12). Fish fed the 60% SBM diet (0.78) had a significantly lower DHA/EPA ratio than fish fed the FM diet (0.93). No significant ($P > 0.05$) differences were observed between fish fed all other experimental diets and fish fed the FM diet (Table 4, Fig. 12).

Table 4. Fatty acid composition in mg/g ($\bar{X}$ ± SEM, n =3) of juvenile black sea bass fed diets with different protein sources (fishmeal, FM; soybean meal, SBM; poultry by-product meal, PBM; meat-and-bone meal, MBM). Means with a common letter were not significantly different.

Fatty Acid	**1 – FM**	**2 - 60% SBM**	**3 - 70% SBM**	**4 - 80% SBM**	**5 - 90% SBM**	**6 - 100% SBM**	**7 - 60% PBM**	**8 - 30% MBM**
14:0	18.21 ± .35 b	22.45 ± .90 a	16.87 ± .25 c	20.23 ± .32 ab	16.67 ± .61 c	15.58 ± 1.06 c	17.68 ± .36 bc	17.34 ± .35c
16:0	68.21 ± 1.29 a	68.63 ± 1.45 a	63.18 ± .93 ab	65.21 ± .70 ab	57.58 ± 1.61 b	54.42 ± 3.65 b	68.47 ± 1.40 a	66.83 ± .66 a
16:1	31.17 ± .58 c	38.30 ± 1.97 a	31.55 ± .57 bc	36.75 ± .39 ab	29.46 ± 2.03 c	29.49 ± 2.03 c	33.24 ± .11 abc	29.12 ± .24 c
18:0	13.53 ± .48 bc	13.4 ± .08 bc	13.79 ± .25 bc	13.69 ± .26 bc	12.56 ± .22 c	12.13 ± .76 c	14.66 ± .25 ab	15.86 ± .20 a
18:1n-11	10.28 ± .17 b	11.81 ± .24 ab	11.17 ± .24 ab	12.17 ± .14 a	11.53 ± .34 ab	11.17 ± .76 ab	11.46 ± .33 ab	11.57 ± .13 ab
18:1n-9	52.50 ± .51 bc	56.74 ± 1.01 b	55.47 ± 1.09 b	55.74 ± .82 b	50.4 ± 1.47 bc	47.81 ± 3.26 c	66.43 ± 1.02 a	64.77 ± .78 a
18:2n-6	12.96 ± .30 c	24.19 ± .61 ab	23.96 ± .60 ab	27.25 ± .27 a	23.56 ± .83 ab	25.77 ± 1.82 a	21.36 ± .83 b	12.83 ± .23 c
18:4n-3	4.92 ± .23 c	7.94 ± .36 a	6.07 ± .42 bc	7.17 ± .02 ab	6.52 ± .34 ab	6.14 ± .49 bc	5.84 ± .15 bc	5.79 ± .08 bc
20:0	5.65 ± .17 b	5.94 .08 b	6.01 ± .16 b	6.31 ± .18 ab	7.15 ± .09 a	6.48 ± .42 ab	6.51 ± .26 ab	6.32 ± .21 ab
20:1	3.41 ± .19 ab	3.93 ± .19 a	3.52 ± .14 ab	3.82 ± .09 ab	3.64 ± .06 ab	3.48 ± .10 ab	3.45 ± .09 ab	3.21 ± .06 b
20:5n-3	23.70 ± 1.99 d	37.03 ± 1.23 a	29.60 ± .61 bcd	35.60 ± .61 ab	30.82 ± 1.13 bc	29.56 ± 2.06 cd	28.15 ± .69 cd	26.96 ± .54 cd
22:1	3.35 ± .03 c	3.61 ± .08 c	3.71 ± .07 bc	3.88 ± .21 bc	4.90 ± .08 a	4.28 ± .28 ab	3.88 ± .04 bc	3.61 ± .01 c
22:5n-3	5.88 ± .09 c	7.07 ± .14 ab	6.32 ± .13 abc	7.11 ± .08 a	6.70 ± .20 abc	6.19 ± .40 bc	6.28 ± .11 abc	6.01 ± .12 c
22:6n-3	21.76 ± 2.28 c	28.87 ± .68 a	24.92 ± .50 bc	29.14 ± .40 a	27.33 ± .83 bc	25.63 ± 1.61 ab	24.80 ± .20 bc	23.71 ± .48 bc
∑SFA	105.60 ± 2.28 ab	110.42 ± 2.34 ab	99.85 ± 1.48 abc	105.44 ± .92 ab	93.97 ± 2.48 bc	88.61 ± 5.86 c	107.33 ± 2.28 ab	106.35 ± 1.26 ab
∑MUFA	100.71 ± 1.24 bcd	114.38 ± 3.41 ab	105.42 ± 1.79 abcd	112.36 ± 1.51 abc	99.93 ± 2.29 cd	96.23 ± 6.44 d	118.46 ± 1.51 a	112.28 ± 1.18 abcd
∑n-3 PUFA	56.25 ± 2.31 c	80.90 ± 2.24 a	66.91 ± 1.63 bc	79.01 ± 0.67 a	71.36 ± 2.37 ab	67.52 ± 4.52 bc	65.07 ± 1.02 bc	62.47 ± 1.22 bc
∑n-6 PUFA	12.96 ± .30 c	24.19 ± .61 ab	23.96 ± .60 ab	27.25 ± .27 a	23.56 ± .83 ab	25.77 ± 1.82 a	21.4 ± .83 b	12.83 ± .23 c
n-3/n-6 PUFA	4.34 ± .11 b	3.43 ± .01 c	2.79 ± .02 de	2.90 ± .03 de	3.03 ± .01 cd	2.62 ± .02 e	3.05 ± .07 cd	4.87 ± .17 a
22:6n-3 /20:5n-3	0.93 ± .07 a	0.78 ± .01 b	0.84 ± .002 ab	0.82 ± .02 ab	0.89 ± .01 ab	0.87 ± .01 ab	0.88 ± .01 ab	0.88 ± .001 ab
Total Fatty acids	275.53	329.91	296.14	324.07	288.82	278.13	312.21	293.93

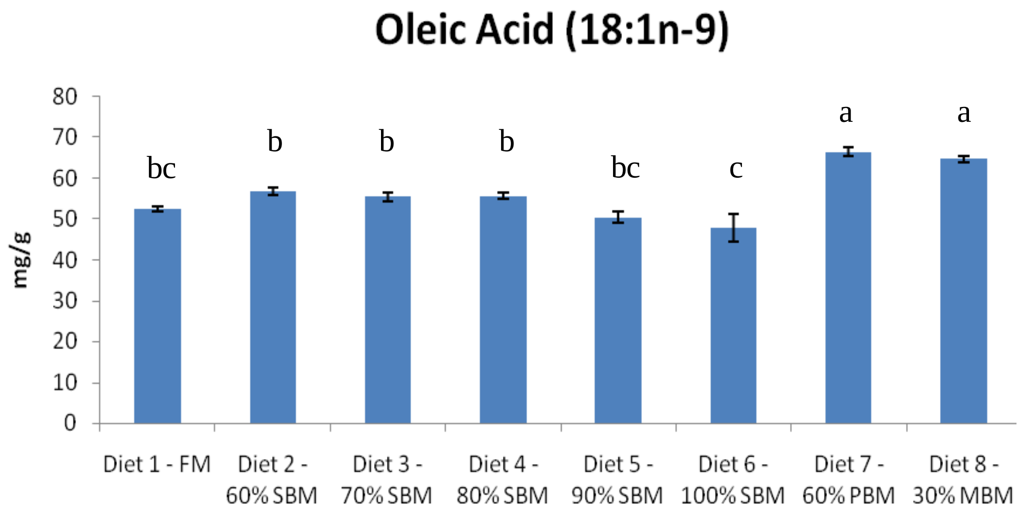

Figure 7. Oleic acid (18:1n-9) concentration ($\bar{X}$ ± SEM, n =3) of juvenile black sea bass fed diets with different protein sources (fishmeal, FM; soybean meal, SBM; poultry by-product meal, PBM; meat-and-bone meal, MBM). Means with a common letter were not significantly different.

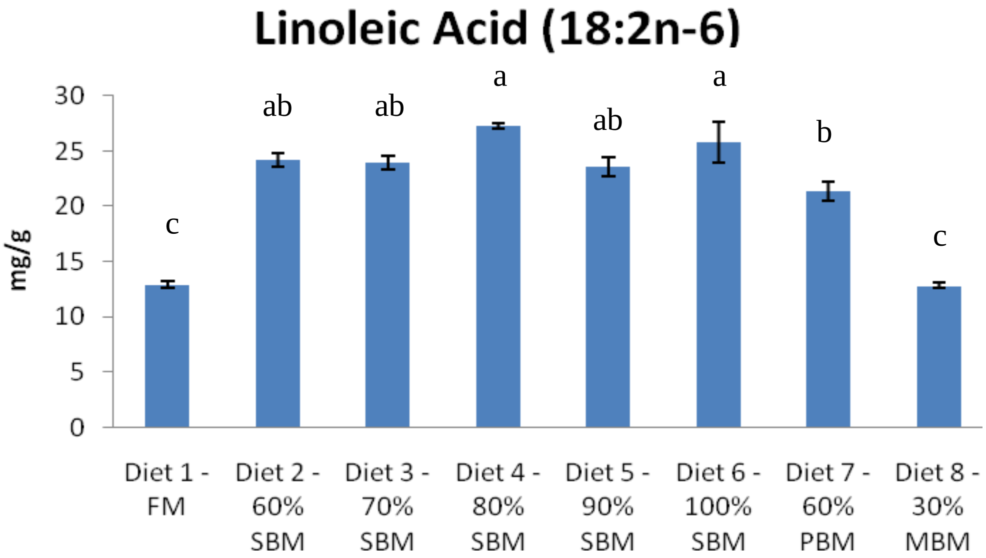

Figure 8. Linoleic (18:2n-6) concentration (± SEM, n =3) of juvenile black sea bass fed diets with different protein sources (fishmeal, FM; soybean meal, SBM; poultry by-product meal, PBM; meat-and-bone meal, MBM). Means with a common letter were not significantly different.

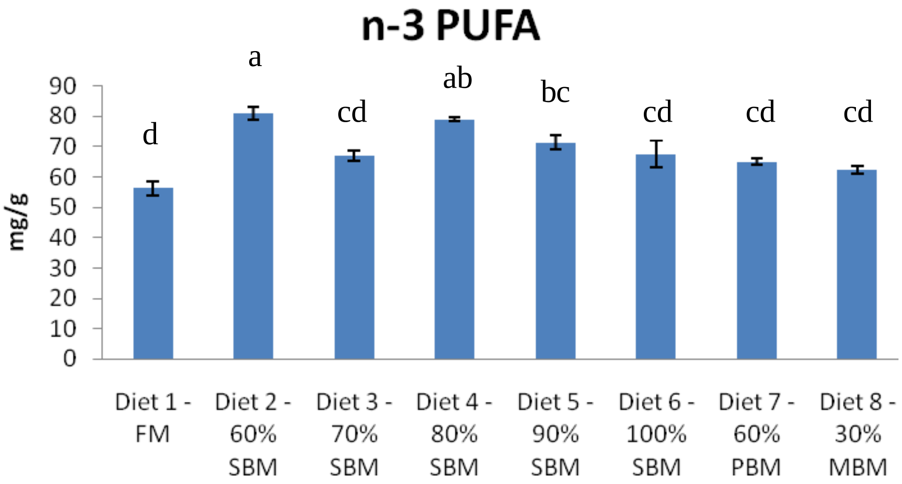

Figure 9. n-3 polyunsaturated fatty acid (PUFA) concentration ($\bar{X}$ ± SEM, n =3) of juvenile black sea bass fed diets with different protein sources (fishmeal, FM; soybean meal, SBM; poultry by-product meal, PBM; meat-and-bone meal, MBM). Means with a common letter were not significantly different.

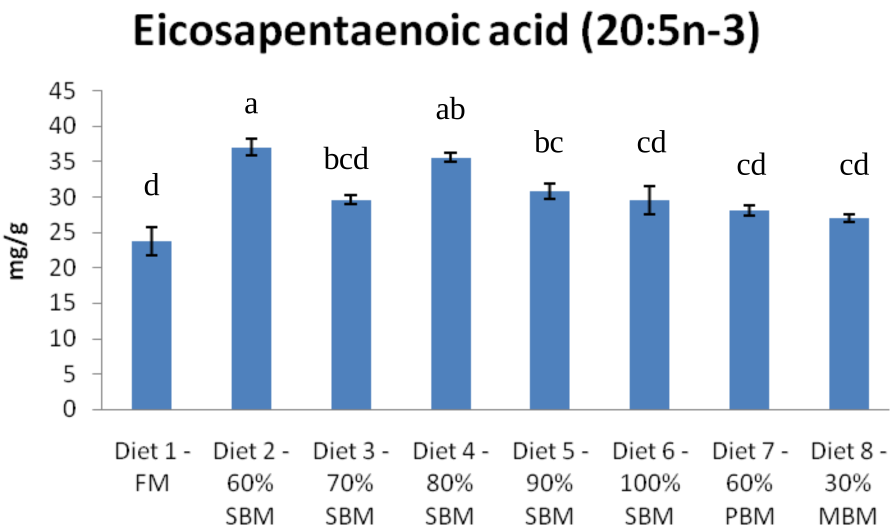

Figure 10. Eicosapentaenoic acid (20:5n-3) (EPA) concentration ($\bar{X}$ ± SEM, n =3) of juvenile black sea bass fed diets with different protein sources (fishmeal, FM; soybean meal, SBM; poultry by-product meal, PBM; meat-and-bone meal, MBM). Means with a common letter were not significantly different.

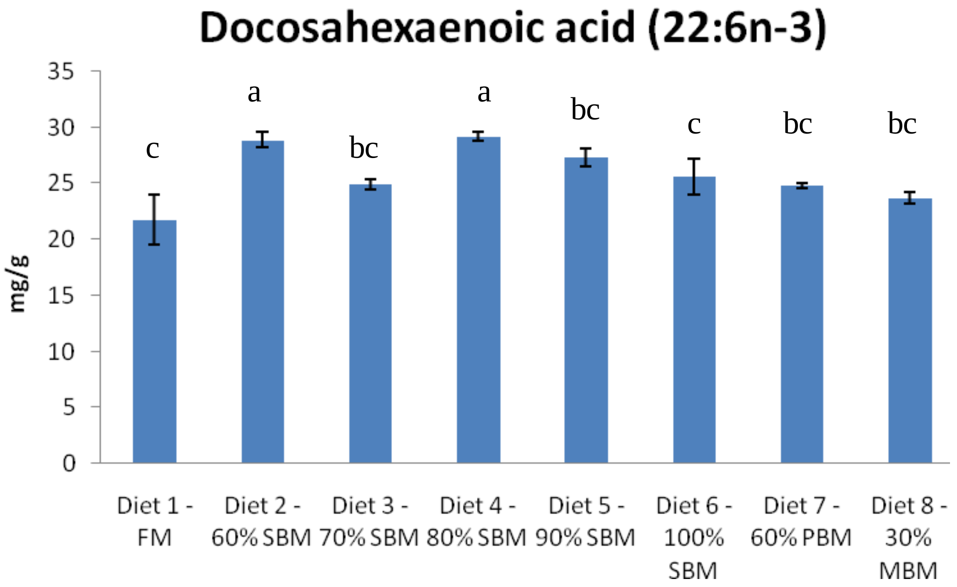

Figure 11. Docosahexaenoic acid (22:6n-3) (DHA) concentration ($\bar{X}$ ± SEM, n =3) of juvenile black sea bass fed diets with different protein sources (fishmeal, FM; soybean meal, SBM; poultry by-product meal, PBM; meat-and-bone meal, MBM). Means with a common letter were not significantly different.

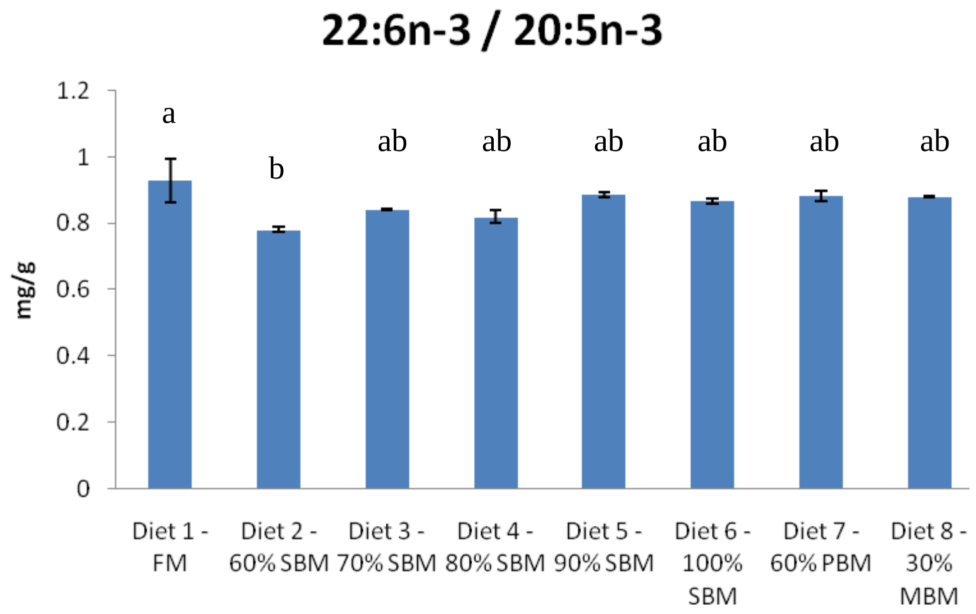

Figure 12. Ratio of 22:6n3 to 20:5n-3 (DHA / EPA) concentration ($\bar{X}$ ± SEM, n =3) of juvenile black sea bass fed diets with different protein sources (fishmeal, FM; soybean meal, SBM; poultry by-product meal, PBM; meat-and-bone meal, MBM). Means with a common letter were not significantly different.

Survival and Growth

Survival and growth data are summarized in Table 5. Survival ranged from 87% - 100% at the conclusion of the experiment (d70) with no significant ($P > 0.05$) differences among treatments (Table 5).

Fish weight

No significant differences ($P > 0.05$) were found in mean fish weight among the experimental groups at the start of the experiment (d0, range = 9.8 g – 10.7 g) or on d14 (range = 11.5 g – 13.1 g) (Fig. 13). On d28, mean fish weights ranged from 13.9 g – 17.1 g, and significant ($P < 0.05$) treatment effects were observed. Fish fed the 70% SBM diet (17.1 g) were significantly larger than fish fed the 90% SBM diet (13.9 g). No significant differences were found between fish fed any of the experimental diets and the fish fed the FM diet. On d42, mean fish weights ranged from 16.2 g – 21.1 g with no significant ($P > 0.05$) differences among treatments. On d56, mean fish weights ranged from 19.8 g – 24.8 g. Mean weight of fish fed the 90% (19.9 g) and 100% SBM (19.8 g) diets were significantly ($P < 0.05$) lower than fish fed the FM diet (24.8 g). On d70, mean fish weights ranged from 22.8 g – 28.5 g. Mean weight of fish fed the 90% (23.4 g) and 100% SBM (22.8 g) diets were significantly ($P < 0.05$) lower than fish fed the FM diet (28.5 g). (Table 5, Fig. 14).

Percent weight gain

Fish fed the FM diet had a percent weight gain of 186.7% (Table 5, Fig. 15). Fish fed the 60% SBM, 70% SBM, 80% SBM, 60% PBM and 30% MBM diets (range = 153.1% -

187.4%) were not significantly different from fish fed the FM diet. Fish fed the 90% SBM (135.0%) and100% SBM diets (115.1%) were significantly ($P < 0.05$) lower in percent weight gain than fish fed the FM diet (Table 5, Fig. 15).

Specific growth rate

No significant differences ($P > 0.05$) in specific growth rate (% body weight / d) were found among fish fed the FM, 60% SBM, 70% SBM, 80% SBM, 60% PBM and 30% MBM diets (range = 1.33 – 1.51 % / d) at the end of the experiment (d70) (Table 5, Fig. 16). Specific growth rates were significantly lower in fish fed the 90% SBM (1.23% / d) and 100% SBM (1.09% / d) diets than in fish fed the FM diet (1.50% / d) (Table 5, Fig. 16).

Feed intake and conversion ratio

Mean feed intakes ranged from 0.39 - 0.47 g / d fish among the treatments with no significant differences ($P > 0.05$) (Table 5). Feed conversion ratio was significantly ($P < 0.05$) lower in fish fed the 70% SBM diet (1.24) compared to fish fed the FM diet (1.55) (Table 5, Fig. 17). In all other treatments, FCR (range = 1.35 – 2.18) was not significantly different from fish fed the FM diet (Table 5, Fig. 17).

Protein efficiency ratio

Protein efficiency ratios ranged from 1.00 – 1.69 among treatments (Table 5, Fig. 18). No significant differences were found ($P > 0.05$) between the protein efficiency ratios (dry / wet) of fish fed any of the experimental diets and fish fed the FM diet.

Table 5: Survival and growth performance of juvenile black sea bass fed diets with different protein sources (fishmeal, FM; soybean meal, SBM, poultry by-product meal, PBM; meat-and-bone meal, MBM) and concentrations. Means with a common letter were not significantly different.

Diet	Survival (%)	Final Weight (g)	Percent Weight Gain (%)	Specific Growth Rate (%/d)	Feed Intake (g/d)	Feed Conversion Ratio (total feed intake (g) / wet weight gain(g)),	Protein Efficiency Ratio (wet weight gain (g) / total dry protein intake, (g)).
FM	87 a	28.5 ± 0.9 a	186.7 ± 9.9 a	1.50 ± 0.05 a	0.47 ± 0.03 a	1.55 ± 0.17 ab	1.40 ± 0.15 abc
60% SBM	96 a	27.5 ± 1.2 a	179.1 ± 10.3 ab	1.46 ± 0.05 a	0.40 ± 0.02 a	1.35 ± 0.04 bc	1.55 ± 0.51 ab
70% SBM	100 a	28.6 ± 1.2 a	168.8± 10.9 ab	1.41 ± 0.06 a	0.39 ± 0.03 a	1.24 ± 0.05 c	1.69 ± 0.06 a
80% SBM	91 a	25.6 ± 1.1 a	153.1 ± 0.5 abc	1.33 ± 0.01 ab	0.43 ± 0.05 a	1.63 ± 0.24 ab	1.38 ± 0.22 abc
90% SBM	93 a	23.4 ± 0.8 a	136.0 ± 6.0 bc	1.23 ± 0.04 b	0.43 ± 0.02 a	1.98 ± 0.15 ab	1.10 ± 0.08 bc
100% SBM	89 a	22.8 ± 0.6 a	115.1 ± 5.7 c	1.09 ± 0.04 b	0.44 ± 0.03 a	2.18 ± 0.10 a	1.00 ± 0.05 c
60% PBM	93 a	27.4 ± 0.9 a	176.6± 13.2 ab	1.45 ± 0.07 a	0.39 ± 0.02 a	1.47 ± 0.09 bc	1.44 ± 0.09 abc
30% MBM	91 a	28.1 ± 0.4 a	187.4 ± 11.6 a	1.51 ± 0.06 a	0.43 ± 0.02 a	1.55 ± 0.04 abc	1.36 ± 0.03 abc

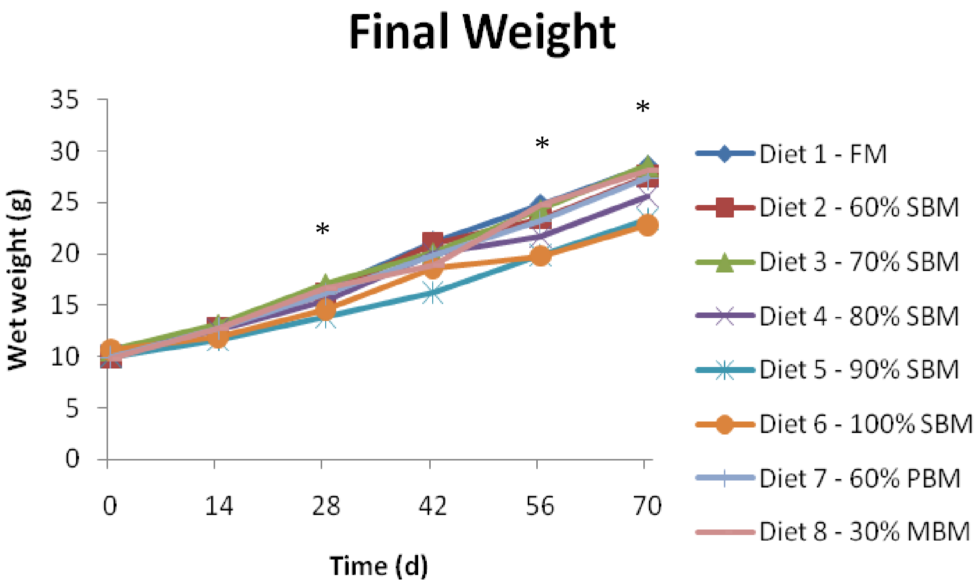

Figure 13. Growth (wet weight) ($\bar{X}$ ± SEM, n = 3) of juvenile black sea bass fed diets with different protein sources (fishmeal, FM; soybean meal, SBM, poultry by-product meal, PBM; meat-and-bone meal, MBM) and concentrations. Significant ($P < 0.05$) treatment effects (*) were observed on d28, d56 and d70.

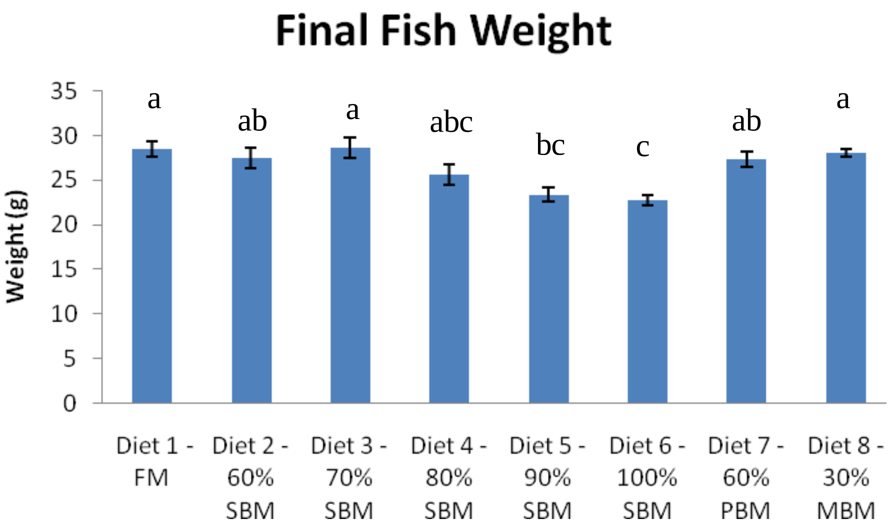

Figure 14. Final weight ($\overline{X}$ ± SEM, n = 3) at the end of the experiment (d70) of black sea bass juveniles fed diets with different protein sources (fishmeal, FM; soybean meal, SBM, poultry by-product meal, PBM; meat-and-bone meal, MBM) and concentrations. Means with a common letter were not significantly different.

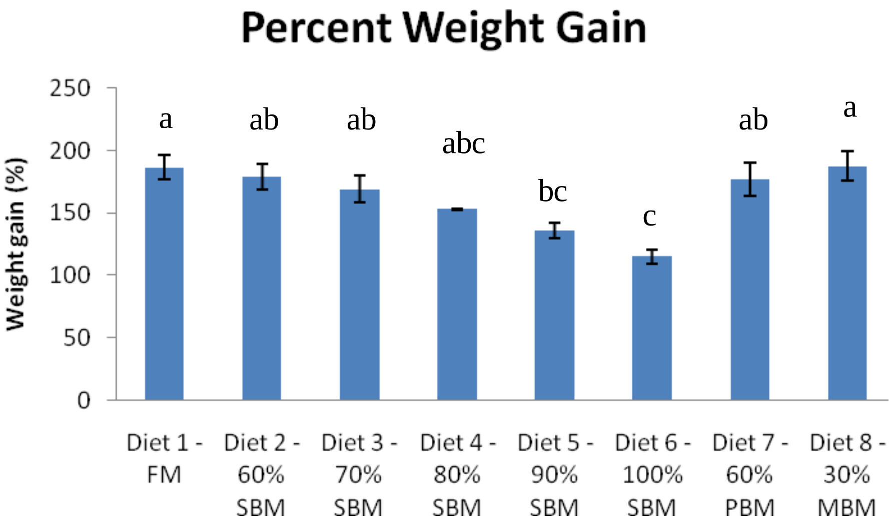

Figure 15. Percent weight gain ($\overline{X}$ ± SEM, n = 3) at the end of the experiment (d70) of juvenile black sea bass fed diets with different protein sources (fishmeal, FM; soybean meal, SBM, poultry by-product meal, PBM; meat-and-bone meal, MBM) and concentrations. Means with a common letter were not significantly different.

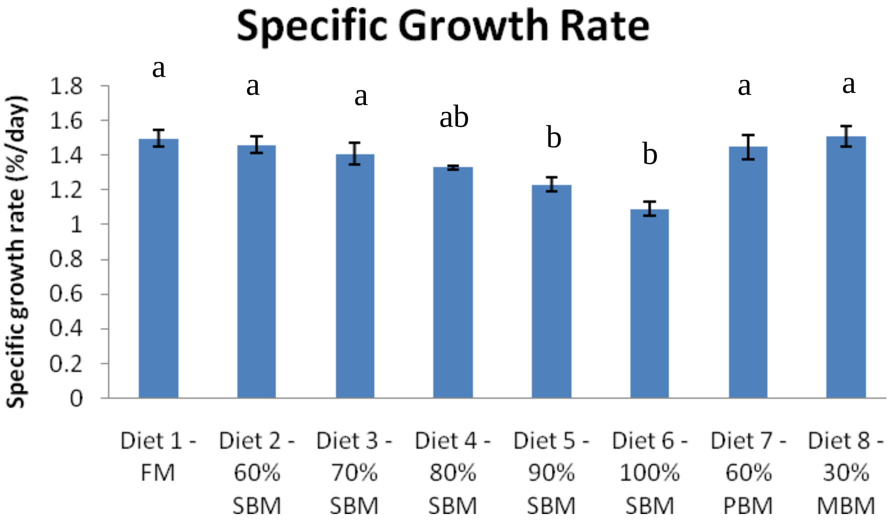

Figure 16. Specific growth rate ($\overline{X}$ ± SEM, n = 3) at the end of the experiment (d70) of juvenile black sea bass fed diets with different protein sources (fishmeal, FM; soybean meal, SBM, poultry by-product meal, PBM; meat-and-bone meal, MBM) and concentrations. Means with a common letter were not significantly different.

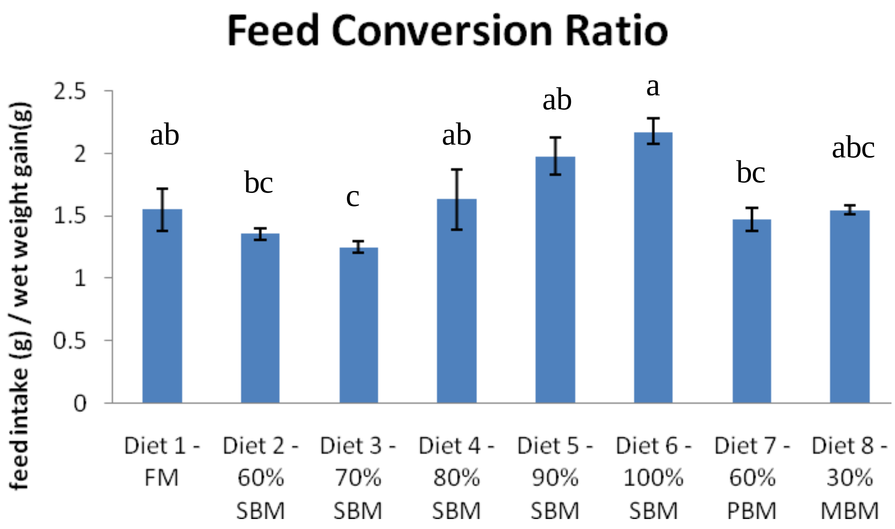

Figure 17. Feed conversion ratio ($\overline{X}$ ± SEM, n = 3) at the end of the experiment (d70) of juvenile black sea bass fed diets with different protein sources (fishmeal, FM; soybean meal, SBM, poultry by-product meal, PBM; meat-and-bone meal, MBM) and concentrations. Means with a common letter were not significantly different.

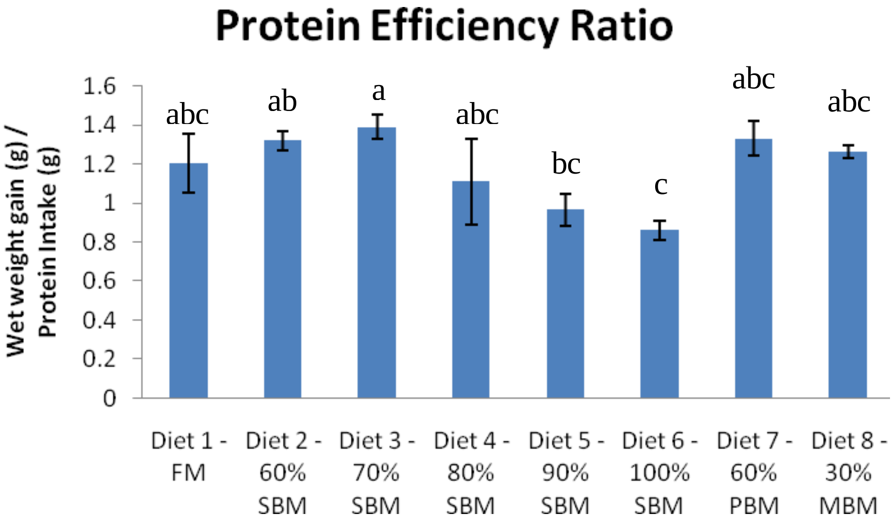

Figure 18. Protein efficiency ratio ($\overline{X}$ ± SEM, n = 3) at the end of the experiment (d70) of black sea bass juveniles fed diets with different protein sources (fishmeal, FM; soybean meal, SBM, poultry by-product meal, PBM; meat-and-bone meal, MBM) and concentrations. Means with a common letter were not significantly different.

Proximate composition of the diets

Moisture content of the diets ranged from 6.68% - 11.2% among treatments. Moisture content of the 70% SBM diet (8.99%), 80% SBM diet (9.31%), 90% SBM diet (10.1%) and 100% SBM diet (11.2%) were significantly ($P < 0.05$) higher than in the FM diet (6.68%) (Table 6). No significant ($P > 0.05$) differences were observed between the moisture content of the other experimental diets and the FM diet (Table 6).

Lipid content ranged from 11.1% - 15.4% among treatments. Lipid content of the 60% PBM diet (15.4%) was significantly ($P < 0.05$) higher than the lipid content of the FM diet (12.6%) (Table 6). No significant ($P > 0.05$) differences were observed between the lipid contents of all other experimental diets and the FM diet (Table 6).

Protein content ranged from 46.3% - 47.9% with no significant ($P > 0.05$) differences between the FM diet and the experimental diets (Table 6). Ash content ranged from 7.57% - 14.4% among treatments. The ash content of all diets were significantly ($P > 0.05$) different from each other (Table 6).

Proximate composition of the fish whole body

Fish whole body moisture content ranged from 66.3% - 69.1% among treatments, with no significant ($P > 0.05$) differences (Table 7). Crude lipid content ranged from 11.0% - 13.0% among treatments. Crude lipid content of all of the SBM diets (60% - 100% replacement) (11.0% - 12.1%) was significantly ($P < 0.05$) lower than in the FM diet (13.0%) (Table 7). Crude lipid contents of the fish fed the 60% PBM (12.4%) and the 30% MBM (12.2%) diets were not significantly different from the FM diet (Table 7).

Crude protein content ranged from 15.8% - 17.1% among treatments. Protein content of the fish fed the 30% MBM diet (15.8%) was significantly ($P < 0.05$) lower than in fish fed the FM diet (16.7%) (Table 7). No significant ($P > 0.05$) differences were observed between the protein content of all other treatment groups (16.2% - 17.1%) and the FM diet (Table 7).

Ash content ranged from 3.77% - 4.57% among treatments. Ash content of the fish fed the 100% SBM diet (3.77%) was significantly ($P < 0.05$) lower than the ash content of the fish fed the FM diet (4.57%) (Table 7). No significant ($P > 0.05$) differences were observed between the ash content of all other treatment groups (4.13 – 4.42%) and the FM diet (Table 7).

Muscle Tissue Proximate Composition

Muscle tissue moisture content ranged from 75.9% - 76.2% with no significant ($P > 0.05$) differences between the treatment groups (Table 8).

Crude lipid content ranged from 3.13% - 3.98% among treatments. Lipid content of the fish fed the 100% SBM diet (3.65%) and the 60% PBM diet (3.98%) was significantly ($P < 0.05$) higher than the lipid content of fish fed the FM diet (3.22%) (Table 8). No significant ($P > 0.05$) differences were observed between the lipid content of all other treatment groups (3.13% - 3.52%) and the FM diet (Table 8).

Protein content ranged from 19.3% - 19.9% with no significant ($P > 0.05$) differences among the treatment groups (Table 8). Ash content ranged from 1.35% – 1.44% among treatments with no significant ($P > 0.05$) differences between fish fed the experimental diets and fish fed the FM diet (Table 8).

Digestive Organ Proximate Composition

Digestive organ moisture content ranged from 29.2% - 42.3% among treatment groups. Moisture content of the fish fed diets containing 70% SBM (38.01%), 80% SBM (39.1%), 90% SBM (38.7%) and 100% SBM (42.3%) were significantly ($P < 0.05$) higher than in the fish fed the FM diet (30.4%) (Table 9).

Lipid content ranged from 47.2% – 66.1% among treatment groups. Lipid content of the fish fed diets containing 70% SBM (50.4%), 80% SBM (49.7%), 90% SBM (51.0%) and 100% SBM (47.2%) were significantly ($P < 0.05$) lower than the lipid content of the fish fed the FM diet (62.3%) (Table 9).

Ash content ranged from 0.53% - 0.77% among treatment groups. Fish fed the SBM diets had significantly ($P < 0.05$) higher ash contents (0.67% - 0.77%) than fish fed the FM diet (0.53%) (Table 9).

Liver Tissue Proximate Composition

Liver tissue moisture content ranged from 58.51% - 62.18% with no significant ($P > 0.05$) differences among the treatment groups (Table 10). Lipid content ranged from 13.8% - 16.3% with no significant ($P > 0.05$) differences among the treatment groups (Table 10). Protein content ranged from 8.93% - 10.33% with no significant ($P > 0.05$) differences between the fish fed the FM diet and fish fed the experimental diets (Table 10).

Table 6. Proximate composition of (% wet basis, $\overline{X}$ ± SEM, n = 3) diets with different protein sources (fishmeal, FM; soybean meal, SBM; poultry by-product meal, PBM; meat-and-bone meal, MBM) and concentrations. Means with a common letter were not significantly different.

Diet	**Moisture**	**Lipid**	**Protein**	**Ash**
100% FM	6.68 ± 0.07 c	12.6 ± 0.51 bc	47.2 ± 0.33 ab	12.3 ± 0.09 b
60% SBM	7.44 ± 0.05 c	13.9 ± 0.76 ab	47.9 ± 0.15 a	9.72 ± 0.03 c
70% SBM	8.99 ± 0.61 b	11.3 ± 0.20 c	47.6 ± 0.17 ab	9.00 ± 0.04 d
80% SBM	9.31 ± 0.01 b	11.3 ± 0.25 c	46.5 ± 0.18 ab	8.59 ± 0.02 e
90% SBM	10.1 ± 0.23 ab	11.1 ± 0.11 c	46.7 ± 0.18 ab	8.04 ± 0.05 f
100% SBM	11.2 ± 0.31 a	11.8 ± 0.39 c	46.3 ± 0.36 b	7.57 ± 0.01 g
60% PBM	7.66 ± 0.08 c	15.4 ± 0.42 a	47.5 ± 0.30 ab	9.81 ± 0.08 c
30% MBM	7.28 ± 0.06 c	14.4 ± 0.12 ab	47.6 ± 0.52 ab	14.4 ± 0.04 a

Table 7. Whole body proximate composition (% wet basis, $\overline{X}$ ± SEM, n = 3) at the end of the experiment (d70) of juvenile black sea bass fed diets with different protein sources (fishmeal, FM; soybean meal, SBM; poultry by-product meal, PBM; meat-and-bone meal, MBM) and concentrations. Means with a common letter were not significantly different.

Diet	**Moisture**	**Lipid**	**Protein**	**Ash**
100% FM	66.8 ± 0.19 a	13.0 ± 0.16 a	16.7 ± 0.06 abc	4.57 ± 0.12 a
60% SBM	66.8 ± 0.51 a	12.1 ± 0.34 bc	17.1 ± 0.10 a	4.22 ± 0.18 ab
70% SBM	66.9 ± 0.56 a	11.6 ± 0.04 cd	16.9 ± 0.08 ab	4.28 ± 0.08 ab
80% SBM	67.5 ± 0.34 a	11.0 ± 0.14 d	16.8 ± 0.13 abc	4.26 ± 0.09 ab
90% SBM	68.6 ± 0.14 a	11.3 ± 0.16 d	16.2 ± 0.09 cd	4.13 ± 0.05 ab
100% SBM	69.1 ± 0.28 a	11.0 ± 0.07 d	16.3 ± 0.09 cd	3.77 ± 0.16 b
60% PBM	66.3 ± 0.26 a	12.4 ± 0.11 ab	16.5 ± 0.13 bcd	4.34 ± 0.09 a
30% MBM	67.4 ± 1.39 a	12.2 ± 0.07 abc	15.82 ± 0.05 d	4.42 ± 0.04 a

Table 8. Muscle tissue proximate composition (% wet basis, $\overline{X}$ ± SEM, n = 3) at the end of the experiment (d70) of juvenile black sea bass fed diets with different protein sources (fishmeal, FM; soybean meal, SBM; poultry by-product meal, PBM; meat-and-bone meal, MBM) and concentrations. Means with a common letter were not significantly different.

Diet	**Moisture**	**Lipid**	**Protein**	**Ash**
FM	75.9 ± 0.33 a	3.22 ± .15 c	19.5 ± 0.13 a	1.40 ± 0.02 abc
60% SBM	76.1 ± 0.35 a	3.16 ± .05 c	19.4 ± 0.22 a	1.37 ± 0.01 bc
70% SBM	75.9 ± 0.25 a	3.52 ± .02 bc	19.6 ± 0.20 a	1.41 ± 0.01 abc
80% SBM	76.1 ± 0.12 a	3.35 ± .10 bc	19.9 ± 0.16 a	1.40 ± 0.01 abc
90% SBM	76.2 ± 0.32 a	3.13 ± .11 c	19.9 ± 0.19 a	1.41 ± 0.01 abc
100% SBM	76.1 ± 0.12 a	3.65 ± .05 ab	19.6 ± 0.07 a	1.35 ± 0.01 c
60% PBM	76.0 ± 0.16 a	3.98 ± .06 a	19.7 ± 0.16 a	1.45 ± 0.02 a
30% MBM	76.1 ± 0.17 a	3.35 ± .04 bc	19.3 ± 0.10a	1.43 ± 0.01 ab

Table 9. Digestive organ proximate composition (% wet basis, $\overline{X}$ ± SEM, n = 3) at the end of the experiment (d70) of juvenile black sea bass fed diets with different protein sources (fishmeal, FM; soybean meal, SBM; poultry by-product meal, PBM; meat-and-bone meal, MBM) and concentrations. Means with a common letter were not significantly different.

Diet	**Moisture**	**Lipid**	**Ash**
FM	30.43 ± 0.85 d	62.33 ± 1.04 ab	0.53 ± 0.02 d
60% SBM	33.84 ± 1.18 bcd	57.42 ± 1.05 b	0.69 ± 0.02 ab
70% SBM	38.01 ± 2.62 abc	50.38 ± 1.32 c	0.75 ± 0.03 ab
80% SBM	39.05 ± 0.78 ab	49.71 ± 1.52 c	0.67 ± 0.03 abc
90% SBM	38.73 ± 1.36 ab	50.95 ± 1.83 c	0.77 ± 0.03 a
100% SBM	42.27 ± 1.25 a	47.21 ± 0.66 c	0.75 ± 0.01 ab
60% PBM	29.22 ± 7.28 d	66.10 ± 0.42 a	0.56 ± 0.02 cd
30% MBM	31.24 ± 1.50 cd	60.28 ± 0.72 b	0.64 ± 0.01 bcd

Table 10. Liver tissue proximate composition (% wet basis, $\overline{X}$ ± SEM, n = 3) at the end of the experiment (d70) of juvenile black sea bass fed diets with different protein sources (fishmeal, FM; soybean meal, SBM; poultry by-product meal, PBM; meat-and-bone meal, MBM) and concentrations. Means with a common letter were not significantly different.

Diet	**Moisture**	**Lipid**	**Protein**
FM	60.5 ± 0.57 a	15.5 ± 0.24 a	9.75 ± 0.12 ab
60% SBM	61.1 ± 1.09 a	16.2 ± 0.48 a	9.45 ± 0.25 ab
70% SBM	60.4 ± 1.46 a	16.3 ± 1.08 a	10.3 ± 0.42 a
80% SBM	61.3 ± 0.19 a	14.6 ± 0.43 a	9.79 ± 0.09 ab
90% SBM	61.6 ± 0.99 a	13.8 ± 1.28 a	9.71 ± 0.27 ab
100% SBM	62.2 ± 0.46 a	14.5 ± 0.36 a	9.73 ± 0.08 ab
60% PBM	58.5 ± 0.33 a	16.2 ± 0.46 a	8.93 ± 0.04 b
30% MBM	59.2 ± 0.50 a	15.6 ± 0.46 a	9.19 ± 0.08 b

DISCUSSION

Amino Acid Composition of the Diets

In the SBM diets, lysine content appeared to be lower than in the FM diet (Table 2), and as SBM increased, lysine decreased. The lysine requirements of black sea bass are unknown at this time. At the end of the study, lysine was higher in the fish whole body than in the diets, indicating that lysine may have been deficient in the diets and that lysine was conserved in the tissues (Table 2). SBM diets for juvenile cobia were also deficient in lysine (Zhou et al., 2005). Lysine supplementation in the SBM diets of kuruma shrimp, *Marsupenaeus,* resulted in a significantly higher growth rate compared to shrimp fed unsupplemented SBM diets (Alam et al., 2005). Lysine is considered to be the first limiting amino acid for many fish species (Wilson, 2002). Reduced growth and feed utilization in Atlantic cod, *Gadus morhua*, fed diets containing SBM compared to fish fed the control diet was partially attributed to the lower lysine level in the SBM diets (Hansen et al., 2007).

Lysine also appeared to be lower in the PBM and MBM diets compared to the FM diet and the fish whole body (Table 2). PBM studies on cuneate drum (Wang et al., 2006) and turbot (Yigit et al., 2006), and MBM studies on cuneate drum (Wang et al., 2006) and yellow croaker (Ai et al., 2006) found similar results. Reduced growth in yellow croaker fed diets with more than 45% of the FM replaced by MBM was partially attributed to deficiencies in lysine, arginine and threonine (Ai et al., 2006). Diets for turbot with 100% of the FM replaced by PBM had 32% less lysine than the control diet, below the requirement for the fish (Ai et al., 2006). The authors concluded that the lower final weight in fish fed the 100% PBM diet could be attributed to the low level of lysine. Reduced growth in gilthead sea bream fed diets with 75% or more of the protein replaced by PBM was partially attributed to low levels of lysine in the diets (Nengas et al., 1999).

In the SBM diets, methionine content appeared to be lower than in the FM diet (Table 2, Fig. 2), and methionine content decreased as the SBM level increased. At the end of the study, methionine content in the fish whole body was higher than in the diets, indicating that methionine may have been deficient in the diets and possibly conserved in the tissues (Table 2). SBM studies on cobia (Zhou et al., 2005), gibel carp (Jose et al., 2006), and Mediterranean yellowtail (Tomas et al., 2005) also found lower levels of methionine in the SBM diets compared to the control diet. Zhou et al. (2006) concluded that in their study of SBM diets for cobia, methionine was the first limiting amino acid. As SBM increased, methionine decreased, causing reduced growth in the fish (Zhou et al., 2006). In Mediterranean yellowtail, methionine consumption was the same for fish fed FM and SBM diets because feed intake was higher in fish fed the SBM diets (Tomas et al., 2005). It was concluded that fish consumed more of the SBM diets because of the increased palatability of the diets caused by the soybean extrusion process (Tomas et al., 2005). The authors hypothesized that the higher feed intake of fish fed diets containing 40% or 50% SBM resulted in excess feed and amino acid consumption, causing the fish to expend energy to digest the extra protein, leading to reduced growth (Tomas et al., 2005).

Methionine also appeared to be lower in the PBM and MBM diets compared to the FM diet and the fish whole body (Table 2). PBM studies on turbot (Yigit et al., 2006), gilthead seabream (Nengas et al., 1999) and cuneate drum (Wang et al., 2006) had similar results. Methionine was the first limiting essential amino acid for gilthead seabream fed PBM diets, but the total sulfur amino acid content exceeded the requirement for gilthead seabream (Nengas et al., 1999). MBM studies on yellow croaker (Ai et al., 2006) and turbot (Yigit et al., 2006) also found a decrease in methionine as MBM increased. Bureau et al. (2000) supplemented MBM diets for rainbow trout with methionine but did not see an effect on growth performance. None of the studies cited above concluded that methionine deficiency was the primary cause of reduced growth in fish fed PBM or MBM diets. Reduced growth in fish fed PBM diets has been attributed to low lysine levels, low n-3 HUFA content, and variability in the quality of PBM (Kureshy et al., 2000, Nengas et al., 1999, Yigit et al., 2006). Low digestibility and high ash content of MBM, high saturated fatty acids, low levels of essential amino acids such as lysine, arginine and threonine, and reduced palatability are possible reasons for fish fed MBM diets to show reduced growth (Ai et al., 2003, Kureshy et al., 2000).

Fatty Acid Composition of the Diets and Fish Whole Body

In general, the fatty acid composition of the fish whole body was reflective of the fatty acid composition of their diets (Table 3, Table 4). Oleic acid (18:1n-9) was higher in the MBM and PBM diets (Table 3, Fig. 1) and in the fish fed those diets (Table 4, Fig. 7). Similar results were seen in yellow croaker (Ai et al., 2006). Oleic acid increased as MBM increased in both diets and fish whole body (Ai et al., 2006). Higher oleic acid levels were also found in Atlantic salmon, *Salmo salar L.*, when fed diets with poultry fat partially replacing fish oil (Higgs et al., 2006). In this study, the high levels of oleic acid did not appear to negatively affect growth of juvenile black sea bass. Catfish, *Ictalurus punctatus*, fed high oleic corn diets grew as well or better than catfish fed diets containing regular corn (Sugiura & Lovell, 1996). The higher oleic acid content in the diets was not reflected in the fish flesh (Sugiura & Lovell, 1996).

Linoleic acid (18:2n-3) was higher in the SBM and PBM diets and also higher in the whole body of fish fed those diets (Table 3, Table 4, Fig. 2, Fig. 8). Studies on yellow perch

(Kasper et al., 2007), pike perch, *Sander lucioperca* (Schulz et al., 2005), and flounder, *Paralichthys olivaceus* (Lee et al., 2000), also found a higher level of linoleic acid in fish fed diets containing SBM or soybean oil. Atlantic salmon fed diets containing poultry fat as a partial substitute for fish oil showed a significant increase in linoleic acid content (Higgs et al., 2005). Studies conducted on channel catfish found that fish fed diets high in linoleic acid had lower weight gain compared to the control (Dupree, 1969; Stickney & Andrews, 1979 in Watanabe, 1982). Optimum weight gain and FCR was achieved for carp, *Cyprinus carpio*, and chum salmon, *Oncorhynchus keta,* when fed diets with 1% linoleic acid (Takeuchi & Watanabe, 1977; Takeuchi et al., 1979, 1980; Watanabe et al., 1975 in Watanabe, 1982). Optimum weight gain and FCR for eel, *Anguilla japonica,* occurred when they were fed diets containing 0.5% linoleic acid (Takeuchi et al., 1980 in Watanabe, 1982). The SBM diets used in this study ranged from 1.2% - 1.5% linoleic acid and the PBM diet contained 1.3% linoleic acid (Table 3, Fig. 2). These values are lower than in the diets used by Schultz et al. (2005) but higher than in the poultry fat diet used by Higgs et al. (2006). The use of high levels of linoleic acid may be partially responsible for the depressed growth of fish fed the 90% and 100% SBM diets. However, because fish fed the PBM diet, which was also high in linoleic acid, did not have depressed growth, other factors were most likely responsible for reduced growth in fish fed diets with 90% or 100% of the FM protein replaced by SBM.

The n-3 PUFA level was the same or higher in fish fed the experimental diets compared to the FM diet (Table 4, Fig. 9), comprising 2.7 – 3.6% of the diets (Table 4, Fig. 3) and exceeding the typical minimum requirements (0.8% - 2.5%) for juvenile marine fish (Sargent et al., 2002). As SBM content was increased in the diets, fish oil was increased to compensate for the low lipid content of SBM, leading to higher n-3 PUFA levels in the SBM diets (Table 1).

Yellow perch fed a SBM diet has the same or higher n-3 PUFA levels compared to fish fed a FM diet (Kasper et al., 2007).

Eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) concentrations were the same or higher in fish fed the experimental diets compared to fish fed the FM diet (Table 4, Fig 10, Fig. 11), and this was reflective of the diets (Table 3, Fig. 4, Fig. 5). The lowest dietary DHA level was 0.87% (90% SBM diet, Table 3, Fig. 5) and the lowest dietary EPA level was 1.3% (90% SBM diet, Table 3, Fig. 4). This is within the range found to be required in other marine finfish species. For example, red sea bream, *Pagrus major*, were found to have a DHA requirement of 0.5% - 1.0% of the dry diet and an EPA requirement of 1.0% of the dry diet (Sargent, 2002). Striped jack, *Pseudocaranx dentex*, require 1.7% DHA in their diets (Sargent, 2002). In this study, the ratio of DHA to EPA in the diets ranged from 0.64 – 0.68 (Table 3, Fig. 6), within the range of 0.5 – 1.0 DHA/EPA required by gilthead sea bream, *Sparus aurata* (Sargent, 2002). Although the DHA or EPA requirements for juvenile black sea bass are not known, based on the requirements of other marine finfish listed above, it appears that sufficient DHA and EPA was provided in all diets.

Survival and Growth

In this study survival remained high throughout the study and ranged from 87% - 100% with no significant differences (Table 5). The majority of mortalities occurred when fish jumped from the tanks and therefore were not related to diets. Atlantic cod (Hansen et al., 2007), rainbow trout, (Bureau et al., 1999), and red drum (Kureshy et al., 2000) also showed no significant differences in survival when fed diets with FM protein replacement levels as high or higher than those investigated in this study. Kasper et al. (2007), however, saw a significant decline in the survival of yellow perch when fed diets with 92% and 100% of the FM protein replaced by SBM protein.

Based on final fish weight, percent weight gain and specific growth rate, which were all significantly lower in fish fed the 90% and 100% SBM diets, compared to the control FM diet (Table 5, Fig. 13, Fig. 14, Fig. 15, Fig. 17), maximum substitution level of SBM for FM was 80%. However, because the 80% SBM diet was also not significantly different from the 90% or 100% SBM diets, we recommend diets with a maximum of 70% of the FM replaced by SBM for juvenile black sea, provided the diets contain squid meal, krill meal and attractants. This represents a higher successful substitution level (70% SBM) than is typically seen in marine finfish. In gilthead sea bream weight gain declined at substitution levels of about 45% SBM replacement of FM (Martinez-llorens et al., 2008). Percent weight gain for juvenile cobia, declined when SBM replaced 50% or more the FM protein (Zhou et al., 2005). Some freshwater fish were able to accept diets with 100% of the FM replaced by SBM without negatively affecting their growth. In rohu, 100% of the FM protein was successfully replaced with SBM in diets, but with methionine supplementation (Khan et al., 2003). No essential amino acid supplements were used in this study but some additional amino acids were provided by squid meal and krill meal (Table 1). The ability of black sea bass to grow on diets with high substitution levels of SBM for FM indicates a superior ability to digest SBM as compared to other marine finfish. Black sea bass may contain specific digestive enzymes that make this possible, but further investigation is needed. In addition, further studies are needed to determine the long-term effects of SBM on black sea bass growth and morphology.

Fish fed the 60% PBM and 30% MBM diets were not significantly different from the fish fed the FM diet in terms of final weight, percent weight gain or SGR (Table 5, Fig. 16). Other studies obtained similar results. Red drum showed no significant difference in percent weight gain when fed diets with 66.7% of the FM replaced by PBM (Kureshy et al., 2000). However, final fish weight and specific growth rate declined in black Sea turbot, *Psetta maeotica*, when fed diets with more than 50% of the FM protein replaced by PBM (Yang et al., 2006). MBM replaced 25%, 40% or 45% of the FM without significantly affecting the specific growth rate of rainbow trout (Bureau et al., 2000) gilthead seabream (Robaina et al., 1997) or yellow croaker (Ai et al., 2006). In red drum, percent weight gain was significantly lower in all MBM diets (16.7% - 66.7% substitution) compared to the FM control diet (Kureshy et al., 2000). Since only one substitution level for MBM and for PBM was investigated in this study, higher substitution levels may be possible in diets for juvenile black sea bass.

In this study, no significant differences were seen in apparent feed intake among the groups fed SBM (Table 5), indicating that palatability was not an issue for juvenile black sea bass fed diets containing SBM. All diets used in this study contained squid meal, krill meal and attractants (Table 1) intended to increase palatability. A significant decline in feed intake was observed in yellow perch when fed diets with 63.5% of the FM replaced by SBM (Kasper et al., 2007). Feed intake of turbot fed diets containing up to 50% soybean meal concentrate (SBMC) did not differ significantly from the control diet, but the feed intake was significantly lower in fish fed diets containing 75% or more SBMC (Day & Gonzalez, 2000).

Palatability was not an issue for fish fed diets containing PBM or MBM, in this study (Table 5). Both diets contained squid meal, krill meal and attractants intended to increase the palatability of the diets (Table 1). Yigit et al. (2006) saw a decline in feed intake in black sea turbot when fed diets containing 75% or 100% of the FM replaced by PBM, but no significant difference was noted at 50% PBM. Feed intake in gilthead sea bream fed diets with up to 40%

of the FM replaced by MBM was not significantly different from the feed intake of fish fed the control diet (Robaina et al., 1997).

In this study, feed conversion ratio of juvenile black sea bass was significantly lower in fish fed the 70% SBM diet compared to fish fed the FM diet (Table 5, Fig. 17). No significant differences were found between all other treatment groups and the FM diet (Table 5, Fig. 17). The higher FCR in for fish fed the FM diet may be due to the addition of cellulose to the diet. 6.5% of the FM was cellulose, whereas no cellulose was added to the 70% SBM diet (Table 1). Juvenile cobia fed diets with 50% or 60% of the FM protein replaced by SBM had higher FCR compared to fish fed a FM control diet (Zhou et al., 2005). Gilthead sea bream fed diets with 60% or 75% SBM had higher FCR compared to fish fed the FM diet (Martinez-llorens et al., 2008), while mangrove red snapper showed no significant difference in FCR with substitution of up to 50% SBM for FM (Catacutan & Pagador, 2004). These studies indicate that fish fed diets with large amounts of SBM typically have equal or higher FCRs compared to fish fed a control diet. Therefore, SBM diets tend to be less efficient than FM diets.

Protein efficiency ratios of BSB fed the SBM diets were not significantly different from fish fed the control FM diet (Table 5, Fig. 18). PER values were lower in the fish fed the higher SBM substitution diets, but due to high variability among the replicates these differences were not significant. No significant differences in PER were found in rohu, fed up to 100% SBM protein diets, but the diets were supplemented with methionine (Khan et al., 2003). PER declined at 40% and 45% SBM in Mediterranean yellowtail (Tomas et al., 2005) and gilthead sea bream (Martinez-llorens et al., 2008) respectively.

Protein efficiency ratios of BSB fed the PBM or MBM diet were not significantly different from fish fed the FM diet (Table 5, Fig. 18). Replacement of up to 50% PBM in diets for black sea turbot (Yigit et al., 2006) and up to 40% MBM in diets for gilthead sea bream (Robaina et al., 1997) resulted in no significant change in PER .

Whole Body Proximate Composition

No significant differences were found in moisture content of the whole fish body among treatment groups (Table 7). Protein replacement studies on yellow croaker (Ai et al., 2006), rainbow trout (Bureau et al., 2000) and rohu (Khan et al., 2003) also found no significant difference in whole body moisture.

Whole body lipid content was significantly lower in fish fed the SBM diets, compared to the FM diet (Table 7). Although not significant, the 70% - 100% SBM diets were lower in lipid compared to the FM diet (Table 6). Mangrove red snapper had a lower whole body lipid level when fed diets containing 12.5%, 37.5% and 50% SBM in place of FM, however, no significant difference was found in the fish fed a diet with 25% of the FM replaced by SBM (Catacutan & Pagador, 2004). No explanation was given for why the 25% SBM diet did not result in lower whole body lipid. Other SBM studies on rohu, (Khan et al., 2003) and gilthead seabream (Martinez-llorens et al., 2008) found no significant differences in whole body lipid.

Whole body lipid content of BSB fed the 60% PBM or 30% MBM diets were not significantly different from the fish fed the FM diet (Table 7). Studies on gilthead seabream (Robaina et al., 1997) and rainbow trout (Bureau et al., 2000) resulted in no significant change in whole body lipid in fish fed PBM or MBM.

Protein content of the whole fish body was lower in fish fed the 30% MBM diet (Table 7). Studies on yellow croaker (Ai et al., 2006), rainbow trout (Bureau et al., 2000) and silver perch (Stone et al., 2000) did not find a significant difference in whole body protein when fed diets with up to 100% of the FM replaced by MBM. No significant differences in protein content were observed between the fish fed the FM diet and all other experimental diets. Dietary protein level was the same for all treatments (Table 7).

Ash content was significantly lower in fish fed the 100% SBM diet compared to the FM control diet (Table 7). No significant differences in ash content were observed between all other experimental diets and the FM diet. Lower ash content indicates a lower mineral content in the 100% SBM diet due to the lack of FM. Studies on mangrove red snapper (Catacutan & Pagador, 2004) and gilthead seabream (Martinez-llorens et al., 2008) found no significant difference in ash content as SBM concentration increased, but they did not replace 100% of the FM with SBM. Rohu fed diets with 100% of the FM replaced by SBM were not significantly different in ash content from fish fed a control diet (Khan et al., 2003).

Muscle Tissue Proximate Composition

No significant differences were found in muscle tissue moisture, protein or ash between fish fed the experimental diets and fish fed the FM diet (Table 8). Fishmeal protein replacement studies on Indian major carp, *Cirrhinus mrigala* (Jose et al., 2006), yellow perch (Kasper et al., 2007) and Mediterrean yellowtail (Tomas et al., 2005) also found no significant differences in muscle moisture, protein or ash. Muscle moisture of black sea turbot fed diets with more than 50% PBM protein decreased significantly (Yigit et al., 2006). Typically, there is an inverse relationship between muscle tissue lipid and muscle moisture. Muscle tissue lipid was significantly higher in the fish fed the 100% SBM diet and the 60% PBM diet (Table 8), but it did not significantly affect the moisture level. No significant differences in muscle lipid were found in yellow perch fed 100% SBM protein diets (Kasper et al, 2007) or in black sea turbot fed 100% PBM protein diets (Yigit et al., 2006).

Digestive Organ Proximate Composition

Moisture was higher in the digestive organs of fish fed the 70%, 80%, 90%, 100% SBM diets (Table 9). Ash was higher in fish fed all SBM diets (Table 9) and lipid was lower in the digestive organs of fish fed the 70%, 80%, 90%, and 100% SBM diets (Table 9). A decline in digestive organ lipid level may indicate an increasing use of lipid for energy by fish fed diets high in SBM (Krogdahl et al., 2003). The authors hypothesized that this is because fish have trouble digesting the SBM and therefore must use lipids to meet their energy requirements (Krogdahl et al., 2003). In this study, lower lipid level in the digestive organs may be because the lipid contents of the SBM diets were lower than the lipid contents of the FM, PBM and MBM diets (Table 6). No significant differences were found in the moisture, lipid or ash content of the digestive organs of fish fed the 60% PBM or 30% MBM diets compared to fish fed the FM diet (Table 9).

Several studies have investigated changes in the intestinal morphology of fish fed SBM diets. While mangrove red snapper (Catacutan & Pagador, 2004) and cobia (Romarheim et al., 2008) fed SBM diets were not found to have any differences in intestinal morphology, changes in the epithelial cells of the distal intestine of rainbow trout were observed when the fish were fed SBM diets (Suzuki & Yamamoto, 2004). These changes can cause the intestinal cells to be less effective at metabolizing nutrients (Iwashita et al., 2008a). It has been hypothesized that anti-nutritional factors in SBM, possibly soya saponins or soya lectins may cause the intestinal changes (Iwashita et al., 2008a). Intestinal changes have also been seen in salmonids fed SBM diets (Krogdahl et al., 2003). Diets with as little as 10% SBM protein replacing FM protein had detrimental effects on the intestine of Atlantic salmon (Krogdahl et al., 2003). As the SBM inclusion level increased, the intestinal changes became more severe (Krogdahl et al., 2003).

Reduced growth in juvenile black sea bass fed diets with 90% or 100% of the FM replaced by SBM may be due antinutritional factors in SBM. Trypsin inhibitor is a protein found in SBM that depresses the growth of fish (Hendricks, 2002). Heat-treatment of SBM inactivates most of the trypsin inhibitor but not all of it (Tomas et al., 2005). The protein efficiency ratio of fish fed the 90% and 100% SBM diets was significantly lower than in fish fed the 70% SBM diet (Table 5, Fig. 20), indicating that protein digestibility was lower in these diets (Hendricks, 2002, Martinez-llorens et al., 2008). Phytic acid or phytate is another antinutrional factor found in SBM but it is not destroyed by heat treatment (Hendricks, 2002). Phytate is a plant storage form of phosphorous and is not digestible by fish (Drew et al., 2007). Mineral supplementation in the diets is intended to increase the phosphorus availability. Supplementation of SBM diets for African catfish, *Clarias gariepinus* (Van Weerd et al., 1999), striped bass, *Morone saxatallis* (Papatryphon & Soares, 2001) and Japanese flounder (Masumoto et al., 2001) with phytase, a digestive enzyme, increases digestibility. Phytase supplementation may improve the growth of black sea bass fed more than 80% SBM diets, but experimentation is needed.

Liver Tissue Proximate Composition

No significant differences were found in liver tissue moisture, lipid or protein between fish fed any of the experimental diets and fish fed the FM diet (Table 10). In other studies on SBM or soybean oil, liver lipid content increased in mangrove red snapper (Catacutan & Pagador, 2004), pike perch, *Sander lucioperca* (Schultz et al., 2005) and cobia (Zhou et al.,

2005) fed soy-based diets. An increase in liver lipid can indicate a deficiency in essential fatty acids (Watanabe, 1993), reduced lipid utilization (Schultz et al., 2005) or a deficiency in dietary phosphorus (Catacutan & Pagador, 2004). Because the majority of the lipids provided in the SBM diets came from fish sources not soybean sources, lipid utilization was not a problem in this study.

CONCLUSIONS

In summary, no significant differences were observed in survival, growth, whole body ash, protein or moisture, muscle tissue proximate composition, or liver tissue proximate composition between fish fed diets with 60%, 70%, or 80% of the FM replaced by SBM and fish fed the FM control diet. Because the 80% SBM diet was also not significantly different from the unsuccessful 90% or 100% SBM diets in terms of growth, we do not recommend this diet. All diets contained 7.5% squid meal, 5% krill meal and attractants (Table 1). Without these ingredients, maximum protein replacement levels may be different. Further studies are needed to confirm this possibility. Based on the results of this study, we recommend that juvenile black sea bass can be successfully raised on diets containing 7.5% squid meal, 5% krill meal and attractants, with up to 70% of the FM protein replaced by SBM.

Fish fed diets with 90% or 100% of the FM replaced by SBM had significantly lower growth compared to fish fed the FM diet. Reduced growth may be attributed to a higher linoleic acid content, a lower level of methionine or lysine, or anti-nutritional factors in the SBM.

No significant differences were observed in survival, growth, whole body moisture, protein or ash, muscle tissue proximate composition, or liver tissue proximate composition between fish fed diets with 60% of the FM replaced by PBM or 30% of the FM replaced by MBM and fish fed the FM diet. Since only one substitution level was tested in these trials using animal by-products, further studies are needed to evaluate even higher substitution levels of PBM or MBM as a FM replacement in diets for juvenile black sea bass. Based on the results of this study, PBM and MBM are potentially viable alternatives to FM in black sea bass diets.

LITERATURE CITED

Ai, Q., Mai, K., Tan, B., Xu, W., Duan, Q., Ma, H. & Zhang, L. (2006) Replacement of fish meal by meat and bone meal in diets for large yellow croaker, *Pseudosciaena crocea. Aquaculture,* 260, 255-263.

Alam, M.S., Teshima, S, Koshio, S., Ishikawa, M., Uyan, O., Hernandez, L.H. & Michael, F. R. (2005) Supplemental effects of coated methionine and/or lysine to soy protein isolate diet for juvenile kuruma shrimp, *Marsupenaeus japonicas*. *Aquaculture,* 248, 13-19.

Alam, M., Teshima, S., Yaniharto, D., Koshio S. & Ishikawa, M. (2002) Influence of different dietary amino acid patterns on growth and body composition of juvenile Japanese flounder, *Paralichthys olivaceus*. *Aquaculture,* 210, 359-369.

Alam, M.S., Watanabe, W.O. & Carroll, P.M. (2008) Dietary protein requirements of juvenile black sea bass, *Centropristis striata*. *Journal of the World Aquaculture Society,* 39, 656-663.

Alam, M. S., Watanabe, W.O., Carroll, P.M. & Rezek, T. (2008) Effects of dietary protein and lipid levels on growth performance and body composition of black sea bass *Centropristis striata* (Linnaeus 1758) during grow-out in a pilot-scale marine recirculating system. *Aquaculture Research,* in press.

Bureau, D.P., Harris, A.M., Bevan, D.J., Simmons, L.A., Azevedo, P.A. & Cho C.Y. (2000) Feather meals and meat and bone meals from different origins as protein sources in rainbow trout (*Oncorhynchus mykiss)*. *Aquaculture,* 181, 281-291.

Catacutan, M. & Pagador, G. (2004) Partial replacement of fishmeal by defatted soybean meal in formulated diets for the mangrove red snapper, *Lutjanus argentimaculatus. Aquaculture Research,* 35, 299-306.

Cheng, Z. J., Hardy, R.W. & Blair, M. (2003) Effects of supplementing methionine hydroxy analogue in soybean meal and distiller's dried grain-based diets on the performance and nutrient retention of rainbow trout [*Oncorhynchus mykiss* (Walbaum)]. *Aquaculture Research,* 34, 1303-1310.

Copeland, K.A., Watanabe W.O. & Dumas, C.F. (2005) Economic evaluation of a small-scale recirculating system for ongrowing of captive wild black sea bass *Centropristis striata* in North Carolina. *Journal of the World Aquaculture Society,* 36, 489-497.

Copeland, K.A., Watanabe W.O. & Carroll, P.M. (2002) Growth and feed utilization of wild-caught black sea bass *Centropristis striata* fed practical diets in a recirculating tank system under a semi-controlled temperature regime. *Journal of the World Aquaculture Society,* 33, 97-109.

Cotton, C. (2002) Optimizing growth for aquaculture of juvenile black sea bass, *Centroprista striata* L.: Effects of temperature, salinity, commercial diet and feeding ration. University of Georgia, Athens, GA. Retrieved on April 1, 2007 from http://www.shellfish.uga.edu/dissthesis.html.

Day, O.J. & Gonzalez, H.G.P. (2000) Soybean protein concentrate as a protein source for turbot *Scophthalmus maximus* L. *Aquaculture Nutrition,* 6, 221-228.

Division of Marine Fisheries, *Black Sea Bass Stock.* Retrieved on May 4, 2007 from www.ncfisheries.net/stocks/seabass.htm

Drew, M.D., Borgeson,T.L. & Thiessen, D.L. (2007) A review of processing feed ingredients to enhance diet digestibility in finfish. *Animal Feed Science and Technology*, 138, 118-136.

Gallagher, M. L. (1994) The use of soybean meal as a replacement for fish meal in diets for hybrid striped bass (*Morone saxatilis x M. chrysops*). *Aquaculture,*126, 119-127.

Hansen, A.-C., Karlsen, O., Rosenlund, G., Rimbach, M. & Hemre, G. –I. (2007) Dietary plant protein utilization in Atlantic cod, *Gadus morhua* L. *Aquaculture Nutrition*, 13, 200-215

Hardy R.W. (2006) Worldwide fish meal production outlook and the use of alternative protein meals in aquaculture. Retrieved on April 16, 2007 from http://www.educacion.uanl.mx/publicaciones/maricultura/viii/pdf/25Hardy.pdf

Hendricks, J.D. (2002) Adventitious toxins In *Fish Nutrition* (Halver, J.E. & Hardy, R.W. eds.), pp. 601 – 649. Elseviser Science, San Diego, CA.

Higgs, D. A., Balfry, S.K., Oakes, J.D., Rowshandeli, M., Skura, B.J. & Deacon, G. (2006) Efficacy of an equal blend of canola and poultry fat as an alternative dietary lipid source for Atlantic salmon (*Salmo salar* L.) in sea water. I: effects on growth performance, and whole body and fillet proximate and lipid composition. *Aquaculture Research,* 37, 180-191.

Iwashita, Y., Suzuki, N., Yamamoto, T., Shibata, J-I., Isokawa, K., Soon, A. H., Ikehata, Y., Furuita, H., Sugita, T. & Goto, T. (2008) Supplemental effect of cholyltaurine and soybean lecithin to a soybean meal-based fish meal-free diet on hepatic and intestinal morphology of rainbow trout, *Oncorhynchus mykiss*. *Fisheries Science*, 74, 1083-1095.

Iwashita, Y., Yamamoto, T., Furuita, H., Sugita, T. & Suzuki, N. (2008). Influence of certain soybean anti-nutritional factors supplemented to a casein-based semipurified diet on intestinal and liver morphology in fingerling rainbow trout, *Oncorhynchus mykiss*. *Fisheries Science;* 74: 1075 – 1082.

Jose. S., Mohan, M.V., Shyama, S., Ramachandran Nair, K. G.& Mathew, P.T. (2006) Effect of soybean-meal-based diets on the growth and survival rate of the Indian major carp,*Cirrhinus mrigala* (Ham.). *Aquaculture Nutrition*, 12, 275-279.

Kasper, C.S., Watkins, B.A. & Brown, P.B. (2007) Evaluation of two soybean meals fed to yellow perch (*Perca flavescens)*. *Aquaculture Nutrition*, 13, 431-438.

Khan, M.A., Jafri, A.K., Chadha, N.K. & Usmani, N. (2003) Growth and body composition of rohu (*Labeo rohita*) fed diets containing oilseed meals: partial or total replacement of fish meal with soybean meal. *Aquaculture Nutrition*, 9, 391-396.

Kikuchi, K. (1999) Use of defatted soybean meal as a substitute for fish meal in diets of Japanese flounder (*Paralichthys olivaceus*). *Aquaculture*, 179, 3-11.

Krogdahl, A., Bakke-McKellep, A.M., & Baeverfjord, G. (2003) Effects of graded levels of standard soybean meal on intestinal structure, mucosal enzyme activities, and pancreatic response in Atlantic salmon (*Salmo salar* L.). *Aquaculture Nutrition*, 9, 361-371.

Kureshy, N., Davis, D.A. & Arnold, C.R. (2000) Partial Replacement of Fish Meal with Meat-and-Bone Meal, Flash Dried Poultry By-Product Meal, and Enzyme-Digested Poultry By-Product Meal in Practical Diets for Juvenile Red Drum. *North American Journal of Aquaculture,* 62, 266-272.

Lee, K.J., Dabrowski, K., Blom, J.H., & Bai, S.C. (2001) Replacement of fish meal by mixture of animal by-products in Juvenile Rainbow Trout Diets. *North American Journal of Aquaculture,* 63, 109-117.

Lee, S.-M., Cho, S. H. & Kim, K. –D. (2000) Effects of dietary protein and energy levels on growth and body composition of juvenile flounder, *Paralichthys olivaceous. Journal of the World Aquaculture Society,* 31, 306-315.

Lewis, H.A. & Kohler,C.C. (2008) Minimizing fish oil and fish meal with plant-based alternatives in sunshine bass diets without negatively impacting growth and muscle fatty acid profile. *Journal of the World Aquaculture Society*, 39, 573-585.

Martinez-llorens, S., Vidal, A. T., Garcia, I. J., Torres & M. P., Cerda, M. J. (2008) Optimum dietary soybean level for maximizing growth and nutrient utilization of on-growing gilthead seabream (*Sparus aurata*). *Aquaculture Nutrition*, in press.

Masumoto, T, Tamura, B. & Shimeno, S. (2001) Effects of phytase on bioavailability of phosphorus in soybean meal-based diets for Japanese flounder, *Paralichthys olivaceus. Fisheries Science*, 210, 359-369.

Musick, J. A. & Mercer, L.P. (1977) Seasonal distribution of black sea bass, *Centropristis striata*, in the mid-Atlantic bight with comments on the ecology and fisheries of the species. *Transactions of the American Fisheries Society*, 106, 12-25.

Muzinic, L.A., Thompson, K.R., Metts, L.S., Dasgupta, S. & Webster, C.D. (2006) Use of turkey meal as partial and total replacement of fish meal in practical diets for sunshine bass (*Morone chrysops x Morone saxatilis*) grown in tanks. *Aquaculture*, 12, 71-81.

Nengas, I., Alexis, M.N., & Davies, S.J. (1999) High inclusion levels of poultry meals and related byproducts in diets for gilthead seabream, *Sparus aurata* L. *Aquaculture*, 179, 13-23.

Papatryphon, E. & Soares, J.H. Jr (2001) The effect of phytase on apparent digestability of four practical plant feedstuffs fed to striped bass, *Morone saxatillis*. *Aquaculture Nutrition*, 7, 161-167

Robaina, L., Moyano, F.J., Izquierdo, M.S., Socorro, J., Vergara, J.M. & Montero, D. (1997) Corn gluten and meat and bone meals as protein sources in diets for gilthead seabream (*Sparus aurata*): Nutritional and histological implications. *Aquaculture*, 157, 347-359.

Romarheim, O.H., Zhang, C., Penn, M., Liu, Y.-J., Tian, L. –X., Skrede, A., Krogdahl & A., Storebakken, T. (2008) Growth and intestinal morphology in cobia (*Rachycentron canadum)* fed extruded diets with two types of soybean meal partly replacing fish meal. *Aquaculture Nutrition*, 14, 174-180.

Saitoh, S., Koshio, S., Harada, H., Watanabe, K., Yoshida, T., Teshima, S. & Ishikawa, M. (2003) Utilization of extruded soybean meal for Japanese flounder *Paralichthys olivaceus* juveniles. *Fisheries Science*, 69, 1075-1077.

Sargent, J.R., Tocher, D.R., Bell & J.G. (2002). The lipids In *Fish Nutrition* (Halver, J.E. & Hardy, R.W. eds) pp. 181-257. Elsevier Science, San Diego, CA.

Schultz, C., Knaus, U., Wirth, M. & Rennert, B. (2005) Effects of varying dietary fatty acid profile on growth performance, fatty acid, body and tissue composition of juvenile pike perch (*Sander lucioperca*). *Aquaculture Nutrition*, 11, 403-413.

Sedberry, G.R. (1988) Food and feeding of black sea bass, *Centropristis striata*, in live bottom habitats in the south Atlantic bight. *Journal of the Elisha Mitchell Scientific Society*, 104, 35-50.

Stone, D.A.J, Allan G.L., Parkinson S. & Rowland S.J. (2000) Replacement of fish meal in diets for Australian silver perch, *Bidyanus bidyanus*:III. Digestibility and growth using meat meal products. *Aquaculture* 186, 311-326.

Sugiura, S. H. & Lovell, R.T. (1996) Use of high oleic corn in catfish feeds. *Journal of the World Aquaculture Society*, 27, 74-81.

Teshima, S.I., Koshio, S., Ishikawa, M., Alam, M.S. & Hernandez, L.H.H. (2004) Effects of protein and lipid sources on the growth and survival of red sea bream Pagrus major and Japanese flounder *Paralichthys olivaceus* receiving micro-bound diets during larval and early juvenile stage. *Aquaculture Nutrition*, 10, 279-287.

Tomas, A., De La Gandara, F., Garcia-Gomez, A., Perez, L., & Jover, M. (2005) Utilization of soybean meal as an alternative protein source in the Mediterranean yellowtail, *Seriola dumerili*. *Aquaculture Nutrition*, 11, 333-340.

Van Weerd, J.H., Khalaf, K.H.A., Aarlsen, F.J. & Tijsses, P.A. (1999) Balance trials with African catfish *Clarias gariepinus* fed phytase-treated soybean meal-based diets. *Aquaculture Nutrition*, 5, 135-142.

Vaugn, D.S., Collins, M.R. & Schmidt, D.J. (1995) Population characteristics of the black sea bass *Centropristis striata* from the Southeastern U.S. *Bulletin of Marine Science*, 56, 250-267.

Wang, Y., Guo J., Bureau, D. P. & Cui, Z., (2006) Replacement of fish meal by rendered animal protein ingredients in feeds for cuneate drum (*Nibea miichthioides). Aquaculture*, 252, 476-483.

Watanabe, T. (1982) Lipid nutrition in fish. *Comparative Biochemistry and Physiology*, 73B, 3-15.

Wilson, R.P. (2002) Amino acids and proteins In *Fish Nutrition* (Halver, J.E. & Hardy, R.W., eds.) pp. 143-179.

Yang, Y., Xie, S., Cui, Y., Lei, W., Zhu, X., Yang, Y. & Yu, Y. (2004) Effect of replacement of dietary fish meal by meat and bone meal and poultry by-product meal on growth and feed utilization of gibel carp*, Carassius auratus gibelio*. *Aquaculture Nutrition,*10, 289-294.

Yigit, M., Erdem, M., Koshio, S., Ergun, S., Turker, A. & Karaali, B. (2006) Substituting fish meal with poultry byproduct meal in diets for black Sea turbot *Psetta maeotica*. *Aquaculture Nutrition,*12, 340-347.

Zhang, S., Xie, S., Zhu, X., Lei, W., Yang, Y. & Zhao, M. (2006) Meat and bone meal replacement in diets for juvenile gibel carp (*Carassius auratus gibelio)*: effects on growth performance, phosphorus and nitrogen loading. *Aquaculture Nutrition,*12, 353-362.

Zhou, Q. C., Mai, K. S., Tan, B. P. & Liu, Y. J. (2005) Partial replacement of fishmeal by soybean meal in diets for juvenile cobia (*Rachycentron canadum). Aquaculture Nutrition*, 11, 175-182.

APPENDIX A

Vitamin Premix:

Product Name : Kadai (Univ. of Kagoshima) Vitamin for Fish

Addition Rate : 4.0 – 4.5%

Ingredients	Content/Kg	Unit
Excipient		
β-carotene		
Vitamin D3	12,906,000.00	IU
Vitamin E	12,832.00	mg
Vitamin K3	1,528.00	mg
Vitamin B1 (coated)	1,925.00	mg
Vitamin B2	6,413.00	mg
Vitamin B6	1,528.00	mg
Vitamin B12	2.80	mg
Niacin	25,658.00	mg
Calcium D-pantothenate	8,983.00	mg
Biotin	192.00	mg
Folic acid	481.00	mg
Para-amino benzoic acid	12,775.00	mg
Inositol	128,304.00	mg
Choline chloride (Silica)	262,310.00	mg
Cellulose	128,679.00	mg

Mineral Premix:

Product Name : Kadai (Univ. of Kagoshima) Mineral for Fish

Addition Rate : 4.0 per dry feed

Ingredients	Content/Kg	Unit
Excipient		
Sodium chloride		
Magnesium sulfate	126,674.00	mg
Sodium monohydrogen phopahte	80,635.00	mg
Potassium monohydrogen phopahte	221,732.00	mg
Calcium monohydrogen phopahte	125,566.00	mg
Ferric citrate	27,460.00	mg
Calcium lactate	302,367.00	mg
Alminium hydroxide	173.00	mg
Zinc sulfate	3,301.00	mg
Copper sulfate	92.00	mg
Manganese sulfate	740.00	mg
Calcium iodate	139.00	mg
Cobaltous sulfate	923.00	mg