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ARTICLE

Evaluation of Poultry By-Product Meal as an Alternative to Fish Meal in the Diet of Juvenile Black Sea Bass Reared in a Recirculating Aquaculture System

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Abstract

A feeding trial was conducted to determine the maximum substitution limits of poultry by-product meal (PBM; 66% crude protein) protein for fish meal (FM; 59% crude protein) protein in the diet of juvenile Black Sea Bass *Centropristis striata* (family Serranidae). Eight isonitrogenous (44% crude protein) and isolipidic (13% crude lipid) diets were formulated to replace FM protein with PBM protein at 0 (control), 40%, 50%, 60%, 70%, 80%, 90%, and 100% in Black Sea Bass diets. Diets were fed twice daily to triplicate groups of juveniles (initial mean weight = 1.2 g) to apparent satiation for 8 weeks in a recirculating aquaculture system. Final survival was excellent (95–100%) in all diet treatments, with no significant differences. No significant differences in body weight gain (BWG) were observed in fish fed the 40–90% PBM protein diets (1,136–1,357%) compared with the control diet (1,307%). However, BWG of fish fed the 100% PBM protein diet (1,045%) was significantly lower than in the control group. Regression analysis with BWG indicated that PBM protein can replace FM protein in Black Sea Bass diets at levels as high as 81.8%, with no reduction in fish growth performance. For fish fed diets with up to 90% PBM protein, feed conversion (1.08–1.17) and protein efficiency ratios (2.01–2.14) were not significantly different from fish fed a control 100% FM-protein-based diet (0.99 and 2.29, respectively). Apparent digestibility coefficients of dietary protein remained high (81.6–87.0%) under all levels of FM replacement with PBM protein. After the feeding trial, whole body and muscle protein content and the concentrations of whole body n-3 polyunsaturated fatty acids showed no significant differences among the treatments at FM protein replacement levels up to 90%. Poultry by-product meal is a promising alternative protein source for sustainable diet development in Black Sea Bass.

In terms of dietary composition, protein is the single largest and most expensive component in fish feed. Fish meal (FM) is a source of high-quality protein and highly digestible essential amino and fatty acids (Cho and Kim 2011), making it a popular source of protein in aquaculture feeds. Worldwide production of FM has been stable at roughly 6.3 million metric tons annually since the 1980s, with Peru and Chile the main producing countries in 2012 (FAO 2012). Once seen as a renewable source, FM costs have increased as demand has increased, while supply has slowly decreased due to overfishing (Tacon et al. 2006; Trushenski et al. 2006). In addition, FM varies greatly in composition and quality among species or with age (Tacon et al. 2006), season, geographic origin,

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and processing methods (Trushenski et al. 2006). Therefore, it is critical to investigate alternative protein sources to FM protein in aquaculture feeds (Trushenski et al. 2006; Gatlin et al. 2007; Alam et al. 2011, 2012; Jirsa et al. 2015; Anderson et al. 2016).

Terrestrial animal protein sources have several advantages, including a similar amino acid profile to FM, availability, and relatively low cost. The two most common chicken ingredients in pet feed are poultry meal and poultry by-product meal (PBM). Poultry meal is the dry rendered ingredient from a combination of clean flesh and skin, with or without accompanying bones, derived from parts of the whole poultry carcass exclusive of the head, feathers, feet, and entrails. Poultry by-product meal is a protein source produced from waste and by-products of processed chickens possibly including heads and feet, but excluding feathers and intestines. Like other animal-based protein feedstuffs it has a high protein content but can vary in compositional quality and lacks certain essential amino acids (Tacon et al. 2006). Poultry by-product meal has been used successfully to replace FM at high levels of dietary inclusion for a number of finfish species. In Gilthead Seabream *Sparus auratus*, 50% of the FM protein was successfully replaced with PBM protein without a reduction in growth (Nengas et al. 1999). In juvenile Red Drum *Sciaenops ocellatus*, 67% of the FM protein was replaced by PBM protein with no reduction in growth (Kureshy et al. 2000). In juvenile Rainbow Trout *Oncorhynchus mykiss* up to 44% of FM protein can be replaced with PBM without a decrease in the tissue omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Pares-Sierra et al. 2014). A disadvantage of PBM protein as a FM protein replacement is reduced digestibility and feed efficiency at high dietary inclusion levels as reported in Atlantic Salmon *Salmo salar* fed diets that replaced 50% of FM protein with PBM protein (Hatlen et al. 2015). In addition, replacement of FM by PBM influences the whole body composition of some fish, such as Greater Amberjack *Seriola dumerili* (Takakuwa et al. 2006) and Humpback Grouper *Cromileptes altivelis* (Shapawi et al. 2007), but not the muscle tissues of Black Sea Turbot *Psetta maeotica* (Yigit et al. 2006).

Black Sea Bass *Centropristis striata* is a commercially important marine finfish species inhabiting coastal waters of the eastern USA, from the Gulf of Maine to Florida, that commands a high market value (National Oceanic and Atmospheric Administration [NOAA] Fish Watch, www.fishwatch.gov). The abundance of Black Sea Bass along the U.S. East Coast has been declining since the 1950s (NOAA 2012; ASMFC 2016) and stringent quotas are in effect for harvesting of wild populations. Potential for limited market supplies and for higher prices of ocean-caught Black Sea Bass in the future are important economic incentives to investigating the feasibility of Black Sea Bass production via aquaculture to help meet market demand (Watanabe 2011; Watanabe et al. 2016).

Hatchery-raised Black Sea Bass juveniles originating from captive wild-caught broodstock have been successfully cultured through the market stage in a recirculating aquaculture system at the University of North Carolina Wilmington (UNCW) Aquaculture Facility (Watanabe et al. 2003; Watanabe 2011). Diets formulated with 44% protein and 15% lipids were found to produce optimal growth of juvenile Black Sea Bass reared in a recirculating aquaculture system (Alam et al. 2008). Previous studies at the UNCW have shown that Atlantic Menhaden *Brevoortia tyrannus* FM protein can be replaced in the diet of Black Sea Bass with plant protein from soybean meal and cottonseed meal at high substitution levels (68% and 100%, respectively) without impairing growth performance (Alam et al. 2012; Anderson et al. 2016). The objective of this study was to determine under controlled laboratory conditions the maximum substitution limits of the animal protein source PBM for FM protein in juvenile Black Sea Bass diets and the effects of replacement on whole body and muscle tissue proximate composition.

METHODS

Experimental Animals and System

This study was conducted at the UNCW Center for Marine Science under the Institutional Animal Care and Use Committee protocol A1516-016. Juvenile Black Sea Bass were cultured from eggs spawned by photothermally conditioned broodstock held at the UNCW Aquaculture Facility (Wrightsville Beach, North Carolina). Broodstock were induced to spawn using luteinizing hormone-releasing hormone analog implants (Watanabe et al. 2003). Eggs were hatched and reared through the juvenile stage in 150-L tanks (Watanabe 2011).

The experimental system consisted of 24 75-L rectangular (76 cm × 32 cm × 43 cm) glass tanks supported by a recirculating seawater system located in a controlled-environment laboratory. Water quality was maintained by a bead filter (Aquadyne, Georgia), a foam fractionator (Top Fathom, Michigan), and a UV sterilizer (Emperor Aquatics, Pottstown, Pennsylvania). Tanks were subjected to a 12 h light : 12 h dark photoperiod supplied by eight 60-W fluorescent lamps in addition to ambient light levels from sunlight entering the laboratory windows.

Experimental Diets

Eight diets were formulated to replace FM protein with pet-feed-grade PBM protein at levels of 0 (control), 40%, 50%, 60%, 70%, 80%, 90%, and 100% (Table 1). All diets were formulated to have the same crude protein level (44%) and lipid level (13%). The analyzed crude protein

TABLE 1. Formulation of diets using varying amounts of poultry by-product meal protein to replace fish meal protein. All values are in grams per 100 g diet unless otherwise noted.

	Percent fish meal replaced							
Diet ingredients and composition	0	40	50	60	70	80	90	100
Ingredient								
Menhaden fish meal[a]	70	42	35	28	21	14	7	0
Poultry by-product meal[b]	0	25.1	31.3	37.6	43.9	50.1	56.4	62.7
Wheat starch	13.9	13.9	13.9	13.9	13.9	13.9	13.9	13.9
Wheat gluten[c]	4	4	4	4	4	4	4	4
Menhaden fish oil[d]	4.6	4.2	4.1	4.0	3.9	3.8	3.7	3.6
Soybean lecithin[e]	1	1	1	1	1	1	1	1
Vitamin premix[f]	3	3	3	3	3	3	3	3
Mineral premix[f]	3	3	3	3	3	3	3	3
Vitamin C	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Calcium phosphate	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Cellulose	0.0	3.3	4.2	5.0	5.8	6.7	7.5	8.3
Total	100	100	100	100	100	100	100	100
Proximate composition (analyzed)								
Crude protein	44.4	43.2	43.4	42.7	43.3	43.0	42.9	42.9
Digestible protein	38.6	36.4	36.1	36.7	35.3	35.7	35.9	35.7
Crude lipid	14.0	13.2	12.3	12.4	13.0	12.8	12.6	12.8
Digestible lipid	12.9	12.0	10.8	11.0	11.6	11.5	11.4	11.5
Ash	16.4	14.3	14.0	12.8	12.7	12.3	11.8	11.0
Energy (kJ/g)[g]	18.5	17.8	17.5	17.4	17.7	17.6	17.3	17.6

[a]Melick Aquafeed, Catawissa, Pennsylvania; 59.5% protein, 12% lipid.
[b]Melick Aquafeed; 67% protein, 15% lipid.
[c]MP Biomedicals, Solon, Ohio; 77% protein.
[d]Virginia Prime Gold; Omega Protein, Houston, Texas.
[e]Soapgoods, Smyrna, Georgia.
[f]Kadai, University of Kagoshima, Japan.
[g]Calculated value based on carbohydrates, proteins, and lipids at 17.2, 23.6, and 39.5 kJ/g, respectively (Blaxter 1989).

levels in the test diets were 44.4%, 43.2%, 43.4%, 42.7%, 43.3%, 43.0%, 42.9%, and 42.9%, respectively (Table 1). Except for wheat gluten as a binder, no additional protein sources, amino acids, or attractants were used. All diets contained the same amount of vitamin and mineral premix (Kadai, Kagoshima University, Kagoshima, Japan), and Atlantic Menhaden fish oil (Virginia Prime Gold; Omega Protein, Houston, Texas) and soybean lecithin were used as lipid sources in addition to the lipid content found in the protein sources. All experimental diets were produced using a meat grinder, and their proximate compositions were analyzed (Table 1) at the UNCW Center for Marine Science Aquaculture Facility (Alam et al. 2012).

Feeding Protocol

To begin the experiment, tanks were randomly stocked at densities of 20 fish per 75-L tank. After fish were acclimated for 3 d, the initial weights of the fish in each tank were determined. Mean ± SE weights were 1.2 ± 0.07 g, with no significant differences ($P > 0.05$) among treatments. Fish were fed a commercial diet containing 57% protein and 15% lipid (Skretting, Vancouver, Canada) during the acclimation period. Treatment diets were fed twice daily (0900 and 1500 hours) to triplicate groups of juvenile Black Sea Bass to apparent satiation (i.e., as much as fish could consume without wastage) for 8 weeks. Temperature, salinity, dissolved oxygen, and pH were monitored twice weekly using a multiparameter probe (YSI, Yellow Springs, Ohio), while ammonia and nitrite were measured once weekly using a portable HACH spectrophotometer (DR 2010; Loveland, Colorado). Temperature was maintained at 24.1 ± 0.03°C (mean ± SE), salinity at 34.7 ± 0.10 g/L, dissolved oxygen at 5.9 ± 0.02 mg/L, and pH at 7.2–7.5. Ammonia and nitrite levels were kept at 0.17 ± 0.01 mg/L and 0.06 ± 0.01 mg/L, respectively. Tanks were siphoned and cleaned daily.

Proximate Composition of Diets and Fish Tissues

At the end of each experiment, 8–10 fish from each tank were collected for biochemical analysis. Five fish were used to determine proximate composition (moisture, ash, lipid, and protein) and fatty acid profiles of the whole

body, and from three to five fish were dissected to analyze the proximate composition of muscle tissue. Moisture content for the whole body and muscle tissues was determined using a freeze-dryer (Labconco Corporation, Kansas City, Missouri) at the UNCW Aquaculture Facility, and moisture content of the diets was determined using an isotemp oven (Fisher Scientific, Waltham, Massachusetts) (AOAC 2000). Ash content of the diets and fish whole body and muscle tissues was determined using a muffle furnace (Barnstead/Thermolyne, Dubuque, Iowa) for 6 h at 600°C (AOAC 2000). Crude protein was determined by the Kjeldahl method with a Labconco Kjeltec System (Rapid Digestor, Distilling Unit-Rapid Still II and Titration Unit; Labconco Corporation) using boric acid to trap ammonia (AOAC 2000). Total crude lipid analysis was conducted using the Bligh and Dyer method (1959) on diets and fish whole body and muscle tissue. The resulting lipid stock solution was stored at −20°C for subsequent fatty acid analysis.

Fatty Acid Analysis of Diets and Whole Bodies

Lipids from diets and fish whole body tissues were converted into fatty acid methyl esters. First, 1 mL of the lipid stock solution was transferred into a conical vial with 250 μL of a 0.001 g/mL solution of C19:0 fatty acid (internal standard). The solvents were then evaporated with nitrogen gas and heat (40°C). Then 1 mL of 0.5 M sodium hydroxide in methanol was added to the vial and heated at 80°C for 30 min. Next, 1.5 mL of boron trifluoride and methanol was added to the vial and heated for another 30 min at the same temperature. Once cool, 1 mL of saturated sodium chloride and 1 mL of hexane were added to the vial, which was then capped and shaken. After two separate layers formed, the top layer was removed and passed through 63-μm silica in a Pasteur pipette into a 25-mL round-bottom flask. This procedure was repeated with 1 mL of hexanes and 1 mL of 20% ether : hexane solution, and then the pipette was rinsed with 1 mL of 50% ether : hexane solution. The solvents were dried using a Rotavap and the sample transferred to a gas chromatography vial with 800 μL of 100% chloroform. The gas chromatography vials were then flashed with nitrogen and stored in the refrigerator. Identification and quantification of the fatty acid methyl esters was done using the gas chromatography–flame ionization detector (Hewlett-Packard) at the UNCW Department of Chemistry and Biochemistry (Rezek et al. 2010).

Apparent Digestibility Coefficient of Protein and Lipids in the Diets

Following the feeding trial and after collecting fish from each treatment for biochemical analysis, the remaining fish were used to conduct a digestibility study for 14 d to determine the apparent digestibility coefficients (ADCs) of crude protein and crude lipid in the experimental diets. During digestibility studies, fish were maintained under experimental conditions as described above. Each diet was reformulated to included 0.5% chromic oxide, which was offset by a reduction of cellulose. These diets were fed twice daily and the tanks were cleaned after the last feeding. The next morning, fecal material produced overnight was collected, rinsed with deionized water, and stored in a freezer (−20°C) for chromic oxide and nutrient (protein and lipid) analyses. Chromic oxide content was determined using a spectrophotometer (Thermo Fisher Scientific, Two Rivers, Wisconsin) through a modified Furukawa and Tsukahara (1966) method. The ADC of protein and lipid was calculated using the following formula:

$$\text{ADC of nutrients (\%)} = 100 \times [1-(F_{\text{nutrient}}/D_{\text{nutrient}} \times D_{\text{chromic}}/F_{\text{chromic}})],$$

where F is the percent of nutrient or chromic oxide in the fecal matter and D is the percent of nutrient or chromic oxide in the diet (Furukawa and Tsukahara 1966).

Statistical analysis.— Statistical analyses were performed using the JMP 13.0 statistical software (SAS Institute, Cary, North Carolina). Treatment means were compared using a one-way ANOVA after the assumptions of the ANOVA were verified by O'Brien's test (1981) for homogeneity of variances. Significant differences between treatment means were further analyzed using a Tukey–Kramer honestly significant difference analysis (Kramer 1956), with $P < 0.05$ considered significant. Broken-line regression analysis (Robbins et al. 1979) was conducted using statistical package JMP 13:0 (SAS Institute, Cary).

RESULTS

Survival and Growth

At the end of the experiment on day 56, survival ranged from 95.0% to 100.0%, with no significant ($P > 0.05$) differences among treatments (Table 2). No significant differences in mean fish weights (range = 1.1–1.3 g) were observed among treatment groups at the beginning of the experiment (day 0) (Figure 1). By day 14, fish mean weights ranged from 4.1 to 4.3 g, with no significant differences. On day 28 and day 42, fish fed the 100% PBM protein diet were significantly smaller (6.6 g and 9.9 g, respectively) than fish fed the control FM diet (7.9 g and 13.0 g, respectively). At the end of the experiment (day 56), fish weight in the 100% PBM protein diet was significantly lower (13.6 g) than in the control FM protein diet (17.8 g) (Table 2).

Body weight gain (BWG) in all treatment groups was at least 1,000% (Table 2). No significant differences in BWG were observed in fish fed the 40–90% PBM protein

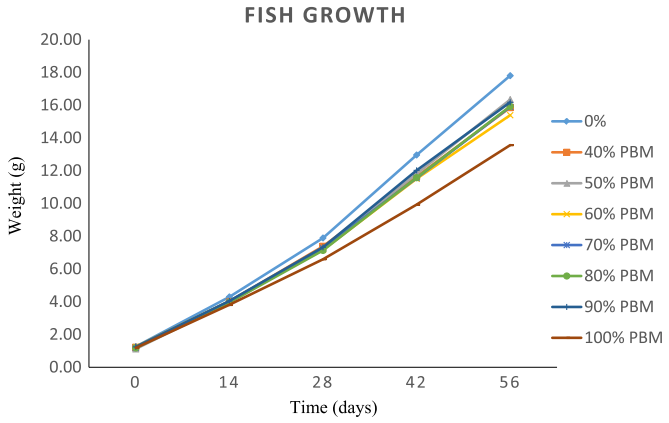

FIGURE 1. Growth (wet weight) of juvenile Black Sea Bass fed diets with increasing concentrations of fish meal protein replaced by poultry by-product meal (PBM) protein for 56 d. Plotted symbols represent means ($N = 3$). [Color figure can be viewed at wileyonlinelibrary.com]

diets (1,136–1,357%) compared with the control diet (1,307%). The BWG of fish fed the 100% PBM protein diet (1,045%) was significantly lower than in the control group. The BWG of fish fed the 90% PBM protein diet (1,196%) was not significantly different from the 100% PBM protein diet (1,045%) (Table 2). A second order polynomial regression analysis between BWG and percent FM replacement did not model the data properly ($Y = 0.0234x^2 + 1292.2$; $R^2 = 0.4345$). Therefore, the maximum replacement level of FM by PBM protein for Black Sea Bass juveniles was estimated by break point regression analysis (Figure 2). Based on BWG, the break point was estimated to be 81.8% FM replacement by PBM protein (Figure 2). Specific growth rate (SGR) of fish fed the PBM protein diets ranged from 2.30% to 2.59% per day, with no significant differences from the control FM protein diet (2.46%/d) (Table 2). Break point analysis using SGR showed the following relationships between SGR (Y) and percent FM replacement (X): $Y = 2.45$ when $X \leq 82.5$ and $Y = -0.0085X + 3.1517$ when $X \geq 82.5$; $R^2 = 0.9988$. Using SGR, the break point occurred at 82.5% FM replacement by PBM protein. Fish fed the 100% PBM protein diet had the lowest SGR (2.30%/d), significantly lower than the 50% PBM protein diet (2.59%/d).

Feed Utilization

No significant differences in feed intake were observed, which ranged from 0.26 to 0.30 g/fish/d (Table 2). Feed conversion ratios (FCRs) for fish fed the 60% PBM protein diet (1.17) and the 100% PBM protein diet (1.19) were significantly higher than fish fed the control FM protein diet (0.99) (Table 2). No significant differences were found among all other treatments (1.08–1.14) compared with the control. The FCR of fish fed the 90% PBM protein diet was not significantly different from fish fed the 100% PBM protein diet. Protein efficiency ratio (PER) was significantly lower for fish fed the 100% PBM protein diet (1.96) compared with fish fed the control FM protein diet (2.29) (Table 2). The PER was not significantly different for fish fed diets containing 40% to 90% PBM protein. The PER was not significantly different between fish fed the 90% and 100% PBM protein diets.

TABLE 2. Survival, growth, and feed utilization (mean ± standard error of the mean [SEM]; $N = 3$) of juvenile Black Sea Bass fed diets with increasing concentrations of fish meal protein replaced by poultry by-product meal (PBM) protein after 56 days. Growth and feed utilization parameters include percent body weight gain (BWG), specific growth rate (SGR), feed intake (FI), feed conversion ratio (FCR), and protein efficiency ratio (PER). Within columns, means with a common letter were not significantly different.

Diet (% PBM)[a]	Survival (%)	Final weight (g)	BWG[b] (%)	SGR[c] (%/d)	FI[d] (g/fish/d)	FCR[e]	PER[f]
0 (control)	98.3 z	17.80 ± 0.76 z	1,307 ± 62.4 zy	2.46 ± 0.05 zy	0.29 ± 0.007 z	0.99 ± 0.07 y	2.29 ± 0.16 z
40	96.7 z	15.85 ± 0.58 zy	1,199 ± 16.2 zyx	2.41 ± 0.03 zy	0.29 ± 0.015 z	1.11 ± 0.01 zy	2.08 ± 0.02 zy
50	98.3 z	16.34 ± 0.50 zy	1,357 ± 34.4 z	2.59 ± 0.02 z	0.29 ± 0.003 z	1.08 ± 0.02 zy	2.13 ± 0.03 zy
60	98.3 z	15.39 ± 0.45 yx	1,136 ± 61.6 yx	2.34 ± 0.08 y	0.29 ± 0.007 z	1.17 ± 0.02 z	2.01 ± 0.04 zy
70	98.3 z	15.90 ± 0.06 zy	1,231 ± 32.8 zyx	2.45 ± 0.05 zy	0.29 ± 0.007 z	1.09 ± 0.03 zy	2.13 ± 0.05 zy
80	100.0 z	15.93 ± 0.34 zy	1,250 ± 41.6 zy	2.47 ± 0.07 zy	0.29 ± 0.003 z	1.09 ± 0.02 zy	2.14 ± 0.05 zy
90	95.0 z	16.18 ± 0.12 zy	1,196 ± 11.5 zyx	2.39 ± 0.02 zy	0.30 ± 0.009 z	1.14 ± 0.02 zy	2.10 ± 0.03 zy
100	98.3 z	13.56 ± 0.45 x	1,045 ± 33.8 x	2.30 ± 0.03 y	0.26 ± 0.015 z	1.19 ± 0.03 z	1.96 ± 0.04 z

[a]Values represent percent of fish meal protein replaced by PBM protein.
[b]Body weight gain (%) = (final wet weight – initial wet weight)/initial wet weight × 100%.
[c]Specific growth rate = [ln (mean final weight) – ln (mean initial weight)]/56 d × 100.
[d]Feed intake was measured as grams per fish per day.
[e]Feed conversion ratio = total feed intake (g)/wet weight gain (g).
[f]Protein efficiency ratio = wet weight gain (g)/total protein intake dry weight (g) over the 56-d study.

Fish Whole Body Proximate Composition

Fish whole body moisture content ranged from 63.5% to 66.2% among treatments (Table 3). Whole body moisture content of fish fed diets with 50–80% PBM protein (65.6–65.8%) and 100% PBM protein (66.2%) was significantly higher than in fish fed the control FM diet (63.5%). Fish whole body ash content ranged from 4.49% to 6.37% (Table 3) among treatments. Whole body ash content of fish fed the 60–90% PBM protein diets (5.39–5.59%) was significantly greater than in fish fed the control FM protein diet (4.49%) but significantly lower than in fish fed the 100% PBM protein diet (6.37%). Whole body crude protein content of fish fed the PBM protein diets (15.6–16.6%) was not significantly different from fish fed the control FM protein diet (16.6%) (Table 3). There were no significant differences in fish whole body crude lipid content among the PBM protein treatment diets (10.5–11.6%) and the control FM protein diet (11.2%) (Table 3).

Muscle Tissue Proximate Composition

No significant treatment differences were observed in fish muscle moisture (75.5–76.4%), ash (1.29–1.34%), or crude protein (18.1–20.1%) content (Table 4). The muscle tissue crude lipid level of fish fed the 50% PBM protein diet (3.4%) was significantly higher than in fish fed the control FM protein diet (2.7%) (Table 4). Crude lipid content in all other treatment diets (2.6–3.0%) was not significantly different.

Fatty Acid Profile of the Diets and Whole Bodies

Fatty acid profile of the diets.— Total saturated fatty acids (SFAs) in the diets ranged from 25.9 to 32.4 mg/g, with only the 100% PBM protein diet (25.9 mg/g) significantly lower than the control FM protein diet (31.3 mg/g) (Table 5). Myristic acid (14:0) generally decreased as PBM protein increased, from 7.7 mg/g in the control FM diet to 2.5 mg/g in the 100% PBM protein diet. No significant differences were observed among treatments in dietary concentration of palmitic acid (16:0) (18.4–21.3 mg/g). Stearic acid (18:0) was significantly higher in diets with 70% and 80% PBM protein (5.1 and 5.0 mg/g, respectively) compared with the control FM protein diet (4.1 mg/g).

Monounsaturated fatty acid (MUFA) concentration ranged from 25.7 to 38.7 mg/g among treatment diets (Table 5). Diets with 40% and 60–100% PBM protein had significantly higher MUFA (33.5 mg/g and 32.0–38.7 mg/g, respectively) than the control FM protein diet (25.7 mg/g). Palmitoleic acid (16:1[n-7]) was significantly lower in diets with 50–100% PBM protein (7.0–9.2 mg/g) compared with the control FM protein diet (11.7 mg/g). Oleic acid (18:1[n-9]) concentration in diets containing PBM protein (17.6–26.9 mg/g) was significantly higher than in the control FM protein diet (9.5 mg/g). Total dietary concentrations of n-6 polyunsaturated fatty acids (PUFAs) (8.5–17.7 mg/g) were mainly related to concentrations of linoleic acid (18:2[n-6]) (6.8–16.9 mg/g) (Table 5). Both showed a similar trend toward increasing concentrations with increasing PBM protein in the diet.

The concentration of n-3 PUFAs in the diet clearly decreased with increasing PBM protein concentration, from 29.6 mg/g in the control FM protein diet to 8.3 mg/g in the 100% PBM protein diet (Table 5). Concentrations of stearidonic acid (18:4[n-3]) (0.9–3.1 mg/g), EPA (20:5[n-3]) (3.5–12.1 mg/g), and DHA (22:6[n-3]) (3.1–12.3 mg/g) (Table 5) also decreased with increasing PBM protein in the diets. The ratio of DHA to EPA in the diets was not significantly different up to 70% PBM protein (0.93–1.01) but was significantly lower in diets with 80–100% PBM protein (0.84–0.91) compared with the control

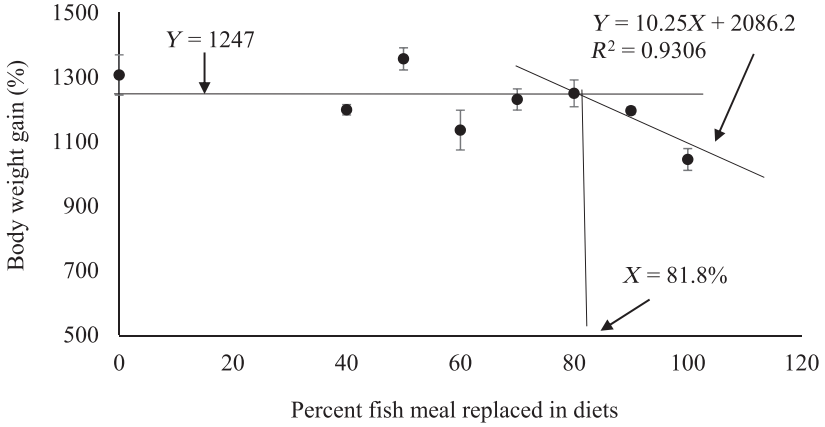

FIGURE 2. Broken-line regression analysis between body weight gain and percent fish meal replacement by poultry by-product meal in the diets of juvenile Black Sea Bass. Plotted symbols represent the mean ± SEM (N = 3).

FM protein diet (1.01) (Table 5). The ratio of n-3 to n-6 PUFAs in the diets decreased with increasing PBM protein from 3.5 mg/g in the control FM protein diet to 0.5 in the 100% PBM protein diet.

Fatty acid profile of the whole body.— Total whole body SFA concentration was significantly higher in fish fed the 50% PBM protein diet (77.0 mg/g) than in fish fed the control FM protein diet (53.9 mg/g) or the 40% PBM protein diet (55.1 mg/g) (Table 6), while no significant differences were observed among all other PBM protein treatments (49.2–64.5 mg/g). Myristic acid (14:0) was significantly lower in fish fed the 60–100% PBM protein diets (4.8–6.1 mg/g) than in fish fed the control FM protein diet (8.7 mg/g). Palmitic acid (16:0) was significantly higher in fish fed the 50% PBM protein diet (54.7 mg/g) than in fish fed the control FM protein diet (36.9 mg/g). No significant differences were observed among all other treatments (35.1–47.2 mg/g). Fish fed the 50% and 100% PBM protein diets had significantly higher stearic acid (18:0) levels (12.4 mg/g) than fish fed the control FM protein diet (8.3 mg/g).

Total MUFAs of the whole body were higher in fish fed the 50, 90, and 100% PBM protein diets (81.3, 73.6, 83.1 mg/g, respectively) compared with the control FM protein diet (46.5 mg/g) (Table 6). With the exception of the 50% PBM protein diet, total MUFAs in the whole body generally increased with increasing PBM protein in the diet. Palmitoleic acid (16:1[n-7]) was significantly higher in fish fed the 50% PBM protein diet (21.4 mg/g) than in fish fed the control FM protein diet (15.4 mg/g), while no significant difference was observed in all other PBM protein diet treatments (13.1–16.0 mg/g). Fish fed the 50% (52.2 mg/g) and 70–100% (41.5–61.7 mg/g) PBM protein diets showed significantly higher oleic acid (18:1[n-9]) concentrations than fish fed a control FM protein diet (24.7 mg/g). Oleic acid concentrations in fish whole body generally increased with increasing PBM protein in the diets. No significant differences were observed in gondoic acid (20:1[n-9]) among treatments (range = 1.0–1.7 mg/g).

Whole body concentration of n-6 PUFAs was significantly higher in fish fed 50% (25.5 mg/g) and 70–100% (19.1–31.6 mg/g) PBM protein compared with fish fed the control FM protein diet (8.9 mg/g), and this was primarily related to linoleic acid (18:2[n-6]) concentrations (Table 6). With the exception of the 50% PBM protein replacement diet, both total n-6 PUFAs and linoleic acid increased in

TABLE 3. Proximate composition (% wet basis; mean ± SEM; $N = 3$) of whole body tissue from juvenile Black Sea Bass fed diets with increasing concentrations of fish meal protein replaced by poultry by-product meal (PBM) protein after 56 d. Within columns, means with a common letter were not significantly different.

Diet (% PBM)[a]	Moisture (%)	Ash (%)	Protein (%)	Lipid (%)
0 (control)	63.5 ± 0.44 y	4.49 ± 0.16 x	16.6 ± 0.34 z	11.2 ± 0.18 zy
40	65.2 ± 0.62 zy	4.90 ± 0.07 x	16.6 ± 0.14 z	11.6 ± 0.06 z
50	65.6 ± 0.27 z	4.86 ± 0.04 x	15.8 ± 0.14 z	11.6 ± 0.23 z
60	66.1 ± 0.19 z	5.59 ± 0.11 y	16.6 ± 0.25 z	11.1 ± 0.04 zy
70	65.8 ± 0.47 z	5.39 ± 0.07 y	15.7 ± 0.24 z	11.3 ± 0.18 zy
80	65.8 ± 0.43 z	5.45 ± 0.13 y	15.6 ± 0.38 z	11.2 ± 0.21 zy
90	65.1 ± 0.33 zy	5.52 ± 0.07 y	16.4 ± 0.22 z	11.0 ± 0.18 zy
100	66.2 ± 0.31 z	6.37 ± 0.07 z	16.2 ± 0.34 z	10.5 ± 0.22 y

[a]Percent of fish meal protein replaced by PBM protein.

TABLE 4. Proximate composition (% wet basis; mean ± SEM; $N = 3$) of muscle tissue from juvenile Black Sea Bass fed diets with increasing concentrations of fish meal protein replaced by poultry by-product meal (PBM) protein after 56 d. Within columns, means with a common letter were not significantly different.

Diet (% PBM)[a]	Moisture (%)	Ash (%)	Protein (%)	Lipid (%)
0 (control)	76.4 ± 0.52 z	1.31 ± 0.04 z	19.1 ± 0.65 z	2.7 ± 0.04 y
40	76.2 ± 0.09 z	1.29 ± 0.03 z	19.3 ± 0.23 z	3.0 ± 0.12 zy
50	75.9 ± 0.24 z	1.32 ± 0.01 z	19.3 ± 0.17 z	3.4 ± 0.02 z
60	75.5 ± 0.51 z	1.30 ± 0.04 z	20.1 ± 0.19 z	2.6 ± 0.10 y
70	76.2 ± 0.31 z	1.34 ± 0.02 z	18.5 ± 0.29 z	2.6 ± 0.13 y
80	75.9 ± 0.31 z	1.31 ± 0.01 z	18.1 ± 0.26 z	2.8 ± 0.16 y
90	76.2 ± 0.02 z	1.31 ± 0.01 z	19.1 ± 0.80 z	2.8 ± 0.06 y
100	76.3 ± 0.31 z	1.33 ± 0.03 z	18.2 ± 0.35 z	2.7 ± 0.10 y

[a]Percent of fish meal protein replaced by PBM protein.

TABLE 5. Fatty acid profile (mg/g dry weight; mean ± SEM; $N = 3$) of diets with increasing concentrations of fish meal protein replaced by poultry by-product meal (PBM) protein for juvenile Black Sea Bass. Within rows, means with a common letter were not significantly different. Abbreviations are as follows: SFA = saturated fatty acid, MUFA = monounsaturated fatty acid, PUFA = polyunsaturated fatty acid, EPA = eicosapentaenoic acid, and DHA = docosahexaenoic acid. For fatty acid notation, the number to the left of the colon is the number of carbon atoms in the compound, the number immediately to the right of the colon is the number of double bonds, and the number after the hyphen indicates the position of the first double bond from the methyl end.

Fatty acid	0% (control)	40% PBM	50% PBM	60% PBM	70% PBM	80% PBM	90% PBM	100% PBM
14:0	7.7 ± 0.39 z	6.0 ± 0.24 y	4.8 ± 0.11 x	4.3 ± 0.09 xw	4.3 ± 0.18 xw	3.5 ± 0.22 wv	3.7 ± 0.21 xw	2.5 ± 0.02 v
16:0	19.6 ± 0.87 z	21.3 ± 0.68 z	18.4 ± 0.19 z	19.1 ± 0.32 z	20.4 ± 0.75 z	19.3 ± 0.99 z	20.2 ± 0.76 z	18.4 ± 0.54 z
16:1(n-7)	11.7 ± 0.57 z	10.8 ± 0.41 zy	9.0 ± 0.16 x	8.8 ± 0.16 x	9.2 ± 0.38 zy	8.2 ± 0.47 xw	9.1 ± 0.27 x	7.0 ± 0.07 w
18:0	4.1 ± 0.16 y	5.0 ± 0.13 zy	4.5 ± 0.02 zy	4.8 ± 0.07 zy	5.1 ± 0.16 z	5.0 ± 0.22 z	5.0 ± 0.39 zy	4.9 ± 0.21 zy
18:1(n-9)	9.5 ± 0.42 w	18.9 ± 0.78 x	17.6 ± 0.11 x	20.1 ± 0.31 x	23.3 ± 0.81 y	23.4 ± 1.13 y	26.9 ± 0.16 z	24.6 ± 0.77 zy
18:1(n-11)	3.4 ± 0.15 z	2.8 ± 0.15 y	2.5 ± 0.05 yx	2.4 ± 0.04 x	2.3 ± 0.09 x	2.2 ± 0.08 xw	2.0 ± 0.02 xw	1.8 ± 0.08 w
18:2(n-6)	6.8 ± 0.33 w	12.4 ± 0.47 x	11.6 ± 0.16 x	12.8 ± 0.18 yx	14.8 ± 0.55 zy	14.6 ± 0.71 y	16.9 ± 0.30 z	14.7 ± 0.40 y
18:4(n-3)	3.1 ± 0.137 z	2.5 ± 0.100 y	2.0 ± 0.044 x	1.8 ± 0.033 xw	1.8 ± 0.035 xw	1.4 ± 0.083 w	1.5 ± 0.122 w	0.9 ± 0.003 v
20:1(n-9)	1.1 ± 0.05 z	1.0 ± 0.03 z	0.8 ± 0.01 y	0.8 ± 0.01 y	0.8 ± 0.02 y	0.7 ± 0.03 yx	0.7 ± 0.05 x	0.6 ± 0.02 x
20:2(n-6)	1.7 ± 0.08 z	1.3 ± 0.04 y	1.1 ± 0.02 x	1.0 ± 0.01 xw	1.0 ± 0.03 xw	0.8 ± 0.04 wv	0.8 ± 0.02 v	0.6 ± 0.01 u
20:5(n-3) (EPA)	12.1 ± 0.58 z	9.2 ± 0.33 y	7.4 ± 0.14 x	6.7 ± 0.11 xw	6.5 ± 0.22 xw	5.3 ± 0.29 w	5.3 ± 0.24 w	3.5 ± 0.05 v
22:5(n-3)	2.1 ± 0.09 z	1.8 ± 0.17 z	1.4 ± 0.01 y	1.3 ± 0.02 yx	1.2 ± 0.03 yx	1.0 ± 0.05 yxw	1.0 ± 0.02 xw	0.8 ± 0.02 w
22:6(n-3) (DHA)	12.3 ± 0.57 z	9.0 ± 0.37 y	7.0 ± 0.07 x	6.3 ± 0.09 x	6.0 ± 0.17 xw	4.8 ± 0.24 wv	4.4 ± 0.05 v	3.1 ± 0.08 u
∑SFA	31.3 ± 1.41 z	32.4 ± 1.05 z	27.6 ± 0.31 zy	28.2 ± 0.48 zy	29.8 ± 1.08 zy	27.8 ± 1.44 zy	28.9 ± 0.94 zy	25.9 ± 0.73 y
∑MUFA	25.7 ± 1.18 w	33.5 ± 1.17 yx	29.9 ± 0.30 xw	32.0 ± 0.52 yx	35.7 ± 1.28 zy	34.5 ± 1.70 zyx	38.7 ± 0.27 z	34.0 ± 0.92 zyx
∑n-3 PUFA	29.6 ± 1.35 z	22.6 ± 0.93 y	17.7 ± 0.27 x	16.0 ± 0.25 x	15.5 ± 0.44 xw	12.5 ± 0.66 wv	12.1 ± 0.30 v	8.3 ± 0.15 u
∑n-6 PUFA	8.5 ± 0.40 w	13.7 ± 0.51 yx	12.7 ± 0.17 x	13.8 ± 0.20 yx	15.9 ± 0.58 zy	15.4 ± 0.75 y	17.7 ± 0.32 z	15.3 ± 0.41 y
n-3/n-6 PUFA	3.5 ± 0.005 z	1.6 ± 0.007 y	1.4 ± 0.005 x	1.2 ± 0.009 w	1.0 ± 0.009 v	0.8 ± 0.007 u	0.7 ± 0.029 t	0.5 ± 0.009 s
DHA/ EPA	1.01 ± 0.007 z	0.98 ± 0.016 zy	0.95 ± 0.009 zyx	0.95 ± 0.004 zyx	0.93 ± 0.008 zyxw	0.91 ± 0.006 yxw	0.84 ± 0.044 w	0.88 ± 0.010 xw

TABLE 6. Fatty acid profile (mg/g dry weight; mean ± SEM; $N = 3$) of whole body tissue from juvenile Black Sea Bass fed diets with increasing concentrations of fish meal protein replaced by poultry by-product meal (PBM) protein after 56 d. Within rows, means with a common letter were not significantly different. For abbreviations and fatty acid notation see Table 5.

Fatty acid	0% (control)	40% PBM	50% PBM	60% PBM	70% PBM	80% PBM	90% PBM	100% PBM
14:0	8.7 ± 0.50 zy	7.6 ± 0.39 yx	9.9 ± 0.17 z	5.8 ± 0.09 xw	6.0 ± 0.57 xw	6.1 ± 0.52 xw	5.5 ± 0.70 xw	4.8 ± 0.33 w
16:0	36.9 ± 1.99 y	38.9 ± 1.03 y	54.7 ± 3.29 z	35.1 ± 0.33 y	37.7 ± 2.20 y	40.9 ± 3.25 zy	43.8 ± 5.33 zy	47.2 ± 2.01 zy
16:1(n-7)	15.4 ± 1.09 y	15.4 ± 0.58 y	21.4 ± 0.62 z	13.1 ± 0.12 y	14.4 ± 1.32 y	15.3 ± 1.39 y	15.1 ± 1.87 y	16.0 ± 0.68 y
18:0	8.3 ± 0.44 x	8.6 ± 0.31 x	12.4 ± 1.05 zy	8.3 ± 0.18 x	9.0 ± 0.36 yx	9.6 ± 0.66 zyx	11.0 ± 1.28 zyx	12.4 ± 0.49 z
18:1(n-9)	24.7 ± 1.74 w	34.0 ± 0.87 xw	52.2 ± 3.36 zy	35.5 ± 0.44 xw	41.5 ± 2.45 yx	46.5 ± 3.91 zyx	53.5 ± 6.42 zy	61.7 ± 2.23 z
18:1(n-11)	5.0 ± 0.28 zy	4.4 ± 0.16 zy	5.9 ± 0.50 z	3.5 ± 0.07 y	3.7 ± 0.37 y	3.7 ± 0.23 y	3.7 ± 0.47 y	4.0 ± 0.12 y
18:2(n-6)	7.3 ± 0.91 v	11.1 ± 0.36 wv	23.1 ± 1.39 zy	13.0 ± 0.06 xwv	17.8 ± 1.86 yxw	20.8 ± 2.31 yx	25.2 ± 2.93 zy	29.9 ± 0.44 z
18:4(n-3)	2.3 ± 0.50 zy	1.3 ± 0.12 y	3.4 ± 0.11 z	1.1 ± 0.03 y	1.7 ± 0.32 zy	1.8 ± 0.25 zy	1.7 ± 0.19 zy	2.3 ± 0.70 zy
20:1(n-9)	1.4 ± 0.11 zy	1.2 ± 0.02 zy	1.7 ± 0.16 z	1.0 ± 0.02 y	1.1 ± 0.06 y	1.2 ± 0.09 y	1.4 ± 0.16 zy	1.5 ± 0.04 zy
20:2(n-6)	1.6 ± 0.37 zy	0.9 ± 0.04 y	2.4 ± 0.10 z	0.8 ± 0.02 y	1.3 ± 0.18 y	1.4 ± 0.18 y	1.5 ± 0.19 y	1.6 ± 0.12 zy
20:5(n-3) (EPA)	8.1 ± 2.51 zy	3.3 ± 0.17 y	12.1 ± 0.41 z	2.9 ± 0.07 y	5.6 ± 1.25 y	5.8 ± 1.07 y	6.1 ± 0.71 y	5.8 ± 0.27 y
22:5(n-3)	2.2 ± 0.66 zy	0.8 ± 0.06 yx	3.0 ± 0.22 z	0.7 ± 0.01 x	1.4 ± 0.27 yx	1.5 ± 0.27 yx	1.7 ± 0.19 zyx	2.0 ± 0.09 zyx
22:6(n-3) (DHA)	8.3 ± 2.98 zy	2.6 ± 0.11 y	12.6 ± 0.91 z	2.3 ± 0.05 y	5.0 ± 1.21 y	5.3 ± 1.11 y	6.0 ± 0.65 y	5.6 ± 0.23 y
∑SFA	53.9 ± 2.94 y	55.1 ± 1.44 y	77.0 ± 4.44 z	49.2 ± 0.41 y	52.7 ± 3.14 y	56.5 ± 4.42 y	60.2 ± 7.32 zy	64.5 ± 2.73 zy
∑MUFA	46.5 ± 3.21 x	55.0 ± 1.33 yx	81.3 ± 4.57 z	53.1 ± 0.36 yx	60.7 ± 4.19 zyx	66.7 ± 5.58 zyx	73.6 ± 8.92 zy	83.1 ± 2.99 z
∑n-3 PUFA	20.9 ± 6.66 zy	8.0 ± 0.41 y	31.0 ± 1.64 z	7.0 ± 0.12 y	13.7 ± 3.04 y	14.4 ± 2.69 y	15.6 ± 1.75 y	15.7 ± 1.27 y
∑n-6 PUFA	8.9 ± 1.28 v	12.0 ± 0.40 wv	25.5 ± 1.49 zy	13.8 ± 0.04 xwv	19.1 ± 2.04 yxw	22.2 ± 2.49 yx	26.7 ± 3.12 zy	31.6 ± 0.40 z
n-3/n-6 PUFA	2.3 ± 0.373 z	0.7 ± 0.012 yx	1.2 ± 0.007 y	0.5 ± 0.008 x	0.7 ± 0.077 yx	0.6 ± 0.056 yx	0.6 ± 0.005 yx	0.5 ± 0.040 x
DHA/ EPA	0.99 ± 0.05 zy	0.78 ± 0.02 x	1.04 ± 0.05 z	0.80 ± 0.01 x	0.89 ± 0.01 yx	0.90 ± 0.04 zyx	0.99 ± 0.01 zy	0.96 ± 0.01 zy

the fish whole body with increasing PBM protein in the diets (Table 6). Concentration of n-3 PUFAs in the whole body (7.0–31.0 mg/g) were not significantly different among treatments, except in fish fed 50% PBM protein (31.0 mg/g), which was higher than in fish fed the other diets with PBM protein (7.0–15.7 mg/g) (Table 6). This pattern was also reflected in the whole body concentrations of constituent n-3 PUFAs stearidonic acid (1.1–3.4 mg/g), EPA (2.9–12.1 mg/g), and DHA (2.3–12.6 mg/g), which were not significantly different among treatments, except for higher levels in fish fed the 50% PBM protein diet (Table 6). The ratio of n-3 to n-6 PUFAs in the whole body was significantly lower in fish fed all of the PBM protein diet treatments (0.5–1.2) than in fish fed the control FM protein diet (2.3) (Table 6).

Digestibility of diets.— The ADC of protein in the diet with 70% PBM protein (81.6%) was lower than in the 60% PBM protein diet (85.9%) and in the control FM protein diets (87.0%) but was not significantly different from the other treatment groups (83.0–84.2%) (Table 7). Lipid ADC ranged from 88.2% to 92.3% among treatments, with no significant differences (Table 7).

TABLE 7. Apparent digestibility coefficients (%; mean ± SEM; $N = 3$) of diets with increasing concentrations of fish meal protein replaced by poultry by-product meal (PBM) protein for juvenile Black Sea Bass. Within columns, means with a common letter were not significantly different.

Diet (% PBM)[a]	Protein (%)	Lipid (%)
0 (control)	87.0 ± 0.92 z	92.3 ± 0.91 z
40	84.2 ± 0.92 zy	91.1 ± 0.66 z
50	83.2 ± 0.45 zy	88.2 ± 1.16 z
60	85.9 ± 0.72 z	89.1 ± 0.50 z
70	81.6 ± 0.75 y	89.4 ± 0.83 z
80	83.0 ± 0.92 zy	89.6 ± 0.50 z
90	83.7 ± 0.17 zy	90.7 ± 0.64 z
100	83.2 ± 1.37 zy	90.2 ± 1.37 z

[a]Percent of fish meal protein replaced by PBM protein.

DISCUSSION

Survival and Growth

Survival of juvenile Black Sea Bass in all diet treatments was excellent throughout the experiment with little or no mortality. This is similar to what has been reported in other carnivorous marine finfish species, such as Humpback Grouper (Shapawi et al. 2007), Cobia *Rachycentron canadum* (Zhou et al. 2011), Greater Amberjack (Takakuwa et al. 2006), Black Sea Turbot (Yigit et al. 2006), and Florida Pompano *Trachinotus carolinus* (Rossi and Davis 2012), which showed high survival when fed diets with FM protein replaced by PBM protein at levels of 20–100%.

The overall growth of the fish was satisfactory for fish reared for 56 d (initial weight = 1.2 g, final weight = 13.9–17.8 g) under all diet treatments. Growth performance was comparable to what was observed in previous studies of juvenile Black Sea Bass of similar size fed formulated diets over similar durations (Alam et al. 2008, 2012; Watanabe 2011). In this study, juvenile Black Sea Bass fed diets with 40–90% PBM protein showed equivalent growth to those fed the control 100% FM protein diet over a 56-d study period. However, growth was significantly lower in fish fed the 100% PBM protein diet than those fed the control FM protein diet, and regression analysis showed a break point at 81.8% of FM replacement when BWG was plotted against percent FM replacement. Shapawi et al. (2007) also reported no significant differences in growth of Humpback Grouper fingerlings fed 50% or 75% feed-grade PBM protein diets but found reduced growth in fingerlings fed a 100% feed-grade PBM protein diet.

Compared to marine species, freshwater finfish show better growth performance when fed diets completely replacing FM protein with PBM protein. For example, Nile Tilapia *Oreochromis niloticus* (El-Sayed 1998) and sunshine bass (female White Bass *Morone chrysops* × male Striped Bass *M. saxatilis*) (Webster et al. 1999, 2000) fed diets completely replacing FM protein with PBM protein showed no reduction in growth performance. On the other hand, the dietary FM protein level for the marine Japanese Sea Bass *Lateolabrax japonicus* can only be reduced to 80% if PBM protein is used as a substitute (Wang et al. 2015). For the marine finfish Totoaba *Totoaba macdonaldi*, PBM protein was a good source of nutrients in juvenile diets at a FM protein replacement level of 67%, whereas fish fed a 100% PBM protein diet showed the slowest growth and highest mortality (Zapata et al. 2016). In the marine Florida Pompano, growth performance was unaffected when PBM protein replaced 67% of the FM protein in the diet (Riche 2015); however, growth performance was reduced when PBM protein replaced 100% of the FM protein (Rossi and Davis 2012). Riche (2015) suggested that methionine was first limiting or colimiting with lysine when PBM supplied all the dietary protein in Florida Pompano. Although amino acid composition of the treatment diets was not analyzed in the present study, the reduction in growth of fish fed diets replacing 100% FM with PBM may have been related to a deficiency of some essential amino acids. Based on the broken-line regression analysis using BWG data, PBM protein can replace up to 81.8% of FM protein in the diet of juvenile Black Sea Bass without adverse effects. These levels of FM replacement using a feed-grade PBM is similar or slightly higher to those typically seen in other marine finfish (67–80%) and suggests that Black Sea Bass have an ability to digest and assimilate high levels of PBM protein.

Feed Utilization

Feed intake in juvenile Black Sea Bass did not differ significantly among treatments, suggesting that palatability was not affected by substitution of PBM protein for FM protein in the treatment diets. The FCR and PER in fish fed diets with up to 90% PBM protein were also not significantly different from fish fed a control 100% FM protein diet. In contrast, fish fed a 100% PBM protein diet showed increased FCR and decreased PER values consistent with decreased growth found in the 100% PBM protein treatment. Poor feed utilization was also observed in Humpback Grouper fed a 100% PBM protein diet (Shapawi et al. 2007). In Black Sea Turbot, lower feed intake and FCR of fish fed 100% PBM protein replacement diets were reported (Yigit et al. 2006). In contrast to these findings, feed utilization was not affected by complete replacement of FM protein by PBM protein in African Catfish *Clarias gariepinus* (Abdel-Warith et al. 2001) or sunshine bass (Webster et al. 1999). The quality of PBM used to replace FM can influence feed utilization and growth of finfish and, hence, the maximum level of substitution resulting in no adverse effects on fish performance (Dong et al. 1993; Bureau et al. 1999).

Whole Body Proximate Composition

Whole body moisture content was significantly higher in Black Sea Bass fed diets with 50–80% and 100% PBM protein. In contrast, Takakuwa et al. (2006) noted lower whole body moisture content in Greater Amberjack fed diets replacing 60% of the FM protein with PBM protein.

In the present study, whole body ash content increased with increasing levels of PBM protein in the diet. This was likely related to high ash (mineral) content in PBM, which often contains bones, heads, and feet and may indicate that Black Sea Bass have a lower digestibility of ash from PBM compared with ash from FM. Similar results were observed in Humpback Grouper fed diets with 75% and 100% PBM protein, which showed higher whole body ash contents compared with the fish fed control diets with high FM (Shapawi et al. 2007). In African Catfish (Abdel-Warith et al. 2001) and Nile Tilapia (El-Sayed 1998) diets, complete replacement of FM protein with PBM protein also produced high whole body ash content.

No significant differences were seen in whole body protein content of juvenile Black Sea Bass in this study, suggesting that assimilation of PBM protein by these fish was comparable to FM protein. Greater Amberjack fed diets replacing 20–60% of FM protein with PBM protein with or without supplemental amino acids likewise showed no differences in whole body protein (Takakuwa et al. 2006). Juvenile Cobia fed diets replacing 15–60% FM protein with pet-food-grade PBM protein (Zhou et al. 2011) and Humpback Grouper fingerlings fed diets with 75% and 100% of a pet-food-grade PBM protein (Shapawi et al. 2007) produced whole body protein content similar to a control FM protein diet. Gilthead Seabream fed diets with 100% poultry meat meal protein (Nengas et al. 1999) also showed no difference in whole body protein from fish fed a control FM protein diet. However, a diet containing 75% of a locally available PBM protein produced a decrease in whole body protein content in this species and showed a low protein digestibility (Nengas et al. 1999). These results emphasize the importance of understanding the quality of PBM and its influence on digestibility and on fish proximate composition.

Whole body lipid content was not affected by the replacement of FM protein by PBM protein up to a level of 100%. Dietary lipid levels were only slightly different between PBM protein diets and the control FM protein diet, but very similar whole body lipid levels among treatments suggest that the lipid in PBM was utilized as efficiently as the lipid in FM. This is similar to other marine finfish, including Greater Amberjack (Takakuwa et al. 2006) and Cobia (Zhou et al. 2011) fed diets with up to 60% PBM protein and Humpback Grouper (Shapawi et al. 2007) fed 75% and 100% PBM protein, for which whole body lipid levels were not affected by PBM protein substitution for FM protein in the diet. Among freshwater finfish, Yang et al. (2006) reported no difference in whole body lipid content of Gibel Carp *Carassius auratus gibelio* fed up to 100% PBM protein. No difference in whole body lipid content was reported in African Catfish (Abdel-Warith et al. 2001), Nile Tilapia (El-Sayed 1998), or sunshine bass (Webster et al. 1999) fed diets replacing 100% of FM protein with PBM protein.

Muscle Tissue Proximate Composition

Moisture, ash, and protein content of the muscle tissue showed no significant differences among fish fed the different diet treatments. These results differ from the trend observed in whole body tissues toward higher ash levels with increasing PBM protein in the diet, indicating that inorganic material from PBM was concentrated in either bone tissue or intestines, rather than in muscle tissue. Yigit et al. (2006) also reported no differences in moisture, ash, or protein content for muscle tissue in Black Sea Turbot fed diets replacing up to 50% of the FM protein with PBM protein. In contrast, replacement of FM protein by PBM protein at levels of 75% and 100% in the diet of Black Sea Turbot caused an increase in muscle moisture and ash content and a decrease in protein content (Yigit et al. 2006). Juvenile Black Sea Bass fed the 50% PBM protein replacement diet had significantly higher muscle lipid levels than the control 100% FM protein diet, while fish fed diets with 40% and 60–100% PBM protein showed no differences in muscle lipid. A possible reason may be the inadvertent selection of bigger fish for lipid analysis in that particular diet treatment.

Black Sea Turbot fed diets with 25–100% PBM protein showed no significant differences in muscle tissue lipid content (Yigit et al. 2006).

Fatty Acid Profile of the Diets and Whole Body

Although total lipid content of the whole body was not significantly different between fish fed PBM protein and FM protein, the composition of fatty acids in the whole body reflected dietary levels of the terrestrial animal and marine protein sources used. Oleic acid (18:1[n-9]) levels increased with increasing PBM protein in the diet, causing a corresponding increase in total MUFA concentrations with increasing PBM protein. Clearly, PBM contains higher MUFAs than FM, and high levels of dietary PBM produced high amounts of MUFAs in the whole body of juvenile Black Sea Bass. This same trend was observed in linoleic acid (18:2[n-6]) and the sum of n-6 PUFAs in the whole body of juvenile Black Sea Bass. Similarly, juvenile Coho Salmon *Oncorhynchus kisutch* fed a diet completely replacing FM protein with PBM protein contained elevated oleic acid, total MUFA, linoleic acid, and total n-6 PUFA levels (Twibell et al. 2012). Increased oleic and linoleic acid levels were also reported in Atlantic Salmon *S. salar* fed diets partially replacing fish oil with poultry fat (Higgs et al. 2006).

In the present study, fish fed the 100% PBM diet showed the lowest growth performance, and this may also be due in part to the relatively low dietary levels of essential fatty acids (Table 5), particularly the long-chain n-3 PUFAs, 20:5(n-3) EPA, and 22:5(n-3) DHA. The lipids present in the poultry meal are generally rich in MUFAs (particularly oleic acid) and total n-6 PUFAs but are low in n-3 PUFAs, EPA, and DHA (Higgs et al. 2006). The PBM protein can be included up to a level of 44% in diets for juvenile Rainbow Trout without a decrease in EPA and DHA in whole body tissues (Pares-Sierra et al. 2014).

Given the trend toward lower dietary n-3 PUFAs with increasing incorporation of PBM protein, the comparable levels of n-3 PUFAs in whole body tissues among all diet treatments are noteworthy and may suggest that dietary n-3 PUFA requirements were met under all diet treatments. This supports the idea that growth inhibition in the 100% PBM protein diet may have been due to an amino acid deficiency. Fish fed the 50% PBM protein diets showed much higher whole body EPA, DHA, and n-3 PUFAs than the fish fed the others diets, but this was possibly due to the inadvertent selection of bigger fish for fatty acid analysis in that diet treatment.

The PBM used in the present study had a higher lipid content than the FM, so less fish oil was added as PBM was increased in the diets to maintain the diets isolipidic. Hence, the diet replacing 100% FM protein with PBM protein contained 1.2% less fish oil than the high FM-based control diet. Since PBM is low in n-3 PUFAs, the substitution of PBM protein for FM protein and the incremental reduction of fish oil reduced EPA and DHA levels in the diets. However, growth performance was not impaired up to a substitution level of 90% PBM protein. The EPA and DHA levels for the diets replacing up to 90% FM protein with PBM protein met or exceeded the minimum recommended dietary levels for other marine fish, such as Red Seabream *Pagrus major* and Yellowtail *Seriola quinqueradiata* (Sargent et al. 2002). Juvenile Coho Salmon fed diets using PBM protein to replace FM protein showed lower EPA, DHA, and total n-3 PUFA concentrations as well as a lower n-3 to n-6 PUFA ratio (Twibell et al. 2012). A significantly lower n-3 to n-6 PUFA ratio in the whole body of Black Sea Bass fed PBM-based diets compared with the fish fed control FM protein diet was also observed in this study (Table 6). Replacing FM protein with PBM protein in the diets of Black Sea Bass also did not affect the ratio of DHA to EPA (0.84–1.01) found in the whole body, a level which was above the dietary requirement for Yellowtail (0.5) (Sargent et al. 2002). Wu et al. (2002) reported that the growth of Malabar Grouper *Epinephelus malabaricus* was enhanced when the DHA to EPA ratio in the diet was greater than 1.0. No recommended level of EPA or DHA in the diet for Black Sea Bass has been published. However, based on the EPA and DHA levels in the diets in this study and the reported requirements for other marine species, sufficient EPA and DHA were provided in all the diets replacing FM protein with PBM protein.

Apparent Digestibility Coefficient of Dietary Protein and Lipid

In the present study, the ADC of protein ranged from 82% to 84%, which is similar to values reported for other species such as Rainbow Trout (81–91%) fed poultry-meal-based diets (Bureau et al. 1999; Sugiura et al. 2000; Cheng and Hardy 2002; Cheng et al. 2004; Gaylord et al. 2008). However, low and variable protein digestibility has been reported by other workers in Rainbow Trout (64–78%) (Dong et al. 1993). Raw materials high in bone tissues result in PBMs with high ash content, which is associated with low protein digestibility (Bureau et al. 1999; Sugiura et al. 2000). The relatively high ADC of protein in the present study could be due to the use of low-ash and high-quality (66% protein) PBM in the diets.

Except for a slightly lower ADC of protein in the 70% PBM diet, the ADC of protein for all treatments suggests that PBM protein is as digestible as FM protein and thus well assimilated by Black Sea Bass. No significant differences were reported in protein ADC of Greater Amberjack fed diets with 60% of the FM protein replaced by PBM protein with and without supplemented amino acids (Takakuwa et al. 2006); however, protein ADC values

were lower than those in the present study indicating that Black Sea Bass are able to digest PBM protein more efficiently than Greater Amberjack. Total replacement of FM protein with PBM protein was achieved in hybrid Striped Bass (female Striped Bass × male White Bass) using available (rather than actual) dietary amino acid concentrations (Gaylord and Rawles 2005). In the present study, growth was significantly lower in Black Sea Bass fed 100% PBM protein, possibly due to a deficiency in available concentrations of some essential amino acids in this diet. Further studies are needed on amino acid availability and amino acid digestibility in PBM-based diets in Black Sea Bass.

Lipid ADC was uniformly high among treatments, suggesting that the lipid in PBM was as digestible by Black Sea Bass as the lipid in FM. This is in accord with what was found in Greater Amberjack fed diets with up to 40% PBM protein with and without additional amino acids (Takakuwa et al. 2006) and in Humpback Grouper fed 75% and 100% feed-grade PBM protein (Shapawi et al. 2007) in which lipid ADC was not different from the control FM diet.

CONCLUSION

The results demonstrated that FM protein can be replaced by feed-grade PBM protein in juvenile Black Sea Bass diets at levels as high as 81.8% without adversely affecting survival, growth, feed utilization, fish biochemical composition, or ADC of protein or lipid. Poultry by-product meal is a highly effective protein source for alternative protein-based feed formulation for Black Sea Bass.

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