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Effects of Chicken By-Product Meal as a Fish Meal Replacer in Diets With or Without Jack Mackerel Meal Inclusion: Growth and Feed Availability for Rockfish (*Sebastes schlegeli*)

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Abstract: The current experiment is conducted to determine the effects of chicken by-product meal (CBM) as a fish meal (FM) replacer in diets, with or without the inclusion of jack mackerel (*Trachurus japonicus*) meal (JMM), on the growth and feed availability of rockfish (*Sebastes schlegeli*). A two-way ANOVA experimental design [FM replacement level (FMRL, 25% and 50%) × JMM inclusion (without and with)] was adopted. The control (Con) diet contained 55% FM. In the Con diet, 25% and 50% of FM were substituted with CBM, either without or with the inclusion of 22% JMM, referred to as the CBM25, CBM50, CBM25J, and CBM50J diets, respectively. A total of 375 juvenile fish were distributed into 15 tanks with triplicates. The 25% FM replacement diets led to significantly ($p < 0.003$ and $p < 0.004$, respectively) greater weight gain and specific growth rate (SGR) of rockfish than the 50% FM replacement diets. Furthermore, the CBM-replaced diets with JMM inclusion led to significantly ($p < 0.003$ and $p < 0.002$, respectively) greater weight gain and SGR of fish compared with those without JMM inclusion. Weight gain and SGR of rockfish fed the Con and CBM25J diets were significantly ($p < 0.001$ for both) greater than those of fish fed the CBM25, CBM50, and CBM50J diets. However, neither dietary FMRL nor JMM inclusion had significant ($p > 0.05$) effects on feed consumption, blood chemistry, or the biochemical composition of rockfish. In conclusion, 25% of FM could be replaced with CBM in the 55% FM-based diet, including JMM, without adverse effects on the growth, feed availability, blood chemistry, or biochemical composition (proximate composition and amino and fatty acid profiles) of rockfish.

Keywords: *Sebastes schlegeli*; fish meal replacement level; jack mackerel meal; feed attractant; biochemical composition

Key Contribution: To address the challenges of sluggish fish meal (FM) production and rising feed costs, feed nutritionists are seeking cost-effective and reliable alternatives, such as chicken by-product meal (CBM), to replace FM. However, low-FM diets often lower palatability, reduce feed consumption and ultimately impair growth performance. Jack mackerel meal (JMM) has shown the strongest attractiveness to rockfish among several protein feed ingredients. In this study, the 25% FM replacement diets led to greater growth performance of rockfish compared to the 50% FM replacement diets. The CBM-replaced diets with JMM inclusion also led to greater growth performance of rockfish compared to those without JMM inclusion. Up to 25% of FM could be substituted with CBM in diets with JMM inclusion without negatively affecting the growth performance and feed availability of rockfish.

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1. Introduction

Seafood, including fish and shellfish, is a significant food source for people worldwide as it is rich in protein, n-3 highly unsaturated fatty acids (HUFAs), vitamins, and minerals [1]. To meet the growing demand and ensure sustainable aquaculture, it is crucial to develop and fortify feeds to increase their nutrient content [2]. Carnivorous fish species, including rockfish (*S. schlegeli*) and red sea bream (*Pagrus major*) require ca. 50% protein content in their feeds [3,4]. Fish meal (FM) is an outstanding source of essential amino acids (EAAs), essential FAs (EFAs), nucleotides, vitamins, and minerals, which is why FM is commonly used as the main protein source in commercial fish feeds [5]. Nonetheless, as the aquaculture industry has flourished, the catch of fish used for FM sources has declined [6], highlighting the need to search for a FM substitute in aquafeeds for sustainable aquaculture.

Terrestrial animal proteins, such as chicken by-product meal (CBM) [7,8], meat and bone meal [9], and porcine blood meal [10] have been extensively researched as replacers for FM in fish feeds because of their high protein content, reasonable price, and absence of anti-nutritional factors [11]. In particular, poultry consumed in the Republic of Korea (*hereafter*, Korea) mainly includes chicken and duck, with chicken accounting for ca. 96.2% of the poultry slaughtered [12]. By-products from chicken processing plants include bones, heads, legs, and organs, accounting for ca. 30% of the total production [13,14]. Inadequate disposal of these uneaten by-products can result in environmental pollution, the spread of diseases, and the loss of valuable biological resources, including proteins, lipids, and enzymes [14].

CBM, a type of poultry by-product meal (PBM), is produced exclusively in chicken processing plants in Korea and is commercially available. Furthermore, CBM contains high crude proteins (65.9–67.0%) and lipids (11.3–14.5%) with year-round availability. Since CBM (USD 0.72/kg, USD 1 = KRW 1300) is more affordable than FM (USD 2.00/kg), it is considered a potential FM replacer in aquafeeds [7,15]. The substitutability of CBM, chicken intestine, and chicken waste meal for FM has been demonstrated in the diets of abalone (*Haliotis discus hannai*) [15] and olive flounder (*Paralichthys olivaceus*) [7], grass carp (*Ctenopharyngodon idella*) [16], and Asian seabass (*Lates calcarifer*) [17]. Specifically, up to 50% and 28.6% of FM could be substituted with CBM and chicken intestine in the 65% and 35% FM-basal feeds of olive flounder and Asian seabass, respectively, without adverse impacts on weight gain and feed consumption [7,17]. Recently, Li and Cho [8] have highlighted that up to 20% of FM could be substituted with CBM in the rockfish feed without compromising growth, feed consumption, and feed utilization when juvenile rockfish grown from 2.5 g to 12.5 g are fed a 55% FM-basal diet or a diet substituting 10%, 20%, 30%, 40%, and 50% FM with CBM.

In general, however, FM replacement with a substitute in diets commonly leads to decreased palatability and reduced feed intake by fish, ultimately resulting in deteriorated growth [18,19]. Thus, manipulation of feed stimulants or feed ingredients showing strong attractiveness to a target fish species on the low-FM diet can improve palatability and increase feed consumption by fish [20,21]. In particular, histidine, inosine monophosphate (IMP), the blend of IMP, L-histidine, and L-glutamic acid, and each of IMP, lactic acid, and glutamic acid in the muscle extracts of jack mackerel (*Trachurus japonicus*) play a significant role in eliciting stimulatory responses in olive flounder, greater amberjack (*Seriola dumerili*), Pacific bluefin tuna (*Thunnus orientalis*), and yellowtail (*Seriola quinqueradiata*), respectively [22–25]. Furthermore, jack mackerel meal (JMM) exhibited the strongest attractiveness to rockfish among 16 protein feed ingredients [26], and the optimum inclusion level of JMM was estimated to be 22% in the 55% FM-basal diet of juvenile rockfish [27]. Therefore, incorporating an optimal level (22%) of JMM into the low-FM diet can contribute positively to the sustainable advancement of rockfish aquaculture.

Rockfish is a commercially valuable fish species in Korea, with an aquaculture production of 14,418 metric tons, accounting for ca. 13% of the total aquaculture production (79,651 metric tons) in 2023 [28]. Therefore, a variety of studies associated with dietary nutrition requirements [3,28–32], digestibility of feed ingredients [33], reproductive cycle [34], optimal feeding frequency [35], stocking density in cages [36], and development of a feed stimulant to enhance feed consumption and growth performance [26] of rockfish have been reported.

However, to address the challenges faced by rockfish aquaculture in Korea, particularly the rising cost and limited availability of FM, using CBM as a FM replacer and incorporating JMM, which is highly attractive to rockfish, in the low-FM diets can provide a sustainable and cost-effective solution for improving feed consumption and growth performance. The present experiment, thus, is designed to assess the effects of replacing 25% and 50% of FM with CBM in diets, with or without the inclusion of JMM, on the growth performance, feed consumption, feed utilization, biochemical composition, and blood chemistry of rockfish.

2. Materials and Methods

2.1. Experimental Conditions of the Feeding Trial

Juvenile rockfish were bought from a commercial hatchery (Buan-gun, Jeollabuk-do, Republic of Korea). Rockfish were acclimated to the experimental conditions for 2 weeks by being provided with a commercial extruded pellet (50% crude protein, 8% crude lipid, 17% ash, 1.5% calcium, 3% fiber, and 2.7% phosphorus; National Federation of Fisheries Cooperatives, Uiryeong-gun, Gyeongsangnam-do, Republic of Korea), which was spherical in shape with a diameter of 4 mm. A total of 375 similar-sized juveniles (initial weight of 8.3 g) were randomly distributed into 15 50 L flow-through tanks in triplicate (25 fish/tank). Each tank was supplied with a blend of underground and sand-filtered seawater at a ratio of 1:1. Aeration was constantly provided to each tank. The water flow rate for each tank was 4.5 L/min. Water quality was monitored with a digital multimeter (AZ-86031; AZ Instrument Corp., Taichung, Taiwan), and water temperature was 22.3 ± 0.68 °C (mean ± SD), salinity was 32.5 ± 0.58 g/L, pH was 7.3 ± 0.07, and dissolved oxygen was 7.4 ± 0.09 mg/L during the 8-week feeding trial. Rockfish were carefully hand-fed to apparent satiation twice a day throughout the 8-week feeding trial. Dead fish were immediately removed when observed, and all tank bottoms were siphon-cleaned daily.

2.2. Design of the Feeding Trial

A two-way [FM replacement level (RL) (25% and 50%) × JMM inclusion (without and with)] ANOVA experimental design was employed to assess the inclusion effect of JMM in diets substituting 25% and 50% FM with CBM on the growth performance of rockfish (Table 1).

Table 1. The two-way ANOVA experimental design.

Two Main Effects		
FMRL (25% and 50%)	×	JMM inclusion (without and with)
The 25% FM replacement diets		The CBM-replaced diets without JMM inclusion
The 50% FM replacement diets		The CBM-replaced diets with JMM inclusion

FMRL: fish meal replacement level; JMM: jack mackerel meal; CBM: chicken by-product meal.

Fifty-five percent FM and 12% fermented soybean meal, 21.5% wheat flour, and 4.5% each of fish and soybean oils were used as the protein, carbohydrate, and lipid sources, respectively, in the control (Con) diet (Table 2). Here, in the Con diet, 25% and 50% of FM were replaced with CBM, either without or with the inclusion of 22% JMM at the expense

of FM, which was estimated to be the optimal dietary inclusion level for the best growth performance of rockfish [27], referred to as the CBM25, CBM50, CBM25J, and CBM50J diets, respectively.

Table 2. Ingredients and chemical compositions of the experimental diets (%, DM basis).

	Experimental Diets				
	Con	**CBM25**	**CBM50**	**CBM25J**	**CBM50J**
Ingredients (%, DM basis)					
Fish meal (FM) [a]	55.0	41.3	27.5	19.3	5.5
Chicken by-product meal (CBM) [b]		16.5	33.0	16.5	33.0
Jack mackerel meal (JMM) [c]				22.0	22.0
Fermented soybean meal [d]	12.0	12.0	12.0	12.0	12.0
Wheat flour	21.5	20.4	18.8	19.3	16.6
Fish oil	4.5	4.5	4.5	4.5	4.5
Soybean oil	4.5	2.8	1.7	3.9	3.9
Vitamin premix [e]	1.0	1.0	1.0	1.0	1.0
Mineral premix [f]	1.0	1.0	1.0	1.0	1.0
Choline	0.5	0.5	0.5	0.5	0.5
Nutrients (%, DM basis)					
Dry matter	95.0	92.3	91.0	93.5	91.8
Crude protein	51.7	51.1	51.2	51.7	51.8
Crude lipid	14.3	14.5	14.7	14.6	14.5
Ash	10.4	10.2	11.4	11.1	11.6
Carbohydrate [g]	23.6	24.2	22.7	22.6	22.1
Estimated gross energy (kcal/g) [h]	4.3	4.3	4.3	4.3	4.3

[a] Fish meal (FM) (crude protein: 74.1%, crude lipid: 7.6%, ash: 14.0%) was imported from Chile (USD 2.00/kg of FM, USD 1 = KRW 1300). [b] Chicken by-product meal (CBM) (crude protein: 65.0%, crude lipid: 13.6%, ash: 16.4%) (USD 0.72/kg of CBM) was purchased from Hwasong Ind. Co., Ltd. (Seogwipo-si, Jeju Special Self-Governing Province, Korea). [c] Jack mackerel meal (JMM) (crude protein: 72.8%, crude lipid: 9.8%, ash: 15.4%) was purchased from Daekyung Oil & Transportation Co., Ltd. (Busan Metropolitan City, Korea) (USD 2.61/ kg of JMM). [d] Fermented soybean meal (crude protein: 55.4%, crude lipid, 1.5%, ash: 6.7%) was purchased from CJ Cheiljedang (Seoul, Korea). [e] Vitamin premix (g/kg mix): L-ascorbic acid, 121.2; DL-α-tocopheryl acetate, 18.8; thiamin hydrochloride, 2.7; riboflavin, 9.1; pyridoxine hydrochloride, 1.8; niacin, 36.4; Ca-D-pantothenate, 12.7; myo-inositol, 181.8; D-biotin, 0.27; folic acid, 0.68; p-aminobenzoic acid, 18.2; menadione, 1.8; retinyl acetate, 0.73; cholecalciferol, 0.003; cyanocobalamin, 0.003. [f] Mineral premix (g/kg mix): $MgSO_4 \cdot 7H_2O$, 80.0; $NaH_2PO_4 \cdot 2H_2O$, 370.0; KCl, 130.0; Ferric citrate, 40.0; $ZnSO_4 \cdot 7H_2O$, 20.0; Ca-lactate, 356.5; CuCl, 0.2; $AlCl_3 \cdot 6H_2O$, 0.15; KI, 0.15; $Na_2Se_2O_3$, 0.01; $MnSO_4 \cdot H_2O$, 2.0; $CoCl_2 \cdot 6H_2O$, 1.0. [g] Carbohydrate was calculated by the difference [100 − (crude protein + crude lipid + ash)]. [h] Estimated gross energy (kcal/g) was calculated as 4 kcal/g for crude protein and carbohydrate and 9 kcal/g for crude lipid [37].

Five experimental diets were formulated to fulfill the protein and lipid requirements of rockfish [3,29]. These diets were isoproteic (51.5%), isolipidic (14.5%), and isocaloric (4.3 kcal/g diet). The ingredients in each diet were thoroughly blended with water at a ratio of 3:1. Then, the blended mixture was pelletized using a pellet extruder (SMC-32; SL Company, Incheon Metropolitan City, Republic of Korea). The pelletized diets were dried at 45 °C for 24 h in an electronic drying machine (SI-2400; SIN IL Drying Machine Co., Ltd., Daegu Metropolitan City, Republic of Korea). All meals were kept in the refrigerator at –20 °C until use.

2.3. Nutrient Composition of the Main Protein Sources and the Experimental Diets

Crude protein and lipid content in FM, CBM, and JMM were 74.1 and 7.6%, 65.0 and 13.6%, and 72.8 and 9.8%, respectively. All EAAs, except for arginine and tryptophan, and non-EAAs (NEAAs), except for glycine and proline in CBM, were relatively lower than those in FM (Table 3). All EAAs in JMM were relatively higher than those in FM. Increased FM replacement with CBM in diets led to increased arginine and tryptophan and increased glycine and proline. All EAAs tended to increase with JMM inclusion in the same FMRL.

Table 3. Amino acid (% of the diet) profiles of the main protein sources and the experimental diets.

	Main Protein Sources				Experimental Diets				
	FM	**JMM**	**CBM**	**Requirement**	**Con**	**CBM25**	**CBM50**	**CBM25J**	**CBM50J**
Essential amino acids (EAAs, %)									
Arginine	4.02	4.19	4.14		2.91	2.99	3.02	3.08	3.12
Histidine	1.61	3.03	1.29		1.21	1.18	1.12	1.34	1.31
Isoleucine	2.48	2.90	1.95		1.60	1.51	1.46	1.71	1.66
Leucine	5.23	5.29	4.08		3.71	3.44	3.21	3.46	3.48
Lysine	5.53	5.96	3.92	2.99 [a]	3.81	3.12	3.00	3.25	3.13
Methionine	2.01	2.10	1.27	1.37 [b]	1.61	1.19	1.16	1.24	1.19
Phenylalanine	2.77	2.86	2.22		2.06	1.90	1.81	1.95	1.84
Threonine	3.14	3.28	2.43		2.24	2.01	1.90	2.06	1.97
Tryptophan	0.45	1.22	0.47		0.31	0.37	0.40	0.42	0.46
Valine	3.04	3.54	2.58		2.17	2.10	2.07	2.33	2.28
∑EAA [c]	30.28	34.37	24.35		21.63	19.81	19.15	20.84	20.44
Non-essential amino acids (NEAAs, %)									
Alanine	4.53	4.57	4.31		3.10	3.12	3.21	3.12	3.25
Aspartic acid	6.40	6.69	4.87		4.76	4.34	4.22	4.36	4.25
Cysteine	0.86	0.90	0.75	0.12 [b]	0.82	0.80	0.81	0.81	0.82
Glutamic acid	8.95	9.28	8.22		7.09	7.15	7.23	7.19	7.34
Glycine	3.83	4.39	6.05		2.65	3.38	3.74	3.51	3.84
Proline	2.82	3.05	3.94		2.26	2.55	2.71	2.58	2.77
Serine	2.94	3.05	2.46		2.25	2.13	2.09	2.15	2.10
Tyrosine	2.06	2.04	1.44		1.40	1.25	1.11	1.16	1.12
∑NEAA [d]	32.39	33.97	32.04		24.33	24.72	25.12	24.88	25.49

[a, b] Data were obtained from Yan et al. [31,32]. [c] ∑EAAs: Total essential amino acids. [d] ∑NEAAs: Total non-essential amino acids.

Total saturated FAs (∑SFAs), including palmitic acid (C16:0) and stearic acid (C18:0), total monounsaturated FAs (∑MUFAs), including oleic acid (C18:1n-9) and linoleic acid (C18:2n-6), in CBM were relatively higher than in FM, but total n-3 highly unsaturated FAs (∑n-3 HUFAs), including eicosapentanoic acid (EPA, 20:5n-3) and docosahexanoic acid (DHA, 22:6n-3) (Table 4) were lower. The ∑MUFAs and ∑n-3 HUFAs in JMM were relatively higher than in FM. Increased FM replacement with CBM in diets led to increased ∑SFAs and ∑MUFAs but decreased ∑n-3 HUFAs, including EPA and DHA. The ∑MUFAs and ∑n-3 HUFAs, including EPA and DHA, tended to increase in the same FMRL with JMM inclusion than those without JMM inclusion.

2.4. Measurement of the Growth Performance and Biological Indices of Rockfish

At the end of the 8-week experiment, all live fish were starved for 24 h and then anesthetized with tricaine methanesulfonate (MS-222) at 100 mg/L. The number and collective weight of live fish from each tank were determined to assess survival and weight gain. Ten fish from each tank were randomly selected to calculate the biological indices. Growth performance, feed utilization, and biological indices of fish were calculated using the following formulas: SGR (%/day) = [Ln final weight of fish (g) − Ln initial weight of fish (g)] × 100/days of the feeding trial (56 days)], feed efficiency (FE) = weight gain of fish (g)/total feed consumption (g), protein efficiency ratio (PER) = weight gain of fish (g)/protein consumption (g), protein retention (PR, %) = protein gain of fish (g) × 100/protein consumption of fish (g), condition factor (CF, g/cm^3) = body weight of fish (g) × 100/total length of fish (cm)^3, viscerosomatic index (VSI, %) = viscera weight of fish (g) × 100/body weight of fish (g), and hepatosomatic index (HSI, %) = liver weight of fish (g) × 100/body weight of fish (g).

Table 4. Fatty acid (% of total fatty acids) profiles of the main protein sources and the experimental diets.

	Main Protein Sources			Experimental Diets				
Fatty Acid (%)	**FM**	**JMM**	**CBM**	**Con**	**CBM25**	**CBM50**	**CBM25J**	**CBM50J**
C14:0	6.41	5.01	0.97	1.69	1.40	1.37	1.39	1.36
C16:0	23.34	21.42	28.12	15.19	17.65	18.11	17.05	17.74
C18:0	5.01	7.44	8.71	4.23	4.71	4.82	4.97	5.02
C20:0	0.32	0.32	0.17	0.26	0.33	0.35	0.34	0.34
C24:0	2.56	3.31	0.04	0.66	0.46	0.36	0.61	0.47
∑SFA [a]	37.64	37.50	38.01	22.03	24.55	25.01	24.36	24.93
C14:1n-5	0.13	0.14	0.24	0.01	0.04	0.07	0.04	0.07
C15:1n-5	0.07	0.07	0.27	0.02	0.02	0.02	0.02	0.02
C16:1n-7	6.73	6.74	5.78	2.26	2.21	2.15	2.24	2.18
C17:1n-7	0.81	0.86	0.19	0.12	0.14	0.12	0.16	0.12
C18:1n-9	15.23	20.36	48.02	22.38	25.97	28.16	28.21	30.51
C20:1n-9	4.17	1.80	1.05	2.11	1.42	1.22	0.88	0.85
C22:1n-9	0.45	0.83	0.06	0.28	0.26	0.21	0.24	0.22
∑MUFA [b]	27.59	30.80	55.61	27.18	30.06	31.95	31.79	33.97
C18:2n-6	4.17	1.73	4.86	35.72	33.76	33.80	31.81	31.88
C18:3n-3	0.57	0.76	0.06	4.43	3.88	2.16	3.67	2.07
C18:3n-6	0.23	0.10	0.02	0.06	0.04	0.06	0.06	0.04
C20:2n-6	0.08	0.17	0.08	0.13	0.12	0.12	0.12	0.12
C20:3n-3	0.08	0.00	0.00	0.03	0.03	0.03	0.03	0.03
C20:4n-6	1.14	0.69	0.36	0.36	0.30	0.28	0.33	0.31
C20:5n-3	10.54	10.73	0.13	4.53	3.52	2.96	3.59	3.02
C22:6n-3	13.30	15.40	0.37	4.61	3.31	2.82	3.52	3.01
∑n-3 HUFA [c]	23.92	26.13	0.50	9.17	6.86	5.81	7.14	6.06
Unknown	4.66	2.12	0.50	0.92	0.43	0.81	0.72	0.62

[a] ∑SFAs: Total saturated fatty acids. [b] ∑MUFAs: Total monounsaturated fatty acids. [c] ∑n-3 HUFAs: Total n-3 highly unsaturated fatty acids.

2.5. Blood Chemistry Analysis of Rockfish

After completion of the 8-week experiment, blood was drawn from the caudal vein of 3 anesthetized fish from each tank using a syringe and heparinized syringe. An automatic chemistry system (Fuji Dri-Chem NX500i, Fujifilm, Tokyo, Japan) was used to assess aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total bilirubin (T-BIL), total cholesterol (T-CHO), triglyceride (TG), total protein (TP), and albumin (ALB) in the plasma samples (kept at –70 °C), which were separated by centrifugation at 2710× *g* for 10 min at 4 °C (Fuji Dri-Chem NX500i, Fujifilm, Tokyo, Japan), and kept at –70 °C until analysis.

Turbidity was measured to analyze lysozyme activity based on the study by Lange et al. [38]. A 100 µL volume of serum was added to a 1.9 mL suspension of *Micrococcus lysodeikticus* (0.2 mg/mL; Sigma, St. Louis, MO, USA) in 0.05 M sodium phosphate buffer at pH 6.2. The reaction was conducted at 25 °C for 60 min at 15 min intervals, and absorbance was evaluated using a microplate reader (Infinite® 200 PRO; TECAN, Männedorf, canton of Zürich, Switzerland) at 530 nm. Lysozyme activity was evaluated as the amount of enzyme required to lyse *M. lysodecticus*.

Superoxide dismutase (SOD) was assessed using an SOD ELISA kit (MBS705758; MyBiosource, Inc., San Diego, CA, USA) that was pre-coated with an antibody specific to SOD. This assay uses a competitive inhibition enzyme immunoassay technique. The intensity of the color developed after the addition of the substrate solution was inversely correlated with the concentration of SOD in the serum sample. Absorbance was assessed at 450 nm, and it was the same as the machine used for lysozyme activity. The concentration was determined by constructing a standard curve.

2.6. Biochemical Composition of the Experimental Feeds and Whole-Body Fish

All experimental diets, the initial 10 fish prior to the feeding experiment, and the remaining (≥8) fish from each tank after the 8-week feeding trial were used to evaluate biochemical composition. The proximate composition of the samples was conducted based on the standard procedure [39]. Dry (all experimental feeds) and wet (the whole-body fish) samples were dried in an oven for 6 h and 24 h at 105 °C, respectively, to assess the moisture content (AOAC Official Method 930.15). The Kjeldahl method (N × 6.25) (Kjeltec™ 2100 Distillation Unit, Foss, Hillerød, Denmark) and ether extraction method (Soxtec™ 2043 Fat Extraction System, Foss, Hillerød, Denmark) were used to evaluate the contents of crude protein and crude lipid in the samples, respectively (AOAC Official Method 954.01 and 920.39, respectively). The ash content was measured by burning the samples in a muffle furnace at 550 °C for 4 h (AOAC Official Method 942.05).

The amino acid (AA) profiles were analyzed based on Rutherfurd and Gilani [40]. The AA profiles (except for methionine, cysteine, and tryptophan) of the samples were examined by ion-exchange chromatography with post-column ninhydrin using an AA analyzer (L-8800 Autoanalyzer: Hitachi, Tokyo, Japan). To determine methionine and cysteine profiles, the samples were oxidized with performic acid at below 5 °C for 24 h to obtain methionine sulfone and cysteic acid. Following oxidation, the samples were freeze-dried using deionized water, hydrolyzed, and examined using the AA analyzer. For tryptophan analysis, high-performance liquid chromatography (S1125 HPLC pump system, Sykam, Germany) was used following 24 h of hydrolysis with NaOH (4.2 M) at 110 °C.

Following Folch et al. [41], lipids in the samples were separated using a mixture of chloroform and methanol (2:1 *v*/*v*) for FA analysis. The FA methyl esters (FAMEs) of the samples were synthesized via transesterification using 14% BF_3-MeOH (Sigma, St. Louis, MO, USA) [42] and further analyzed by gas chromatography (Truce GC; Thermo, Waltham, MA, USA) equipped with a flame ionization detector and an SP^{TM}-2560 capillary column (100 × 0.25 mm inner diameter, 0.20 μm film thickness; Supelco, Bellefonte, PA, USA). The sample of FA profiles was calculated by measuring the area of each identified peak and expressing the results as a percentage of the respective area ratio. Each FA peak was determined by comparing it with a known FAME standard (37-component FAME mix; Supelco™, St. Louis, MO, USA). No internal standard was used in this analysis.

2.7. Statistical Analysis

A two-way ANOVA and Duncan's multiple range test [43] were performed in SPSS version 24.0 (SPSS Inc., Chicago, IL, USA) to determine the effects of dietary treatments. All data met normality (Shapiro–Wilk test) and homogeneity of variances (Levene's test) conditions. Percentage data were arcsine-transformed prior to statistical analysis. $p < 0.05$ was considered statistically significant.

3. Results

3.1. Survival and Growth Performance of Rockfish

The survival of rockfish ranged from 98.7% to 100% and was not significantly ($p > 0.9$ and $p > 0.1$, respectively) affected by either dietary FMRL or JMM inclusion (Table 5).

The 25% FM replacement diets (21.5 g/fish and 2.26%/day, respectively) achieved significantly ($p < 0.003$ and $p < 0.004$, respectively) greater weight gain and SGR of rockfish compared to the 50% FM replacement diets (18.8 g/fish and 2.11%/day). Furthermore, the CBM-replaced diets with JMM inclusion (21.5 g/fish and 2.27%/day, respectively) achieved significantly ($p < 0.003$ and $p < 0.002$, respectively) greater weight gain and SGR of rockfish compared to the CBM-replaced diets without JMM inclusion (18.8 g/fish and 2.10%/day).

Weight gain and SGR of fish fed the Con and CBM25J diets were significantly ($p < 0.001$ for both) greater than those of fish fed the CBM25, CBM50, and CBM50J diets.

Table 5. Survival (%), weight gain (g/fish), and specific growth rate (SGR, %/day) of rockfish fed the experimental diets for 8 weeks.

Experimental Diets	Initial Weight (g/Fish)	Final Weight (g/Fish)	Survival (%)	Weight Gain (g/Fish)	SGR [1] (%/day)
Con	8.3 ± 0.07	30.1 ± 0.25	100.0 ± 0.00	21.9 ± 0.31 [a]	2.30 ± 0.028 [a]
CBM25	8.5 ± 0.05	28.2 ± 0.12	100.0 ± 0.00	19.7 ± 0.17 [b]	2.14 ± 0.017 [b]
CBM50	8.2 ± 0.07	26.1 ± 0.75	100.0 ± 0.00	17.9 ± 0.73 [b]	2.06 ± 0.047 [b]
CBM25J	8.3 ± 0.05	31.6 ± 0.39	98.7 ± 1.33	23.3 ± 0.36 [a]	2.38 ± 0.018 [a]
CBM50J	8.3 ± 0.09	28.0 ± 0.96	98.7 ± 1.33	19.7 ± 0.91 [b]	2.16 ± 0.054 [b]
p-value			$p > 0.5$	$p < 0.001$	$p < 0.001$
Main effect: FMRL					
25%			99.3 ± 0.67	21.5 ± 0.82 [A]	2.26 ± 0.055 [A]
50%			99.3 ± 0.67	18.8 ± 0.65 [B]	2.11 ± 0.039 [B]
Main effect: JMM inclusion					
Without			100.0 ± 0.00	18.8 ± 0.52 [B]	2.10 ± 0.029 [B]
With			98.7 ± 0.84	21.5 ± 0.92 [A]	2.27 ± 0.055 [A]
Two-way ANOVA					
FMRL			$p > 0.9$	$p < 0.003$	$p < 0.004$
JMM inclusion			$p > 0.1$	$p < 0.003$	$p < 0.002$
Interaction			$p > 0.9$	$p > 0.1$	$p > 0.1$

FMRL: fish meal replacement level; JMM: jack mackerel meal. Values (means of triplicate ± SE) in the same column sharing the same superscript lowercase letter are not statistically different ($p > 0.05$). Values with different uppercase letters indicate statistical differences ($p < 0.05$) based on two-way ANOVA. [1] Specific growth rate (SGR, %/day) = [Ln final weight of fish (g) − Ln initial weight of fish (g)] × 100/days of the feeding trial].

3.2. Feed Availability and Biological Indices of Rockfish

Feed consumption by rockfish was not significantly ($p > 0.1$ for both) changed by either FMRL or JMM inclusion (Table 6).

The 25% FM replacement diets (1.05) achieved significantly ($p < 0.05$) higher FE of rockfish than the 50% FM replacement diets (0.96). Furthermore, the CBM-replaced diets with JMM inclusion (1.09) achieved significantly ($p < 0.003$) higher FE of rockfish than those without JMM inclusion (0.92). FE of rockfish fed the Con and CBM25J diets was significantly ($p < 0.001$) higher than that of rockfish fed the CBM25 and CBM50 diets but not significantly ($p > 0.05$) different from that of rockfish fed the CBM50J diet. The 25% FM replacement diets (2.04) achieved significantly ($p < 0.05$) higher PER of rockfish than the 50% FM replacement diets (1.85). Furthermore, the CBM-replaced diets with JMM inclusion (2.10) achieved significantly ($p < 0.004$) higher PER of rockfish than those without JMM inclusion (1.80). The PER of rockfish fed the Con and CBM25J diets was significantly ($p < 0.002$) higher than that of rockfish fed the CBM25, CBM50, and CBM50J diets. Furthermore, the CBM-replaced diets with JMM inclusion (37.11%) achieved significantly ($p < 0.008$) higher PR of rockfish than the CBM-replaced diets without JMM inclusion (32.29%). The PR of rockfish fed the Con and CBM25J diets was significantly ($p < 0.005$) higher than that of rockfish fed the CBM25 and CBM50 diets but not significantly ($p > 0.05$) different from that of rockfish fed the CBM50J diet.

The CF, VSI, and HSI of rockfish were not significantly ($p > 0.05$) altered by either FMRL or JMM inclusion.

3.3. Blood Chemistry of Rockfish

Plasma AST, ALT, ALP, T-BIL, T-CHO, TG, TP, and ALB of fish were not significantly ($p > 0.05$) altered by either FMRL or JMM inclusion (Table 7). Furthermore, neither FMRL nor JMM inclusion had a significant ($p > 0.05$) impact on serum lysozyme and SOD activity in rockfish.

Table 6. Feed consumption (g/fish), feed efficiency (FE), protein efficiency ratio (PER), protein retention (PR, %), condition factor (CF, g/cm^3), viscerosomatic index (VSI, %), and hepatosomatic index (HSI, %) of rockfish fed the experimental diets for 8 weeks.

Experimental Diets	Feed Consumption (g/Fish)	FE [1]	PER [2]	PR [3] (%)	CF [4] (g/cm^3)	VSI [5] (%)	HSI [6] (%)
Con	19.16 ± 0.368	1.14 ± 0.006 ^{a}	2.21 ± 0.012 ^{a}	40.57 ± 1.284 ^{a}	1.65 ± 0.032	9.86 ± 0.271	2.79 ± 0.118
CBM25	20.68 ± 0.803	0.96 ± 0.037 ^{bc}	1.87 ± 0.073 ^{bc}	33.46 ± 0.811 ^{bc}	1.60 ± 0.008	9.52 ± 0.024	3.06 ± 0.166
CBM50	20.33 ± 0.370	0.88 ± 0.053 ^{c}	1.72 ± 0.103 ^{c}	31.13 ± 2.162 ^{c}	1.58 ± 0.018	9.73 ± 0.306	2.85 ± 0.063
CBM25J	20.39 ± 0.220	1.15 ± 0.035 ^{a}	2.21 ± 0.058 ^{a}	38.11 ± 1.06 ^{a}	1.62 ± 0.030	10.20 ± 0.309	2.89 ± 0.021
CBM50J	19.13 ± 0.342	1.03 ± 0.032 ^{ab}	1.98 ± 0.063 ^{b}	36.12 ± 1.12 ^{ab}	1.60 ± 0.040	9.46 ± 0.179	2.57 ± 0.148
p-value	$p > 0.1$	$p < 0.001$	$p < 0.002$	$p < 0.005$	$p > 0.6$	$p > 0.2$	$p > 0.1$
Main effect: FMRL							
25%	20.54 ± 0.378	1.05 ± 0.049 ^{A}	2.04 ± 0.087 ^{A}	35.79 ± 1.199	1.61 ± 0.014	9.86 ± 0.205	2.97 ± 0.084
50%	19.73 ± 0.352	0.96 ± 0.043 ^{B}	1.85 ± 0.079 ^{B}	33.62 ± 1.559	1.59 ± 0.020	9.60 ± 0.170	2.71 ± 0.095
Main effect: JMM inclusion							
Without	20.50 ± 0.403	0.92 ± 0.033 ^{B}	1.80 ± 0.065 ^{B}	32.29 ± 1.157 ^{B}	1.59 ± 0.010	9.63 ± 0.145	2.95 ± 0.092
With	19.76 ± 0.337	1.09 ± 0.034 ^{A}	2.10 ± 0.063 ^{A}	37.11 ± 0.822 ^{A}	1.61 ± 0.023	9.83 ± 0.229	2.73 ± 0.097
Two-way ANOVA							
FMRL	$p > 0.1$	$p < 0.05$	$p < 0.05$	$p > 0.1$	$p > 0.4$	$p > 0.3$	$p > 0.05$
JMM inclusion	$p > 0.1$	$p < 0.003$	$p < 0.004$	$p < 0.008$	$p > 0.6$	$p > 0.4$	$p > 0.09$
Interaction	$p > 0.3$	$p > 0.5$	$p > 0.6$	$p > 0.9$	$p > 0.9$	$p > 0.07$	$p > 0.6$

FMRL: fish meal replacement level; JMM: jack mackerel meal. Values (means of triplicate ± SE) in the same column sharing the same superscript lowercase letter are not statistically different ($p > 0.05$). Values with different uppercase letters indicate statistical differences ($p < 0.05$) based on two-way ANOVA. [1] Feed efficiency (FE) = weight gain of fish (g)/total feed consumption (g). [2] Protein efficiency ratio (PER) = weight gain of fish (g)/protein consumption (g). [3] Protein retention (PR, %) = protein gain of fish (g) × 100/protein consumption (g). [4] Condition factor (CF, g/cm^3) = body weight of fish (g) × 100/total length of fish (cm)3. [5] Viscerosomatic index (VSI, %) = viscera weight of fish (g) × 100/body weight of fish (g). [6] Hepatosomatic index (HSI, %) = liver weight of fish (g) × 100/body weight of fish (g).

Table 7. Plasma and serum parameters of rockfish fed the experimental diets for 8 weeks (means of triplicate ± SE).

Experimental Diets	Plasma Parameters								Serum Parameters	
	AST (U/L)	**ALT (U/L)**	**ALP (U/L)**	**T-BIL (mg/dL)**	**T-CHO (mg/dL)**	**TG (mg/dL)**	**TP (g/dL)**	**ALB (g/dL)**	**Lysozyme Activity (U/mL)**	**SOD (ng/mL)**
Con	154.0 ± 8.89	29.7 ± 1.67	293.0 ± 14.29	1.1 ± 0.30	299.0 ± 21.08	358.7 ± 23.41	5.4 ± 0.20	1.5 ± 0.10	502.0 ± 24.04	3.6 ± 0.18
CBM25	143.3 ± 7.13	24.3 ± 2.03	205.3 ± 18.84	1.1 ± 0.09	252.7 ± 6.17	315.7 ± 18.67	4.8 ± 0.18	1.3 ± 0.06	386.7 ± 43.81	4.0 ± 0.25
CBM50	145.7 ± 14.52	26.7 ± 2.33	236.3 ± 43.54	0.9 ± 0.09	240.7 ± 14.40	421.3 ± 45.24	4.5 ± 0.18	1.3 ± 0.13	408.9 ± 51.14	4.0 ± 0.06
CBM25J	133.3 ± 12.45	33.0 ± 12.77	229.0 ± 50.90	0.7 ± 0.06	285.7 ± 24.70	407.0 ± 55.19	4.8 ± 0.21	1.3 ± 0.19	386.7 ± 43.81	3.6 ± 0.24
CBM50J	138.7 ± 10.33	23.3 ± 2.60	230.0 ± 39.72	0.9 ± 0.17	254.7 ± 23.82	393.3 ± 18.50	4.8 ± 0.17	1.4 ± 0.21	408.9 ± 51.14	3.8 ± 0.41
p-value	$p > 0.7$	$p > 0.7$	$p > 0.5$	$p > 0.4$	$p > 0.2$	$p > 0.2$	$p > 0.09$	$p > 0.8$	$p > 0.2$	$p > 0.8$
Main effect: FMRL										
25%	138.3 ± 6.80	28.7 ± 6.10	217.2 ± 24.84	0.9 ± 0.09	269.2 ± 13.57	361.3 ± 33.11	4.8 ± 0.12	1.3 ± 0.09	392.5 ± 24.38	3.8 ± 0.24
50%	142.2 ± 8.12	25.0 ± 1.73	233.2 ± 26.39	0.9 ± 0.09	247.7 ± 12.84	407.3 ± 22.74	4.7 ± 0.13	1.4 ± 0.11	427.0 ± 26.83	3.9 ± 0.26
Main effect: JMM inclusion										
Without	144.5 ± 7.25	25.5 ± 1.48	220.8 ± 22.32	1.0 ± 0.06	246.7 ± 7.50	368.5 ± 32.21	4.7 ± 0.12	1.3 ± 0.07	397.8 ± 30.53	4.0 ± 0.16
With	136.0 ± 7.33	28.2 ± 6.22	229.5 ± 28.87	0.8 ± 0.09	270.2 ± 16.84	400.2 ± 26.21	4.8 ± 0.12	1.4 ± 0.13	421.7 ± 21.08	3.7 ± 0.31
Two-way ANOVA										
FMRL	$p > 0.7$	$p > 0.5$	$p > 0.7$	$p > 0.8$	$p > 0.2$	$p > 0.2$	$p > 0.5$	$p > 0.7$	$p > 0.4$	$p > 0.6$
JMM inclusion	$p > 0.4$	$p > 0.7$	$p > 0.8$	$p > 0.08$	$p > 0.2$	$p > 0.4$	$p > 0.4$	$p > 0.7$	$p > 0.5$	$p > 0.4$
Interaction	$p > 0.8$	$p > 0.3$	$p > 0.7$	$p > 0.2$	$p > 0.6$	$p > 0.1$	$p > 0.5$	$p > 0.9$	$p > 0.7$	$p > 0.7$

FMRL: fish meal replacement level; JMM: jack mackerel meal.

3.4. Biochemical Composition of the Whole Body of Rockfish

Neither FMRL nor JMM inclusion showed significant ($p > 0.05$) changes in the proximate composition of the whole body of the fish (Table 8).

Table 8. Proximate composition (% of wet weight) of the whole-body rockfish fed the experimental diets for 8 weeks (means of triplicate ± SE).

Experimental Diets	Moisture	Crude Protein	Crude Lipid	Ash
Con	68.7 ± 0.78	17.8 ± 0.45	8.1 ± 0.13	4.4 ± 0.08
CBM25	70.3 ± 0.36	17.4 ± 0.27	7.2 ± 0.18	4.2 ± 0.18
CBM50	70.3 ± 0.42	17.5 ± 0.14	7.3 ± 0.50	4.5 ± 0.16
CBM25J	69.4 ± 0.48	17.0 ± 0.05	8.0 ± 0.26	4.4 ± 0.16
CBM50J	69.8 ± 0.42	17.7 ± 0.17	7.1 ± 0.39	4.7 ± 0.12
p-value	$p > 0.2$	$p > 0.3$	$p > 0.1$	$p > 0.3$
Main effect: FMRL				
25%	69.8 ± 0.34	17.2 ± 0.16	7.6 ± 0.22	4.3 ± 0.11
50%	70.1 ± 0.28	17.6 ± 0.11	7.2 ± 0.29	4.6 ± 0.10
Main effect: JMM inclusion				
Without	70.3 ± 0.25	17.5 ± 0.14	7.2 ± 0.24	4.4 ± 0.12
With	69.6 ± 0.30	17.3 ± 0.17	7.5 ± 0.30	4.5 ± 0.11
Two-way ANOVA				
FMRL	$p > 0.5$	$p > 0.09$	$p > 0.2$	$p > 0.1$
JMM inclusion	$p > 0.1$	$p > 0.5$	$p > 0.4$	$p > 0.3$
Interaction	$p > 0.6$	$p > 0.1$	$p > 0.2$	$p > 0.6$

FMRL: fish meal replacement level; JMM: jack mackerel meal.

The AA and FA profiles of the whole body of rockfish were not significantly ($p > 0.05$) altered by either FMRL or JMM inclusion (Tables 9 and 10, respectively).

Table 9. Amino acid (% of wet weight) profiles of whole-body rockfish fed experimental diets for 8 weeks (means of triplicate ± SE).

	Experimental Diets						**Main Effect: FMRL**		**Main Effect: JMM Inclusion**		**Two-Way ANOVA**		
	Con	**CBM25**	**CBM50**	**CBM25J**	**CBM50J**	***p*-Value**	**25%**	**50%**	**Without**	**With**	**FMRL**	**JMM Inclusion**	**Interaction**
Essential amino acids (%)													
Arginine	1.1 ± 0.03	1.1 ± 0.04	1.1 ± 0.05	1.1 ± 0.08	1.1 ± 0.02	$p > 0.7$	1.1 ± 0.04	1.1 ± 0.03	1.1 ± 0.03	1.1 ± 0.04	$p > 0.7$	$p > 0.8$	$p > 0.2$
Histidine	0.4 ± 0.17	0.4 ± 0.05	0.4 ± 0.10	0.4 ± 0.06	0.4 ± 0.02	$p > 0.9$	0.4 ± 0.04	0.4 ± 0.04	0.4 ± 0.05	0.4 ± 0.03	$p > 0.6$	$p > 0.9$	$p > 0.9$
Isoleucine	0.8 ± 0.12	0.7 ± 0.07	0.6 ± 0.02	0.7 ± 0.11	0.7 ± 0.08	$p > 0.6$	0.7 ± 0.06	0.6 ± 0.05	0.6 ± 0.04	0.7 ± 0.06	$p > 0.4$	$p > 0.2$	$p > 0.6$
Leucine	1.3 ± 0.10	1.3 ± 0.10	1.2 ± 0.06	1.3 ± 0.10	1.2 ± 0.06	$p > 0.9$	1.3 ± 0.06	1.2 ± 0.04	1.2 ± 0.05	1.2 ± 0.05	$p > 0.4$	$p > 0.9$	$p > 0.9$
Lysine	1.4 ± 0.10	1.5 ± 0.11	1.4 ± 0.08	1.5 ± 0.12	1.4 ± 0.08	$p > 0.9$	1.5 ± 0.07	1.4 ± 0.05	1.4 ± 0.06	1.4 ± 0.07	$p > 0.4$	$p > 0.9$	$p > 0.9$
Methionine	0.6 ± 0.05	0.6 ± 0.06	0.6 ± 0.03	0.6 ± 0.05	0.6 ± 0.04	$p > 0.9$	0.6 ± 0.03	0.6 ± 0.02	0.6 ± 0.03	0.6 ± 0.03	$p > 0.7$	$p > 0.9$	$p > 0.9$
Phenylalanine	0.7 ± 0.08	0.7 ± 0.06	0.7 ± 0.03	0.7 ± 0.04	0.7 ± 0.05	$p > 0.8$	0.7 ± 0.03	0.7 ± 0.03	0.7 ± 0.03	0.7 ± 0.03	$p > 0.6$	$p > 0.8$	$p > 0.7$
Threonine	0.8 ± 0.04	0.8 ± 0.07	0.8 ± 0.06	0.8 ± 0.05	0.8 ± 0.04	$p > 0.9$	0.8 ± 0.04	0.8 ± 0.03	0.8 ± 0.04	0.8 ± 0.03	$p > 0.7$	$p > 0.5$	$p > 0.9$
Tryptophan	0.1 ± 0.01	0.1 ± 0.01	0.1 ± 0.02	0.1 ± 0.02	0.1 ± 0.02	$p > 0.9$	0.1 ± 0.01	0.1 ± 0.01	0.1 ± 0.01	0.1 ± 0.01	$p > 0.4$	$p > 0.8$	$p > 0.8$
Valine	0.9 ± 0.10	0.8 ± 0.08	0.7 ± 0.01	0.8 ± 0.01	0.8 ± 0.05	$p > 0.4$	0.8 ± 0.04	0.7 ± 0.03	0.7 ± 0.04	0.8 ± 0.03	$p > 0.2$	$p > 0.1$	$p > 0.7$
Non-essential amino acids (%)													
Alanine	1.2 ± 0.06	1.3 ± 0.08	1.2 ± 0.07	1.2 ± 0.07	1.2 ± 0.04	$p > 0.9$	1.3 ± 0.05	1.2 ± 0.04	1.3 ± 0.05	1.2 ± 0.04	$p > 0.5$	$p > 0.5$	$p > 0.7$
Aspartic acid	1.7 ± 0.12	1.8 ± 0.09	1.7 ± 0.10	1.7 ± 0.13	1.6 ± 0.08	$p > 0.9$	1.7 ± 0.07	1.7 ± 0.06	1.7 ± 0.06	1.7 ± 0.07	$p > 0.4$	$p > 0.7$	$p > 0.9$
Cysteine	0.3 ± 0.03	0.3 ± 0.02	0.3 ± 0.03	0.2 ± 0.02	0.3 ± 0.03	$p > 0.9$	0.2 ± 0.01	0.3 ± 0.02	0.3 ± 0.02	0.2 ± 0.02	$p > 0.8$	$p > 0.8$	$p > 0.7$
Glutamic acid	2.4 ± 0.12	2.5 ± 0.26	2.3 ± 0.29	2.4 ± 0.14	2.3 ± 0.12	$p > 0.9$	2.4 ± 0.13	2.3 ± 0.14	2.4 ± 0.18	2.4 ± 0.09	$p > 0.5$	$p > 0.9$	$p > 0.9$
Glycine	1.5 ± 0.05	1.6 ± 0.12	1.6 ± 0.15	1.6 ± 0.12	1.5 ± 0.04	$p > 0.8$	1.6 ± 0.08	1.6 ± 0.08	1.6 ± 0.09	1.5 ± 0.06	$p > 0.7$	$p > 0.5$	$p > 0.5$
Proline	0.8 ± 0.06	0.9 ± 0.07	0.9 ± 0.10	0.9 ± 0.10	0.8 ± 0.06	$p > 0.9$	0.9 ± 0.06	0.8 ± 0.05	0.9 ± 0.05	0.8 ± 0.06	$p > 0.7$	$p > 0.6$	$p > 0.6$
Serine	0.7 ± 0.06	0.9 ± 0.10	0.9 ± 0.12	0.8 ± 0.07	0.8 ± 0.04	$p > 0.5$	0.8 ± 0.06	0.8 ± 0.06	0.9 ± 0.07	0.8 ± 0.04	$p > 0.7$	$p > 0.3$	$p > 0.8$
Tyrosine	0.3 ± 0.03	0.5 ± 0.14	0.5 ± 0.12	0.4 ± 0.09	0.4 ± 0.05	$p > 0.6$	0.4 ± 0.07	0.4 ± 0.06	0.5 ± 0.08	0.4 ± 0.04	$p > 0.8$	$p > 0.5$	$p > 0.9$

FMRL: fish meal replacement level; JMM: jack mackerel meal.

Table 10. Fatty acid (% of total fatty acids) profiles of whole-body rockfish fed experimental diets for 8 weeks (means of triplicate ± SE).

	Experimental Diets						**Main Effect: FMRL**		**Main Effect: JMM Inclusion**		**Two-Way ANOVA**		
	Con	**CBM25**	**CBM50**	**CBM25J**	**CBM50J**	***p*-Value**	**25%**	**50%**	**Without**	**With**	**FMRL**	**JMM Inclusion**	**Interaction**
C14:0	1.97 ± 0.147	1.74 ± 0.038	1.71 ± 0.023	1.72 ± 0.046	1.68 ± 0.032	$p > 0.09$	1.73 ± 0.027	1.69 ± 0.019	1.72 ± 0.021	1.70 ± 0.027	$p > 0.3$	$p > 0.5$	$p > 0.8$
C16:0	15.10 ± 0.273	15.39 ± 0.234	15.90 ± 0.779	14.82 ± 0.286	14.62 ± 0.323	$p > 0.3$	15.11 ± 0.210	15.26 ± 0.474	15.65 ± 0.381	14.72 ± 0.198	$p > 0.7$	$p > 0.07$	$p > 0.4$
C18:0	4.32 ± 0.130	4.36 ± 0.127	4.65 ± 0.199	4.48 ± 0.144	4.73 ± 0.251	$p > 0.4$	4.42 ± 0.090	4.69 ± 0.145	4.50 ± 0.124	4.61 ± 0.141	$p > 0.1$	$p > 0.5$	$p > 0.9$
C20:0	0.17 ± 0.026	0.16 ± 0.026	0.16 ± 0.020	0.15 ± 0.012	0.16 ± 0.020	$p > 0.9$	0.15 ± 0.013	0.16 ± 0.013	0.16 ± 0.015	0.16 ± 0.011	$p > 0.6$	$p > 0.8$	$p > 0.8$
C24:0	0.75 ± 0.049	0.69 ± 0.061	0.61 ± 0.066	0.72 ± 0.064	0.69 ± 0.115	$p > 0.7$	0.70 ± 0.040	0.65 ± 0.062	0.65 ± 0.044	0.71 ± 0.059	$p > 0.5$	$p > 0.4$	$p > 0.7$
∑SFAs [a]	22.31 ± 0.294	22.33 ± 0.410	23.03 ± 1.042	21.89 ± 0.385	21.88 ± 0.251	$p > 0.6$	22.11 ± 0.270	22.46 ± 0.543	22.68 ± 0.524	21.89 ± 0.205	$p > 0.5$	$p > 0.2$	$p > 0.5$
C14:1n-5	0.03 ± 0.012	0.04 ± 0.015	0.05 ± 0.020	0.04 ± 0.017	0.05 ± 0.003	$p > 0.8$	0.04 ± 0.010	0.05 ± 0.009	0.05 ± 0.011	0.04 ± 0.008	$p > 0.6$	$p > 0.7$	$p > 0.9$
C15:1n-7	0.03 ± 0.012	0.03 ± 0.012	0.03 ± 0.009	0.03 ± 0.009	0.03 ± 0.009	$p > 0.9$	0.03 ± 0.007	0.03 ± 0.006	0.03 ± 0.007	0.03 ± 0.006	$p > 0.6$	$p > 0.8$	$p > 0.8$
C16:1n-7	3.62 ± 0.165	3.66 ± 0.205	4.08 ± 0.271	3.78 ± 0.101	4.10 ± 0.289	$p > 0.3$	3.72 ± 0.106	4.09 ± 0.177	3.87 ± 0.179	3.94 ± 0.155	$p > 0.1$	$p > 0.7$	$p > 0.8$
C17:1n-7	0.30 ± 0.015	0.30 ± 0.015	0.30 ± 0.049	0.28 ± 0.069	0.33 ± 0.026	$p > 0.9$	0.29 ± 0.032	0.32 ± 0.025	0.30 ± 0.023	0.30 ± 0.035	$p > 0.5$	$p > 0.9$	$p > 0.6$
C18:1n-9	30.80 ± 2.771	32.83 ± 1.167	32.90 ± 1.626	33.78 ± 1.549	33.33 ± 1.764	$p > 0.8$	33.31 ± 0.839	33.12 ± 1.077	32.87 ± 0.895	33.56 ± 1.055	$p > 0.9$	$p > 0.6$	$p > 0.8$
C20:1n-9	1.24 ± 0.043	1.07 ± 0.159	1.53 ± 0.101	1.34 ± 0.035	1.40 ± 0.349	$p > 0.4$	1.21 ± 0.094	1.47 ± 0.165	1.30 ± 0.132	1.37 ± 0.158	$p > 0.2$	$p > 0.7$	$p > 0.3$
C22:1n-9	0.10 ± 0.012	0.09 ± 0.017	0.08 ± 0.012	0.09 ± 0.015	0.08 ± 0.012	$p > 0.7$	0.09 ± 0.010	0.08 ± 0.007	0.09 ± 0.010	0.09 ± 0.009	$p > 0.4$	$p > 0.9$	$p > 0.9$
∑MUFAs [b]	36.12 ± 3.028	38.02 ± 0.774	38.97 ± 2.062	39.35 ± 1.788	39.32 ± 1.730	$p > 0.7$	38.69 ± 0.920	39.14 ± 1.206	38.50 ± 1.008	39.33 ± 1.113	$p > 0.7$	$p > 0.6$	$p > 0.7$
C18:2n-6	29.73 ± 3.297	27.93 ± 0.828	24.51 ± 0.326	28.34 ± 2.783	28.39 ± 2.292	$p > 0.5$	28.14 ± 1.302	26.45 ± 1.350	26.22 ± 0.862	28.37 ± 1.612	$p > 0.3$	$p > 0.2$	$p > 0.3$
C18:3n-3	3.15 ± 0.413	2.91 ± 0.280	2.54 ± 0.214	2.93 ± 0.274	2.43 ± 0.165	$p > 0.4$	2.92 ± 0.175	2.49 ± 0.123	2.73 ± 0.178	2.68 ± 0.182	$p > 0.1$	$p > 0.8$	$p > 0.7$
C18:3n-6	0.22 ± 0.058	0.26 ± 0.009	0.23 ± 0.029	0.23 ± 0.055	0.22 ± 0.040	$p > 0.9$	0.24 ± 0.026	0.23 ± 0.022	0.24 ± 0.015	0.22 ± 0.031	$p > 0.6$	$p > 0.6$	$p > 0.7$
C20:2n-6	0.42 ± 0.015	0.40 ± 0.032	0.39 ± 0.017	0.37 ± 0.026	0.39 ± 0.038	$p > 0.7$	0.38 ± 0.020	0.39 ± 0.019	0.39 ± 0.016	0.38 ± 0.021	$p > 0.8$	$p > 0.5$	$p > 0.6$
C20:3n-3	0.08 ± 0.015	0.07 ± 0.015	0.07 ± 0.015	0.07 ± 0.009	0.08 ± 0.026	$p > 0.9$	0.07 ± 0.008	0.08 ± 0.014	0.07 ± 0.009	0.08 ± 0.013	$p > 0.5$	$p > 0.7$	$p > 0.7$
C20:4n-6	0.48 ± 0.038	0.48 ± 0.038	0.55 ± 0.017	0.47 ± 0.043	0.49 ± 0.047	$p > 0.6$	0.48 ± 0.026	0.52 ± 0.026	0.52 ± 0.024	0.48 ± 0.029	$p > 0.2$	$p > 0.4$	$p > 0.5$
C20:5n-3	2.54 ± 0.238	2.35 ± 0.135	2.26 ± 0.176	2.33 ± 0.072	2.31 ± 0.260	$p > 0.8$	2.34 ± 0.069	2.29 ± 0.141	2.31 ± 0.102	2.32 ± 0.121	$p > 0.7$	$p > 0.9$	$p > 0.8$
C22:6n-3	2.90 ± 0.058	2.75 ± 0.244	2.87 ± 0.020	2.82 ± 0.217	2.72 ± 0.219	$p > 0.9$	2.79 ± 0.147	2.79 ± 0.104	2.81 ± 0.113	2.77 ± 0.140	$p > 0.9$	$p > 0.8$	$p > 0.5$
∑n-3 HUFAs [c]	5.52 ± 0.209	5.17 ± 0.366	5.20 ± 0.170	5.22 ± 0.136	5.12 ± 0.113	$p > 0.7$	5.19 ± 0.175	5.16 ± 0.093	5.18 ± 0.181	5.17 ± 0.082	$p > 0.8$	$p > 0.9$	$p > 0.7$
Unknown	2.05 ± 0.268	2.49 ± 1.087	4.58 ± 1.001	1.21 ± 0.587	1.76 ± 0.681								

FMRL: fish meal replacement level; JMM: jack mackerel meal. [a] ∑SFAs: Total saturated fatty acids. [b] ∑MUFAs: Total monounsaturated fatty acids. [c] ∑n-3 HUFAs: Total n-3 highly unsaturated fatty acids.

4. Discussion

The 50% FM replacement diets resulted in inferior weight gain and SGR of rockfish compared to the 25% FM replacement diets in this study. Likewise, elevated substitution levels of chicken plasma powder for FM in diets led to a linearly reduced growth performance of largemouth bass (*Micropterus salmoides*) when largemouth bass were supplied with a 51% FM-basal diet or one of diets substituting 5%, 10%, and 15% FM with chicken plasma powder [44]. Wang et al. [45] also revealed that increased FM replacement with PBM in the diet linearly lowered the growth of large yellow croaker (*Larimichthys crocea*) when the fish were fed a 40% FM-based diet or one of diets replacing 25%, 50%, 75%, and 100% FM with PBM. Similarly, increased FM replacement with PBM lowered the growth of gilthead seabream (*Sparus aurata*) when gilthead seabream were fed a 58% FM-based diet or one of diets replacing 50% and 100% FM with PBM [18].

Weight gain and SGR of rockfish fed the CBM-replaced diets with JMM inclusion were superior compared to rockfish fed the CBM-replaced diets without JMM inclusion in this study, indicating that JMM inclusion in the CBM-replaced diets had a significantly improvement effect on growth performance of rockfish compared to the CBM-replaced diets without JMM inclusion. Similarly, incorporating 6%, 9%, and 12% krill meal into the low-FM diets achieved superior and comparable weight gain in olive flounder compared to fish fed a 56% FM-basal diet when juvenile fish were supplied with a 56% FM-basal diet or the low-FM diets substituting 50% FM with a blend of soy protein concentrate (SPC), tankage meal, and PBM supplemented with 3%, 6%, 9%, and 12% krill meal [46]. Likewise, the low-FM diets with shrimp hydrolysate, tilapia hydrolysate, or krill hydrolysate inclusion achieved similar growth performance of olive flounder compared to a 55% FM-based diet when juvenile fish were supplied with a 55% FM-basal diet or diets replacing 50% FM with SPC without or with inclusion of 3% each of shrimp hydrolysate, tilapia hydrolysate, or krill hydrolysate [47]. Nevertheless, slightly, but not significantly, improved weight gain and SGR of rockfish fed the CBM25J diet compared to rockfish fed the Con diet based on multiple comparison data in this study implied that up to 25% of FM could be replaced with CBM in diets with 22% JMM inclusion without compromising the growth performance of rockfish. In comparison with the results from Li and Cho's study [8], in which up to 20% of FM could be substituted with the same CBM in the rockfish feed without compromising growth and feed availability, the substitutability of CBM for FM in the 55% FM-basal feeds of rockfish increased from 20% to 25% with JMM inclusion in this study.

JMM inclusion in the low-FM diets replacing 25% and 50% of FM with CBM led to increased levels of all EAAs, which responded well based on the AA profiles of the main protein sources in this experiment. This might be one of the reasons why incorporating JMM into the CBM-replaced diets produced superior rockfish growth compared to the CBM-replaced diets without JMM inclusion. Similarly, Karapanagiotidis et al. [18] revealed that the growth performance of gilthead seabream fed a diet substituting 50% FM by PBM without AA supplementation was inferior to fish fed a 58% FM-based diet, whereas growth performance of gilthead seabream fed a diet substituting 50% FM by PBM with AA (lysine and methionine) supplementation was comparable to fish fed a 58% FM-based diet. Likewise, a 60% FM replacement with meat meal in diets supplemented with AA achieved comparable growth of olive flounder compared to an 80% FM-based diet, but the growth of fish supplied with a diet substituting 40% of FM with meat meal without AA supplementation was inferior to fish supplied with the 80% FM-based diet when olive flounder were supplied with the 80% FM-based diet or one of diets replacing 20%, 40%, 60%, 80%, and 100% FM with meat meal supplemented with AA (methionine, lysine, and tryptophan) or the diet replacing 40% FM with meat meal without AA supplementation [48]. The lysine requirement (2.99% of the diet) [31] of rockfish was fulfilled in all experimental

diets in this experiment. However, the methionine requirement (1.37% of the diet in the presence of 0.12% cysteine) [32] of rockfish did not seem to be fulfilled in all the experimental diets, except for the Con diet. However, several studies have proved that cysteine can supply approximately 40–50% of the dietary methionine requirement of Asian seabass [49], grouper (*Epinephelus coioides*) [50], and yellowtail kingfish (*S. lalandi*) [51]. Cysteine exceeded 0.80% in all the CBM-replaced diets in this experiment, which was higher than the cysteine (0.12% of the diet) reported in Yan et al.'s study [32]. Therefore, relatively low methionine in all the CBM-replaced diets did not bring about deteriorated growth performance in rockfish. However, increased CBM replacement for FM in diets led to reduced growth performance of rockfish, which might have resulted from the unbalanced AA content of CBM. All EAAs in CBM, except for arginine and tryptophan, were lower than in FM, which was reflected in the experimental diets. Nutritional factors, including imbalances in EAAs, can affect the regulation of the insulin-like growth factor (IGF) and growth hormone (GH) systems, leading to decreased growth performance of gilthead seabream [52]. For example, dietary optimal phenylalanine content has been shown to improve growth performance and GH, hepatic IGF-1, and GH receptor 1 expression in the pituitary of hybrid grouper (*E. fuscoguttatus* × *E. lanceolatus*) [53]. Although the lysine requirement was fulfilled in all experimental diets in this study and the methionine content was relatively lower than the dietary requirement, except for the Con diet, it did not negatively affect the growth performance of rockfish. However, the optimal requirements for most EAAs still remain unknown. This uncertainty makes it difficult to precisely evaluate how deficiencies in individual EAAs can affect the growth of rockfish.

Dietary ∑n-3 HUFA is an important factor because it affects the growth performance and immunity of marine fish [54,55]. ∑n-3 HUFAs, including EPA and DHA, in the CBM-replaced diets with JMM inclusion were higher than those without JMM inclusion, which might explain why the CBM25 diet attained inferior growth performance compared to the CBM25J diet and the CBM50 diet achieved slightly, but not significantly, poorer growth performance compared to the CBM50J diet in this experiment. Similarly, poorer growth was found in black seabream (*Acanthopagrus schlegelii*) and hybrid grouper (*E. fuscoguttatus* × *E. lanceolatu*) supplied with diets containing low ∑n-3 HUFAs compared to fish fed the diets containing proper levels of ∑n-3 HUFAs [54,56].

Feed consumption of rockfish was unaffected by either dietary FMRL or JMM inclusion in this experiment. However, increased FM replacement with CBM in the diet lowered the FE and PER of rockfish, whereas the inclusion of JMM in the CBM-replaced diets improved FE and PER. Likewise, elevated FM replacement with chicken waste meal and PBM in diets lowered feed utilization in Asian seabass and gilthead seabream and large yellow croaker, respectively [17,18,45]. No remarkable differences in feed consumption, FE, PER, and PR of rockfish fed the CBM25J diet compared to rockfish fed the Con diet in this experiment implied that 25% of FM could be substituted with CBM in diets with JMM inclusion without causing any detrimental effects on feed intake and feed utilization of rockfish. Similarly, the inclusion of protein hydrolysates in the low-FM diets improved the growth and FE of olive flounder and red sea bream [47].

CF, a length–weight relationship, is used as an indicator to assess the health of fish [57]. In addition, VSI and HSI are strongly influenced by lipid deposition in the whole body of fish and are used to evaluate growth performance [58,59]. The biological indices (CF, VSI, and HSI) of rockfish were not affected by either FMRL or JMM inclusion in this experiment, probably indicating that 25% and 50% of FM could be replaced by CBM in diets regardless of the inclusion of JMM without deteriorating the biological indices of rockfish. Likewise, VSI, HSI, intraperitoneal fat ratio, and CF of hybrid grouper were not changed by dietary PBM substitution for FM [60]. Similarly, FM substitution with CBM in diets did not change

the biological indices of olive flounder [7] and rockfish [8]. However, the replacement of FM with chicken plasma powder, MM, and MBM, PBM, and spray-dried chicken plasma in diets affected the biological indices of largemouth bass, Ussuri catfish (*Pseudobagrus ussuriensis*), gilthead seabream, and largemouth bass, respectively [9,17,44,61]. Specifically, increased PBM replacements for FM in the gilthead seabream diets led to reduced visceral fat and lowered VSI, probably resulting from decreased lipid content in the low FM diets [17]. However, as all experimental diets were isolipidic at 14.5% and isocaloric at 4.3 kcal/g in this experiment, this might not have altered the biological indices of rockfish.

Plasma parameters can be used to estimate the health status of fish [62,63]. Lysozyme activity is a significant marker of innate immunity in fish [64]. The antioxidant system, including SOD, maintains an equilibrium to protect mitochondria and cellular function from oxidative damage in vivo [65]. None of the blood chemistry parameters of rockfish were altered by either dietary FMRL or JMM inclusion in this experiment, indicating that 25% and 50% FM substitution with CBM in diets with or without JMM inclusion did not have any adverse impact on the blood chemistry of rockfish. Similarly, plasma (glucose, TP, T-CHO, and TG) parameters of olive flounder and red sea bream were not affected by the inclusion of protein hydrolysates in the low-FM diets, but serum lysozyme activity of both fish and SOD of olive flounder were affected [47]. Moreover, increased FM substitution with chicken plasma powder in the diet did not alter the plasma parameters of largemouth bass but lowered serum lysozyme activity [44]. Likewise, the incorporation of krill meal in the low-FM diets did not influence plasma parameters (TG, T-CHO, TP, AST, and ALT) of olive flounder, but it affected lysozyme activity and SOD [46].

None of the biochemical composition (proximate composition, and AA and FA profiles) of rockfish was affected by either dietary FMRL or JMM inclusion in this experiment, indicating that 25% and 50% of FM replacement with CBM in diets with or without the inclusion of JMM did not deteriorate the biochemical composition of rockfish. In particular, no discernible difference in the proximate composition of the whole-body rockfish might be related to the fact that the proximate composition of fish is influenced by internal factors, such as fish size and life cycle stage, and external factors, such as nutritional and environmental conditions [66]. Likewise, the proximate composition of the whole body and dorsal muscle of hybrid grouper was not affected by dietary FM replacements with PBM [60]. Similarly, the inclusion of krill hydrolysate concentrate or tuna hydrolysate liquid in the low-FM diets did not affect the chemical composition of the whole body of red sea bream and olive flounder [47]. Furthermore, increasing ∑n-3 HUFAs in the diets had a positive effect on growth but did not affect the whole-body composition of rockfish in this study. This may be because FAs were used for tissue biosynthesis, converted into energy, or allocated to specific tissues necessary for growth and development rather than being stored in the whole body [67]. Jeong et al. [68] also proved that the biochemical composition of olive flounder was not altered by dietary manipulation of JMM.

5. Conclusions

The 25% FM replacement diets resulted in significantly greater weight gain and SGR of rockfish compared to the 50% FM replacement diets. Additionally, the CBM-replaced diets with JMM inclusion also resulted in significantly greater weight gain and SGR of rockfish than those without JMM inclusion. Weight gain and SGR of rockfish fed the Con and CBM25J diets were significantly higher than those of fish fed the CBM25, CBM50, and CBM50J diets. With the inclusion of JMM, 25% of FM could be replaced with CBM in the 55% FM-based diet without compromising the growth and feed availability of rockfish. However, the feasibility of JMM inclusion in the CBM-replaced diets needs to be evaluated in a long-term feeding trial before practical application.

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