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Nutritional Characteristics of Black Rockfish (*Sebastes schlegeli*) Fed a Diet of Fish Skin

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Abstract

This study investigated the effects of diets substituted with different levels (0, 5, 10, and 20%) of flounder skin meal (FSM) on the nutritional composition of black rockfish *Sebastes schlegeli*. Fish (10.05 ± 0.44 g) were fed to apparent satiation twice daily for 8 weeks. Adding FSM decreased crude lipid levels and increased crude protein and ash. The abundant fatty acids in the FSM-added group were C16:0, C18:1-cis (n9), and C22:6n-3. The major amino acids in the samples were glutamic acid, aspartic acid, glycine, leucine, alanine, lysine, and arginine. The abundant free amino acids in the FSM-added group were taurine, glutamic acid, alanine, leucine, and arginine. Six free sugars were found in all groups. Glucose was predominant, followed by mannose, rhamnose, fucose, fructose, and ribose. Among the three organic acids in the whole body of black rockfish, lactic acid was predominant, followed by citric acid and oxalic acid. Total organic acid content in the control was significantly higher than those of FSM substitution groups.

Keywords: Fish skin; Black rockfish; Organic acid; Free sugar; Fatty acid; Amino acid

Introduction

Numerous studies have been investigated vegetable and animal proteins that could replace fishmeal in fish feed. In particular, there have been many studies on the use of vegetable protein sources such as soybean meal [1-4], cottonseed meal, and rapeseed meal [5,6], which have a relatively stable supply compared to fishmeal, to replace fishmeal as a source of protein. However, plant resources are constantly in competition with livestock and human consumption, and the recent development of plant extract fuels such as bioethanol will eventually lead to an increase in the price of the plant resources usable as protein resources [7]. The by-products of the processing of terrestrial livestock such as cows, chickens, and pigs could be used as animal protein sources, since they have a relatively high protein content and qualitatively similar amino acid composition to fishmeal, and are inexpensive and stably supplied. Various studies have been conducted on their use as protein sources to replace fishmeal in fish feed [8-13]. However, the rise of safety issues due to serious infectious diseases like mad cow disease, swine fever, and avian influenza has gradually restricted the use of livestock by-products lately. Thus, as there are economic and safety issues with using terrestrial protein sources to replace fishmeal, securing economic and safe protein sources from marine products rather than terrestrial products is necessary. Many researchers have investigated by-products obtained from processing marine animal as potential protein sources, including shrimp by-products [14], tuna muscle by-products [15], shrimp and fish by-products [16], squid liver meal mixing soybean meal with by-products of squid processing [11], fish bone and crab by-products [17-19], and fish by-products [20]. Of the fishery by-products, even though fish skins obtained from the consumption of raw fish are a good protein source because of high collagen content, by-products such as bones and internal organs are only partially used and mostly discarded.

Therefore, this study was conducted to investigate improvement of quality and physiological function on cultured black rockfish fed diets substituted with different levels (0, 5, 10, and 20%) of flounder skin meal (FSM).

Materials and Methods

The skin of *Paralichthys olivaceus*, which has the highest farming yield and raw fish consumption in Korea, is easy to secure in large quantities due to its low use, thickness, and high collagen content, and was obtained from nearby fish markets. The fish skin was washed with fresh water and was subjected to hot air drying (50-60°C) followed by grinding via a high speed grinder (ZM-1000, Retsch Co., Japan) to prepare flounder skin meal (FSM).

After acclimation for 2 months in a square stock tank (running water system, 6.0 m×6.0 m×1.2 m) at the Fisheries Science Institute, Chonnam National University, Korea., 45 juvenile fish (mean body weight, 10.05 ± 0.44 g) were randomly selected from the stock tank and transferred to separate 300-L rectangular tanks (running water system, 1.0 m×0.8 m×0.8 m). The flow rate of filtered seawater in each tank was adjusted to 5 L/min. Mean water temperature, salinity, and dissolved oxygen were 20.2 ± 2.3°C, 32.0 ± 1.2 psu, and 6.3 ± 0.4 mg/L, respectively, and were measured using a YSI-85 (YSI, Ohio, USA) probe. The rearing trial was conducted in triplicate for each tested diet. The fish were fed twice a day (at 0800 h and 1600 h), until apparent satiation, for 8 weeks. The amount of feed given to each tank was recorded daily to calculate feeding efficiency.

Ingredients and proximate compositions of the experimental diets in response to FSM substitution and the results of vitamin C analyses are shown in Table 1. Proximate analyses were carried out to evaluate

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Ingredient	g/100 g			
	Control (0)	5	10	20
White fish meal	41	41	41	41
Casein	20	15	10	-
Flounder skin meal (FSM)	-	5	10	20
L-ascorbic acid	0.02	0.02	0.02	0.02
Wheat flour	22.58	23.28	23.98	25.28
Feed oil (squid liver oil)	8.4	7.7	7	5.7
α-potato starch	3	3	3	2
Vitamin premix[1)] (vitamin C free)	2	2	2	2
Mineral Premix[2)]	2	2	2	1
Choline Chloride	1	1	1	1
Total	100	100	100	100
Vitamin C in diets	212.41	197.16	204.67	203.12
Proximate analysis				
Protein	46.71	47.21	47.11	46.11
Lipid	9.21	9.51	10.11	10.81
Ash	8.21	8.61	8.71	8.71

[1)]Vitamin premix (mg/g mixture): retinol acetate, 0.81 mg; cholecalciferol 0.012 mg; vitamin E, 22.5 mg; vitamin K_3, 2.5 mg, thiamine, 5.5 mg; riboflavin, 10 mg; pyridoxine, 6 mg; niacin, 37.5 mg; folic acid, 2 mg; biotin 0.05 mg; inositol 50 mg. All ingredients were diluted with alpha-cellulose to 1g.

[2)]Mineral premix (mg/g mixture): Mn, 3.2 mg; Zn, 3.2 mg; Fe, 3.0 mg; Cu, 0.36 mg; $MgSO_4$, 100 mg; KCl (47%), 60 mg; $Al(OH)_3$, 1.06 mg; $Ca(IO_3)_2$, 0.475 mg; $CoSO_4$, 0.475 mg. All ingredients were diluted with alpha-cellulose to 1g.

*Dry matter

Table 1: Ingredient and proximate composition of experimental diets with various levels of FSM.

the nutritional composition of the prepared diets, and the vitamin C content of the diets were analyzed using the 2,4-dinitrophenyl hydrazine (DNP) colorimetric method [21].

FSM is a high protein meal containing more than 80% crude proteins but is lacking in essential amino acids compared to fishmeal. When fishmeal is replaced by FSM, unknown factors present in essential amino acids and fishmeal may affect the experimental fish, making it difficult to evaluate the influences of FSM substitution on experimental fish. Therefore, to maintain proper balances of the essential amino acids and to minimize the effects of the unknown factors in the fishmeal, white fishmeal (FF Skagen LT Supreme, Denmark) was fixed at the same level throughout the experimental diets. Casein, a purified protein, was used to control the protein content of each experimental diet. Squid liver oil (Ihwa, Korea) rich in DHA and EPA, which are essential fatty acids for the black rockfish, was used as a lipid source. Wheat flours (CJ, Korea) and α-potato starch were employed as carbohydrate sources to control energy and bind the diets. To find the relationship between the level of vitamin C and FSM substitution on the fish body, vitamin C (200 mg/kg) was added based upon the vitamin C requirements of the black rockfish reported by Bai et al. [22]. There were a total of 4 experimental groups, including a control group with fishmeal and casein only and 3 experimental groups with 5, 10, or 20% of the casein replaced by FSM.

Analyzes of proximate composition, fatty acid, total amino acid, free amino acid, organic acid, and free sugar in this study were carried out based on AOAC methods [23] and some modifications of Hwang et al. [24].

All mean values were analyzed via one-way analysis of variance (ANOVA). When differences were found among data, Duncan's multiple range test was used to compare the mean difference by using the SPSS software package version 17 (Statistical Package for Social Sciences, SPSS Inc., Chicago, IL, USA). Differences were considered significant at $p<0.05$.

Results

Proximate compositions with various levels of FSM are shown in Table 2. Crude protein was significantly higher in the FSM groups than the control group. FSM 5% was much higher than FSM 10% and FSM 20% ($P<0.05$). Crude lipid was significantly lower in the FSM groups than the control group, especially in FSM 20% compared to that of FSM 5% and FSM 10% ($P<0.05$).

The fatty acid composition of the whole body is shown in Table 3. Saturates were observed to be significantly lower in all FSM groups than the control group ($P<0.05$), and no significant differences were found in monoenes ($P<0.05$). Significantly higher polyenes were observed in the FSM groups than the control group, especially in FSM 20% ($P<0.05$). The control group was also significantly lower in n-3 than FSM 5% and FSM 20% ($P<0.05$), whereas FSM 10% was not significantly different from the control group. There were no significant differences in n-6 between the experimental groups. The n-3/n-6 ratio was not significantly different between the control group and FSM 5% and FSM 10%, while FSM 20% was significantly higher than the control group ($P<0.05$).

The whole-body amino acid contents are shown in Table 4. The total amino acids and EAA were significantly higher in FSM 20% than the other groups ($P<0.05$). FSM 20% was significantly higher in all amino acids compared to the control group ($P<0.05$), whereas FSM 5% and FSM 10% were not significantly different from the control group.

The whole-body free amino acid contents are shown in Table 5. The total free amino acids were significantly lower in the FSM groups than the control group, and the FSM groups tended to decreased significantly in a dose-dependent manner ($P<0.05$).

Seven kinds of free sugars were analyzed, and fucose, rhamnose, glucose, mannose, fructose, and ribose were detected but not galactose (Table 6). The total free sugars were not significantly different in the control group, FSM 5%, and FSM 10%, while they were significantly lower in FSM 20% ($P<0.05$).

Six kinds of organic acids were analyzed, and lactic acid, oxalic acid, and citric acid were found, but not malic acid, tartaric acid, or maleic acid (Table 7). The total organic acid content was significantly lower in the FSM groups than the control group ($P<0.05$).

Discussion

In the previous result, it was confirmed that there was a high collagen content (approximately 20% dry weight) in *Paralichthys olivaceus* skin [25]. Flounder skin meal (FSM) replaced various fractions of the casein

Proximate composition (g/100 g)	FSM substitution level (%)			
	Control (0)	5	10	20
Moisture	68.14 ± 1.28^{a}	67.93 ± 0.47^{a}	68.73 ± 0.05^{ab}	69.54 ± 0.07^{b}
Crude protein	14.28 ± 0.07^{a}	15.10 ± 0.03^{d}	14.75 ± 0.08^{b}	14.93 ± 0.04^{c}
Crude lipid	10.53 ± 0.22^{c}	9.72 ± 0.02^{b}	9.20 ± 0.25^{b}	8.25 ± 0.53^{a}
Ash	3.77 ± 0.07^{a}	4.11 ± 0.08^{c}	3.92 ± 0.08^{b}	4.04 ± 0.03^{bc}

Data are mean ± SD. Values with different superscripts are significantly different (*P<0.05*).

[ns]Not significant.

Table 2: Proximate composition (%) of whole body in black rockfish (*S. schlegeli*) fed the test diets with various levels of FSM for 8 weeks.

Fatty acid	FSM substitution level (%)			
	Control (0)	5	10	20
C12:0	0.31 ± 0.01^{d}	0.26 ± 0.01^{c}	0.24 ± 0.01^{b}	0.19 ± 0.00^{a}
C13:0	0.05 ± 0.01^{ns}	0.05 ± 0.00	0.05 ± 0.00	0.05 ± .00
C14:0	7.42 ± 0.05^{b}	6.91 ± 0.23^{a}	7.14 ± 0.17^{ab}	7.33 ± 0.07^{b}
C15:0	0.96 ± 0.07^{a}	1.00 ± 0.02^{ab}	1.00 ± 0.01^{ab}	1.04 ± 0.02^{b}
C16:0	24.07 ± 0.06^{b}	23.23 ± 0.46^{a}	23.69 ± 0.22^{ab}	23.44 ± 0.15^{a}
C17:0	0.79 ± 0.01^{bc}	0.79 ± 0.00^{c}	0.75 ± 0.03^{ab}	0.72 ± 0.02^{a}
C18:0	6.69 ± 0.10^{b}	6.81 ± 0.13^{b}	6.63 ± 0.16^{b}	6.24 ± 0.07^{a}
C20:0	1.64 ± 0.04^{c}	1.03 ± 0.03^{b}	0.96 ± 0.02^{a}	0.92 ± 0.05^{a}
C21:0	0.57 ± 0.02^{ns}	0.56 ± 0.11	0.55 ± 0.01	0.50 ± 0.05
C22:0	0.81 ± 0.01^{a}	0.81 ± 0.01^{a}	0.76 ± 0.01^{b}	0.72 ± 0.01^{a}
C23:0	1.12 ± 0.02^{a}	1.25 ± 0.06^{b}	1.34 ± 0.03^{c}	1.55 ± 0.03^{d}
C24:0	1.43 ± 0.02^{a}	1.50 ± 0.01^{c}	1.45 ± 0.01^{ab}	1.47 ± 0.03^{bc}
Saturates	45.87 ± 0.23^{b}	44.21 ± 0.60^{a}	44.58 ± 0.21^{a}	44.18 ± 0.06^{a}
C14:1	0.32 ± 0.01^{d}	0.27 ± 0.01^{a}	0.30 ± 0.01^{b}	0.31 ± 0.00^{c}
C15:1	0.02 ± 0.00^{ns}	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00
C16:1	6.29 ± 0.03^{b}	5.99 ± 0.10^{a}	6.33 ± 0.02^{b}	6.46 ± 0.07^{c}
C17:1	0.45 ± 0.01^{a}	0.45 ± 0.01^{a}	0.46 ± 0.00^{a}	0.49 ± 0.01^{b}
C18:1n9t	0.53 ± 0.19^{ns}	0.37 ± 0.17	0.46 ± .12	0.39 ± 0.10
C18:1n9c	20.43 ± 0.15^{b}	20.45 ± 0.25^{b}	20.40 ± 0.08^{b}	19.89 ± 0.13^{a}
C20:1	4.65 ± 0.11^{ab}	5.15 ± 0.34^{b}	4.72 ± 0.51^{ab}	4.23 ± 0.20^{a}
C22:1n9	0.96 ± 0.01^{a}	0.97 ± 0.01^{ab}	0.99 ± 0.01^{bc}	0.99 ± 0.01^{c}
C24:1	0.89 ± 0.11^{ns}	1.06 ± 0.17	1.04 ± 0.17	1.14 ± 0.16
Monoenes	34.55 ± 0.28^{ns}	34.73 ± 0.52	34.71 ± 0.61	33.92 ± 0.49
C18:2n6t	0.17 ± 0.01^{ns}	0.17 ± 0.01	0.17 ± 0.01	0.12 ± 0.08
C18:2n6c	0.43 ± 0.01^{ns}	0.42 ± 0.01	0.43 ± 0.01	0.43 ± 0.01
C20:2	0.77 ± 0.00^{a}	0.83 ± 0.02^{b}	0.78 ± 0.02^{a}	0.76 ± 0.02^{a}
C22:2	0.07 ± 0.01^{a}	0.11 ± 0.01^{b}	0.10 ± 0.02^{ab}	0.09 ± 0.02^{ab}
C18:3n6	0.33 ± 0.01^{a}	0.36 ± 0.01^{b}	0.33 ± .01^{a}	0.35 ± 0.01^{b}
C18:3n3	1.31 ± 0.01^{ns}	1.32 ± 0.04	1.30 ± 0.02	1.30 ± 0.01
C20:3n6	0.03 ± 0.01^{ns}	0.02 ± 0.00	0.02 ± 0.00	0.03 ± 0.01
C20:3n3	0.15 ± 0.01^{ns}	0.17 ± 0.04	0.15 ± 0.01	0.16 ± 0.02
C20:4n6	0.26 ± 0.00^{a}	0.28 ± 0.01^{b}	0.29 ± 0.00^{c}	0.31 ± 0.00^{d}
C20:5n3	0.00 ± 0.00^{a}	0.28 ± 0.08^{c}	0.19 ± 0.02^{b}	0.18 ± 0.02^{b}
C22:6n3	16.04 ± 0.47^{a}	17.09 ± 0.87^{ab}	16.95 ± 0.42^{a}	18.17 ± 0.40^{b}
Polyenes	19.57 ± 0.47^{a}	21.06 ± 0.87^{b}	20.71 ± 0.41^{b}	21.91 ± 0.47^{c}
n3	17.51 ± 0.48^{a}	18.87 ± 0.88^{bc}	18.59 ± 0.45^{ab}	19.82 ± 0.43^{c}
n6	1.22 ± 0.01^{ns}	1.25 ± 0.03	1.24 ± 0.02	1.24 ± 0.08
n3/n6	14.31 ± 0.33^{a}	15.07 ± 1.00^{ab}	15.04 ± 0.52^{ab}	16.06 ± 0.73^{b}

Data are mean ± SD. Values with different superscripts are significantly different (*P<0.05*).

^{ns}Not significant.

Table 3: Fatty acid composition of whole body in black rockfish (*S. schlegeli*) fed the test diets with various levels of FSM for 8 weeks (g/100 g).

substitution in the fishmeal, and this was fed to the fish for 8 weeks to confirm the substitution effect.

Comparing the results of such fishmeal substitution is difficult because of the various protein sources and fish utilized. However, the nutritional characteristics of the alternative protein sources affect lipid metabolism in the body. In particular, as FSM contains relatively higher lipid content (17%) compared to white and brown fishmeal (7-8%), it would affect lipid accumulation and metabolism. In the FSM substitution group, the protein content in the whole body increased and the lipid content decreased, and the ash content increased significantly compared to the control group. The body composition of fish is affected by various factors such as intraspecific strain differences, water temperature, and increased body weight, and is influenced the most by the amount of feed supplied and the mix proportions of the feed [26,27].

Among whole-body fatty acid responses to the FSM substitution in the feed, saturated fatty acids significantly decreased while polyunsaturated fatty acids increased, especially n-3 HUFA, which is an essential fatty acid in black rockfish [28]. The white fishmeal used in this study contained about 0.7% n-3 HUFA, and fish oil, like the squid liver oil used as a lipid source, has more than 20% n-3 HUFA with an appropriate EPA/DHA ratio [29]. Accumulation of n-3 HUFA in fish bodies increased because of the FSM substitution. This seems to be because of FSM-specific amino acids rather than the differences in n-3 HUFA in the feed.

Amino acids in fish were significantly higher only in FSM 20% than the control group. Although such differences may occur due to the FSM substitution, this is not certain because significant differences in the amino acids were not observed between the control group and the experimental groups. However, the free amino acids in the fish body were significantly decreased in the FSM groups compared to the control group, suggesting that the FSM substitution influenced amino acid metabolism in the fish. In marine animals, free amino acids provide chemical signals for behaviors, communication, and metabolism through sensory organs [30]. Moreover, free amino acids act as substrates for protein biosynthesis or aerobic catabolism and provide osmolality stably during embryonic stages through intrinsic nutrients in marine fish [31,32].

Lactic acids were the most abundant organic acids, especially in the FSM substitution groups. Lactic acids are known to greatly differ based on the amount of activity at the time of harvesting and the storage conditions [33]. However, lactic acids showed significant differences in the FSM substitution group compared to the control group in the present study. Given that the amount of activity at the time of harvesting and storage conditions were similar in this study, lactic acids seem to have an influence on the energy metabolism of glycolysis with respect

	FSM substitution level (%)			
	Control (0)	5	10	20
Aspatic acid	2.97 ± 0.07^{a}	3.20 ± 0.30^{a}	3.17 ± 0.04^{a}	3.58 ± 0.12^{b}
*Threonine	1.54 ± 0.05^{a}	1.66 ± 0.16^{ab}	1.73 ± 0.21^{ab}	1.82 ± 0.04^{b}
Serine	1.85 ± 0.06^{a}	2.02 ± 0.19^{ab}	2.01 ± 0.10^{ab}	2.23 ± 0.07^{b}
Glutamic acid	3.78 ± 0.09^{a}	4.12 ± 0.39^{a}	4.08 ± 0.15^{a}	4.60 ± 0.16^{b}
Proline	1.59 ± 0.03^{a}	1.73 ± 0.17^{a}	1.67 ± 0.06^{a}	1.97 ± 0.09^{b}
Glycine	4.37 ± 0.11^{a}	4.89 ± 0.45^{a}	4.58 ± 0.11^{a}	5.62 ± 0.33^{b}
Alanine	3.11 ± 0.09^{a}	3.41 ± 0.31^{a}	3.18 ± 0.10^{a}	3.79 ± 0.17^{b}
Cystine	0.11 ± 0.00^{a}	0.12 ± 0.03^{ab}	0.13 ± 0.01^{ab}	0.15 ± 0.01^{b}
*Valine	1.61 ± 0.05^{a}	1.74 ± 0.18^{ab}	1.74 ± 0.03^{ab}	1.93 ± 0.06^{b}
*Methionine	0.81 ± 0.02^{a}	0.86 ± 0.10^{a}	0.86 ± 0.01^{a}	0.97 ± 0.03^{b}
*Isoleucine	1.23 ± 0.02^{a}	1.32 ± 0.13^{a}	1.31 ± 0.01^{a}	1.46 ± 0.04^{b}
*Leucine	2.22 ± 0.05^{a}	2.37 ± 0.23^{a}	2.34 ± 0.01^{a}	2.60 ± 0.07^{b}
*Tyrosine	0.57 ± 0.03^{a}	0.61 ± 0.11^{a}	0.67 ± 0.04^{a}	0.76 ± 0.01^{b}
*Phenylalanine	0.96 ± 0.02^{a}	1.07 ± 0.11^{a}	1.04 ± 0.05^{a}	1.19 ± 0.04^{b}
*Histidine	0.81 ± 0.02^{a}	0.91 ± 0.07^{b}	0.86 ± 0.01b^{a}	0.93 ± 0.02^{b}
*Lysine	1.72 ± 0.04^{a}	1.86 ± 0.21^{a}	1.94 ± 0.03^{a}	2.25 ± 0.08^{b}
Ammonia	2.28 ± 0.05^{a}	2.42 ± 0.17^{ab}	2.32 ± 0.07^{a}	2.58 ± 0.11^{b}
*Arginine	1.42 ± 0.04^{a}	1.48 ± 0.18^{a}	1.52 ± 0.02^{a}	1.76 ± 0.07^{b}
Total	32.96 ± 0.82^{a}	35.80 ± 3.44^{a}	35.17 ± 0.76^{a}	40.18 ± 1.49^{b}
*EAA	12.32 ± 0.31^{a}	13.28 ± 1.35^{a}	13.35 ± 0.34^{a}	14.90 ± 0.45^{b}

Data are mean ± SD. Values with different superscripts are significantly different (*P<0.05*).

^{ns}Not significant.

*Essential amino acid.

Table 4: Total amino acid content of whole body in black rockfish (*S. schlegeli*) fed the test diets with various levels of FSM for 8 weeks (g/100 g).

	FSM substitution level (%)			
	Control (0)	5	10	20
Phosphoserine	0.77 ± 0.03^{b}	0.84 ± 0.01^{c}	0.78 ± 0.02^{b}	0.62 ± 0.02^{a}
Taurine	3.31 ± 0.04^{a}	3.27 ± 0.17^{a}	3.41 ± 0.04^{a}	3.57 ± 0.09^{b}
Aspartic acid	3.31 ± 0.02^{d}	2.51 ± 0.02^{c}	1.61 ± 0.04^{b}	1.35 ± 0.02^{a}
Hydroxyproline	1.68 ± 0.05^{b}	1.23 ± 0.27^{a}	1.89 ± 0.13^{b}	1.70 ± 0.18^{b}
Threonine	3.18 ± 0.04^{a}	2.26 ± 0.06^{b}	1.54 ± 0.05^{c}	1.13 ± 0.02^{d}
Serine	2.19 ± 0.02^{d}	1.84 ± 0.09^{c}	1.39 ± 0.04^{b}	0.91 ± 0.02^{a}
Asparagine	0.88 ± 0.06^{b}	0.30 ± 0.02^{a}	0.30 ± 0.01^{a}	0.31 ± 0.00^{a}
Glutamic acid	5.46 ± 0.10^{d}	3.91 ± 0.05^{c}	2.57 ± 0.07^{b}	2.36 ± 0.05^{a}
Proline	1.95 ± 0.04^{d}	1.51 ± 0.10^{c}	1.19 ± 0.03^{b}	0.75 ± 0.03^{a}
Glycine	2.75 ± 0.03^{c}	2.97 ± 0.11^{d}	2.57 ± 0.09^{b}	2.38 ± 0.01^{a}
Alanine	5.95 ± 0.05^{d}	4.92 ± 0.08^{c}	3.79 ± 0.13^{b}	3.02 ± 0.01^{a}
Citrulline	0.34 ± 0.03^{d}	0.22 ± 0.04^{c}	0.11 ± 0.01^{b}	0.05 ± 0.00^{a}
Valine	3.10 ± 0.02^{d}	2.07 ± 0.05^{c}	1.53 ± 0.05^{b}	0.99 ± 0.02^{a}
Methionine	0.86 ± 0.02^{d}	0.48 ± 0.01^{c}	0.41 ± 0.02^{b}	0.32 ± 0.02^{a}
Isoleucine	2.46 ± 0.01^{d}	1.62 ± 0.02^{c}	1.10 ± 0.04^{b}	0.73 ± 0.01^{a}
Leucine	7.33 ± 0.01^{d}	4.33 ± 0.03^{c}	3.34 ± 0.13^{b}	2.54 ± 0.01^{a}
Tyrosine	1.35 ± 0.06^{b}	1.62 ± 0.06^{c}	1.44 ± 0.06^{b}	0.99 ± 0.03^{a}
Phenylalanine	2.84 ± 0.01^{d}	1.61 ± 0.02^{c}	1.33 ± 0.03^{b}	0.95 ± 0.01^{a}
β-aminoisobutyric acid	1.06 ± 0.07^{b}	0.53 ± 0.02^{a}	0.61 ± 0.12^{a}	0.55 ± 0.03^{a}
γ-amino-n-butyric acid	0.03 ± 0.01^{b}	0.01 ± 0.00^{a}	0.01 ± 0.00^{a}	0.02 ± 0.00^{ab}
Histidine	0.95 ± 0.01^{d}	0.56 ± 0.02^{c}	0.45 ± 0.02^{b}	0.20 ± 0.01^{a}
1-methylhistidine	0.06 ± 0.00^{b}	0.03 ± 0.01^{a}	0.02 ± 0.00^{a}	0.01 ± 0.01^{a}
Carnosine	0.21 ± 0.01^{c}	0.13 ± 0.03^{a}	0.14 ± 0.01^{a}	0.07 ± 0.02^{a}
Anserine	0.29 ± 0.06^{ns}	0.29 ± 0.05	0.31 ± 0.07	0.32 ± 0.01
Tryptopan	0.51 ± 0.09^{b}	0.22 ± 0.08^{a}	0.17 ± 0.07^{a}	0.09 ± 0.04^{a}
Hydroxylysine	0.11 ± 0.01^{b}	0.05 ± 0.01^{a}	0.04 ± 0.01^{a}	0.05 ± 0.02^{a}
Ornitine	0.19 ± 0.01^{c}	0.09 ± 0.01^{b}	0.06 ± 0.01^{a}	0.06 ± 0.00^{a}
Lysine	2.99 ± 0.07^{d}	0.77 ± 0.03^{c}	0.49 ± 0.02^{b}	0.33 ± 0.02^{a}
Ammonia	2.20 ± 0.03^{c}	1.86 ± 0.16^{a}	1.42 ± 0.03^{a}	1.55 ± 0.02^{b}
Ethanolamine	0.36 ± 0.08^{ns}	0.40 ± 0.03	0.34 ± 0.03	0.38 ± 0.04
Arginine	8.71 ± 0.12^{d}	5.84 ± 0.18^{c}	5.25 ± 0.16^{b}	3.57 ± 0.05^{a}
Total	67.37 ± 0.26^{d}	48.31 ± 0.99^{c}	39.61 ± 1.29^{b}	31.88 ± 0.22^{a}

Data are mean ± SD. Values with different superscripts are significantly different (*P<0.05*).
^{ns}Not significant.

Table 5: Free amino acid content of whole body in black rockfish (*S. schlegeli*) fed the test diets with various levels of FSM for 8 weeks (g/100 g).

	FSM substitution level (%)			
	Control (0)	5	10	20
Fucose	150.20 ± 15.39^{ns}	155.82 ± 10.22	132.53 ± 14.89	145.44 ± 15.64
Rhamnose	354.54 ± 35.57^{d}	284.62 ± 27.03^{c}	136.03 ± 32.66^{b}	82.35 ± 0.55^{a}
Glucose	280.99 ± 5.25^{a}	424.50 ± 31.76^{b}	464.12 ± 27.22^{b}	289.80 ± 19.75^{a}
Mannose	190.91 ± 11.39^{a}	217.63 ± 32.91^{a}	319.56 ± 14.43^{b}	207.09. ± 17.14^{a}
Fructose	76.05 ± 8.64^{a}	87.12 ± 2.18^{ab}	105.61 ± 7.69^{bc}	109.35 ± 15.86^{c}
Ribose	81.90 ± 6.05^{c}	75.70 ± 7.02^{bc}	65.63 ± 4.73^{b}	49.10 ± 7.70^{a}
Total	1,134.59 ± 82.29^{b}	1,245.38 ± 111.11^{b}	1,223.49 ± 101.61^{b}	883.14 ± 76.63^{a}

Data are mean ± SD. Values with different superscripts are significantly different (*P<0.05*).
^{ns}Not significant.

Table 6: Free sugar content of whole body in black rockfish (*S. schlegeli*) fed the test diets with various levels of FSM for 8 weeks (mg/L).

	FSM substitution level (%)			
	Control (0)	5	10	20
Lactic acid	12.12 ± 0.34^{c}	10.11 ± 0.09^{b}	7.79 ± 1.63^{a}	9.09 ± 0.13^{ab}
Oxalic acid	0.19 ± 0.01^{b}	0.15 ± 0.02^{a}	0.13 ± 0.01^{a}	0.15 ± 0.03^{a}
Citric acid	0.72 ± 0.03^{a}	0.68 ± 0.02^{ab}	0.65 ± 0.03^{a}	0.71 ± 0.02^{b}
Total	13.03 ± 0.12^{c}	10.90 ± 0.18^{b}	8.57 ± 1.60^{a}	9.95 ± 0.16^{ab}

Data are mean ± SD. Values with different superscripts are significantly different (P<0.05).
^{ns}Not significant.

Table 7: Organic acid content of whole body in black rockfish (*S. schlegeli*) fed the test diets with various levels of FSM for 8 weeks (mg/L).

to the FSM substitution.

The glucose content in the free sugars was significantly higher in FSM 5% and FSM 10% than the control group; but no significant differences between control and FSM 20%, indicating that FSM substitution affect glucose metabolism, resulting in differences in body glucose. Ribose differed in a similar manner, leading to glucose levels decreased in response to the FSM substitution. Free ribose is abundant in the muscles of living fish but is also released from inosine, which is produced by ATP decomposition after death. Consequently, ribose content can depend on the pretreatment conditions of the samples immediately after the instant killing [1]. As ribose, along with fructose, is a quantitatively important factor in glycolysis, it is considered to be somewhat associated with glucose metabolism.

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