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Evaluation of a Newly Developed Fish Meal Analogue (BAIFA-M) in Immature Korean Rockfish, *Sebastes schlegeli*, Reared in Cage Culture System

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Four experimental diets were formulated to contain white fish meal (WFM) and/or BAIFA-M as the main animal protein source to determine the optimum BAIFA-M level to replace WFM protein. The diets contained 100 % WFM + 0 % BAIFA-M in the diet M_0 (control) · 70 % WFM+30 % BAIFA-M in the diet M_{30}, 55 % WFM+45 % BAIFA-M in the diet M_{45}, ; 40 % WFM+60 % BAIFA-M in the diet M_{60}. The four groups of rockfish (232 g), receiving the four different diets, were fed for 8 weeks. Groups receiving M_0, M_{30} and M_{45} diets did not show significant (P>0.05) difference in growth and feed efficiency; however, the group fed on M_{60} showed significantly slower growth and lower feed efficiency than the others. Body composition showed no significant difference among the four dietary groups. WFM protein can be replaced by BAIFA-M up to 45 % in the diet of the rockfish.

Key words : Fish meal analogue, Korean rockfish, Animal protein source

Introduction

Korean rockfish, *Sebastes schlegeli*, is one of the important commercial marine aquaculture species in Korea. Actually, this species has several desirable characteristics for aquaculture including high tolerance both to high and low water temperatures, easiness of seedling production due to viviparous and reproductive style, and withstanding in high stocking density. Farming of this species has been rapidly developed since 1987, and aquaculture production in 1999 reached 10,181 mt which is second largest in finfish mariculture production followed by olive flounder, *Paralichthys olivaceus* (MMAF, 2000). Fish meal, which has a good palatability and a high nutritional value in protein content and quality, has been being used as the main protein source of fish feeds from the past. Nevertheless, due to fish meal is expensive and one of the main water pollution sources, nutritionists have anxiously been to seek the cheap and high-quality protein sources as the fish meal replacer during the past decades (Fowler, 1991; Hardy and Masumoto, 1990; Watanabe et al., 1993; Luzier and Summerfelt, 1992; Luzier et al., 1995; Lee et al., 1996; Lee and Bai, 1997a, b; Kim and Bai, 1999).

In recent years, the commercial products of fish meal analogue that was made mainly by mixing the animal protein sources have been developed (Rodreguez-Serna et al., 1996; Kim and Bai, 1997; Park et al., 1999). Our laboratory has been developed the commercial fish meal analogue (BAIFA-M). BAIFA-M was formulated by a specific ratio of mixing six animal protein sources such as blood meal, squid liver powder, meat and bone meal, leather meal, feather meal and poultry by-products, containing 69.1% crude protein and 12.3% crude ash (Table 2). This is accordance to the results of Hardy and Masumoto (1990) who had reported that this new product of fish meal analogue should contain no less than 35% protein had no measurable antiproteinase activity, and match amino acid profiles of fish meal, less than 15% ash, and no less than 0.5% of available phosphorus. Specification for fish meal analogue including in diet formulation requires consideration of essential fatty acid requirement and preventing degradation of the fish meal analogue during storage. From

Table 1. Composition and proximate analyses of the experimental diets (% of the DM basis)[1]

Ingredients	Experimental diet			
	M_0	M_{30}	M_{45}	M_{60}
White fish meal[1]	45.00	31.50	24.75	18.00
Fish meal analogue[2]	-	13.62	20.44	27.25
Corn gluten meal[3]	15.00	15.00	15.00	15.00
Soybean meal[3]	12.00	12.00	12.00	12.00
Wheat flour[4]	21.50	21.50	21.55	21.95
Fish oil[4]	3.50	2.70	2.20	1.67
EPA-DHA (45%)[5]	1.00	1.00	1.00	1.00
Vitamin premixture[6]	1.00	1.00	1.00	1.00
Mineral premixture[7]	1.00	1.00	1.00	1.00
Cellulose	-	0.68	1.06	1.13
Proximate analysis				
Moisture	30.6	29.8	30.1	30.1
Crude protein	48.4	49.0	49.4	49.3
Crude lipid	10.5	9.20	8.45	8.20
Crude ash	9.84	8.88	8.60	8.03

[1]Han Chang Fish meal Co., Korea.
[2]Mixture of the following ingredients : blood meal, squid liver powder, meat and bone meal, leather meal, feather meal and poultry by-products.
[3]Kum Sung Feed Co., Pusan, Korea.
[4]Young Nam Flourmills Co., Pusan, Korea.
[5]E-Hwa Oil & Fat Ind. Co., Ltd., Pusan, Korea.
[6]Contains (as mg/kg in diets) : Ascorbic acid, 300; dl-Calcium pantothenate, 150; Coline bitartrate, 3000; Inositol, 150; Menadione (Vit. K_3), 6; Niacin, 150; Pyridoxine · HCl (Vit. B_6), 15; Riboflavin (Vit. B_2), 30; Thiamine mononitrate, 15; dl-α-Tocopherol acetate, 201; Vit. A, 6; Biotin, 0.45; Folic acid, 1.62; Vit. B_{12}, 0.018; Cholecalciferol, 0.72.
[7]Contains (as mg/kg in premixture) : NaCl, 437.4; $MgSO_4 \cdot 7H_2O$, 1379.8; $NaH_2PO_4 \cdot 2H_2O$, 877.8; $Ca(H_2PO_4)_2 \cdot 2H_2O$, 1366.7; KH_2PO_4, 2414; $ZnSO_4 \cdot 7H_2O$, 226.4; Fe-Citrate, 299; Ca-lactate, 3004; $MnSO_4$, 0.016; $FeSO_4$, 0.0378; $CuSO_4$, 0.0031; $CoSO_4$, 0.00033; Calcium iodate, 0.0006; MgO, 0.00135; $NaSeO_3$, 0.00025

the above this product, replacement of BAIFA-M up to 40% did not show differences in growth rates and nutrient utilization in juvenile Korean rockfish of 2.8 g initial weight for 6 weeks (Kim and Bai, 1997). However, fish meal analogue (BAIFA-M) has not been tested in young adult Korean rockfish.

Therefore, based on our previous experimental results, this study was conducted to determine the potential use of a newly developed fish meal analogue (BAIFA-M) as a fish meal replacer and to determine the replacing level of BAIFA-M in diets for young adult Korean rockfish, *Sebastes schlegeli*.

Materials and Methods

Experimental design and diets

Compositions and proximate analyses of the experimental diets are shown in Table 1. Fish meal analogue (BAIFA-M) was formulated by a specific ratio of mixing six animal protein sources such as blood meal, squid liver powder, meat and bone meal, leather meal, feather meal and poultry by-products. Percentage of the graded level of replacement of WFM by BAIFA-M on the basis of crude protein were as followings : diet M_0 (control), 100% WFM+0% BAIFA-M; diet M_{30}, 70% WFM+30% BAIFA-M; diet M_{45}, 55% WFM+45% BAIFA-M; diet M_{60}, 40% WFM+60% BAIFA-M. Four experimental diets were formulated on isonitrogenous and isocaloric basis of 48.0% crude protein and 16.5 kJ/g diet. Estimated available energy of experimental diets were adjusted to have 16.5 kJ/g (NRC, 1993). Proximate analyses of six animal protein sources and the fish meal analogue (BAIFA-M) are shown in Table 2. And, the essential amino acids composition of the experimental diets are shown

Table 2. Proximate (crude) composition of 6 animal protein sources and the fish meal analogue (% of dry matter basis)[1]

Composition	Protein	Lipid	ash	Moisture
Leather meal	68.5	16.7	7.8	4.1
Meat & bone meal	55.5	18.0	23.0	4.2
Feather meal	88.7	8.2	3.5	9.8
Squid liver powder	51.2	20.6	6.6	10.1
Poultry by-product	68.0	16.7	15.1	3.6
Blood meal	90.5	0.7	2.3	8.2
Analogue (BAIFA-M)[2]	69.1	14.4	12.3	5.2

[1]Values are means from duplicate groups of feedstuffs. .
[2]The specific ratio of mixing the above six animal protein sources cannot be given in this because this ratio is one the important formulation in the patent which is pending in Korea.

in Table 3. Procedures for diet preparation and storage were as previously described by Kim and Bai (1997). The experimental diets were stored at -20℃ until used.

Fish and feeding trial

Experimental fish averaging 232 ± 5.6g (mean ± SD) were provided by Yong Chang Fishery in Tongyong City, Kyongnam-Do, Korea. Prior to the feeding trial, fish were fed the commercial diets to adjust to the experimental conditions for 2 weeks in the experimental sea cage (1.5 × 1.5 × 3m) culture system. The experimental diets were fed for a total of 8 weeks. Triplicate groups of 25 fish per diet treatment were fed one of the experimental diets at a rate of 1.5 ~ 2.5% total body weight per day on a dry matter basis. Water temperature was 19.6 ± 1.6℃ (mean ± SD) during the experimental period, and fish were fed twice a day at 10:00h and 17:00h. Fish in each cage were collectively weighed every 4 weeks and the amount of diet fed was adjusted accordingly.

Sample Collections and Analyses

At the end of the feeding trial, all fish were weighed and counted to calculate weight gain (WG), feed efficiency (FE), specific growth rate (SGR), protein efficiency ratio (PER), condition factor (CF) and survival. Fish were then stored at 20℃ until analysis for hepatosomatic index (HSI) and whole-body composition. Three fish from each cage were randomly selected to determine HSI and CF. Blood samples were obtained from the caudal vein with a syringe. Hematocrit (PCV) was de-

Table 3. Essential amino acid (EAA) composition of the protein sources used in fish meal analogue (BAIFA-M)[1]

Essential amino acid	Diet			
	M_0	M_{30}	M_{45}	M_{60}
Arginine	2.87	2.65	2.54	2.48
Histidine	1.07	1.05	1.01	0.97
Lysine	2.98	3.04	3.12	3.20
Leucine	2.65	2.45	2.54	2.63
Isoleucine	1.88	1.85	1.89	1.93
Methionine[2]	1.62	1.66	1.59	1.54
Phenylalanine[3]	2.79	2.68	2.61	2.54
Threonine	1.48	1.51	1.55	1.60
Trypthophan	0.57	0.61	0.70	0.78
Valine	2.09	2.23	2.45	2.61

[1]Values are calculated from the ingredients used in this study; amino acid content of the ingredients were analyzed by the National Fisheries Research and Development Institute (NFRDI).
[2]In the presence of cysteine
[3]In the presence of tyrosine

termined on three fish randomly selected per cage by the microhaematocrit method (Brown, 1980), and hemoglobin was measured with the same fish by the cyan-methemoglobin procedure using Drabkin's solution. Hemoglobin standard prepared from human blood (Sigma Chemical, St. Louis MO) was used. Crude protein, ash and moisture of whole-body and the experimental diets were analyzed by the AOAC method (1995) and crude fat was determined by using the Soxtec system 1046 (Tecator AB, Sweden) after freeze-drying sample.

Statistical Analysis

All data were subjected to ANOVA using Statistix 3.1 (Analytical Software, St. Paul, MN, USA). When a significant treatment effect was observed, a Least Significant Difference (LSD) test was used to compare means. Treatment effects were considered significant at P<0.05.

Results and Discussion

Growth performance data of young adult Korean rockfish are shown in Table 4. Weight gain (WG, g) of fish fed diets M_{30} and M_{45} were not significantly different compared with that of fish fed diet M_0 (control diet; P>0.05). However, WG of fish fed diets M_{60} were significantly lower than that of fish fed the control diet (P<0.05). Feed efficiency (FE) of fish fed diets M_{30} and M_{45} were not significantly different from that of fish fed the control diet (P>0.05); fish fed diet M_{60} showed lower FE than the fish fed the control diet (P<0.05). Under our experimental conditions, the replacement of 45% of fish meal analogue (BAIFA-M) originating from fish meal in the control diet containing a high level of fish meal resulted in minimal changes in growth and feed efficiency. However, the replacement of 60% of the dietary fish meal protein resulted in reduced performance of the fish. The poor growth may have been caused by reduced palatability or by nutritional changes in the diets. These values are comparable to our previous results observed in Korean rockfish of this size with the similar diet and feeding strategy (Kim and Bai, 1997). Utilization of fish meal analogue (BAIFA-M) containing animal by-product protein sources has been investigated by the same authors in juvenile Korean rockfish. From the above

Table 4. Effects of the experimental diets on growth performance in the Korean rockfish[1]

	Diet				Pooled SEM[2]
	M_0	M_{30}	M_{45}	M_{60}	
Initial weight (g)	232	232	233	232	0.01
Final weight (g)	308	303	301	285	2.75
WG (g)[3]	76^a	71^a	68^a	53^b	1.19
FE (%)[4]	60.5^a	58.3^a	57.8^a	50.1^b	1.10
SGR (%)[5]	0.96^a	0.93^a	0.92^a	0.80^b	0.01
PER[6]	1.58^a	1.53^a	1.49^a	1.37^b	0.12
Survival	97.3	97.3	97.3	96.0	0.52
HSI[7]	2.57	2.59	2.63	2.97	0.09
Hemoglobin (g/dl)	7.82	7.78	7.95	7.68	0.06
Hematocrit (%)	38.6	38.2	38.0	38.0	0.54
CF[8]	1.59	1.80	1.80	1.60	0.05

[1]Values are means from triplicate groups of fish where the means in each row with a different superscript are significantly different (P<0.05)
[2]Pooled standard error of mean : $SD/\sqrt{n}$
[3]Weight gain (g) : final wt. - initial wt.
[4]Feed efficiency : (wet weight gain / dry feed intake) ×100
[5]Specific growth rate : (($\log_e$ final wt. - $\log_e$ initial wt.) / days) ×100
[6]Protein efficiency ratio : wet weight gain / protein intake (g)
[7]Hepatosomatic index : liver wt. × 100 / body wt.
[8]Condition factor : (wet body weight / total body length^3) ×100

growth performance of Korean rockfish, replacement of 45% fish meal by BAIFA-M doesn't has any adverse effects on the experimental fish. Under the reported conditions, the nutritional value of several alternate nutrient source for fish meal are not inferior to that of fish meal and their value can be increased significantly by an appropriate blend of other ingredients and addition of limiting nutrients. However, there is a realization that a considerable difference between fish species and protein sources is the reality (Watanabe et al., 1993; Tacon and Jackson, 1985; Smith et al., 1988; Pongmaneerat et al., 1993). Specific growth rate (SGR) and protein efficiency ratio (PER) obtained in this study (Table 4) were lower than those reported by Kim and Bai (1997). They found that the SGR and PER of Korean rockfish (average 2.8 g) were ranged from 1.29% to 1.50% per day and 2.02 ~ 2.46, respectively. There was a general decrease in protein efficiency corresponding to increase in fish meal analogue (BAIFA-M) in the diets. These results may indicate differences in digestibility and nutrient limitations of fish meal analogue (Meilahn et al., 1996). In the biological measurement, hepatosomatic index (HSI) of fish were not significantly different among all the dietary treatments (P>0.05). HSI values obtained in this study were lower compared to those (3.13 ~ 3.59) from 2.8 g fish (Kim and Bai, 1997) and similar to those (2.07 ~ 2.69) from average 25.1g initial size of Korean rockfish (Kim and Bai, 1999). Mortality was minimal, and no significant differences in survival rate observed among all the treatments means. These results may indicate that the replacement of fish meal protein by fish meal analogue (BAIFA-M) in Korean rockfish diets has no negative effects in HSI and survival rate compared to the control group. No significant difference was observed in hematocrit in the present experiment, approximately 38%. This result is similar to 35 ~ 42% from the average 12.6 g (Bai et al., 1996) and lower than 46 ~ 48% from the average 2.8 g initial size (Kim and Bai, 1999) in Korean rockfish. Hemoglobin values ranged from 7.68 to 7.95 g/dl in this experiment are similar to 8.0 ~ 8.3 g/dl from 12.6g (Bai et al., 1996) and 7.5 ~ 8.0 g/dl from 25.1g (Kim and Bai, 1999), but higher than the data 5.5 ~ 6.6 g/dl from 2.8 g initial size (Kim and Bai, 1997) in Korean rockfish. Usually, hemoglobin values of healthy fish is 10 g/dl (Post, 1993). Many researchers indicated that it can be changed by essential nutrient, environment and growth conditions in fish species.

Results of the whole-body analysis are shown in Table 5. Proximate analyses of whole-body are affected by environmental conditions, different genealogy, growth rates, feeding regimens and diet formulations (Nandeesha et al., 1995). Murai et al. (1985) reported that crude fat contents for growth increased when crude protein and crude ash are low. However, proximate analysis of fish in this study feeding trial were not significantly different among fish fed different diets (P>0.05).

In conclusion, these results indicate that up to 45% of fish meal protein could be replaced by fish meal analogue (BAIFA-M) containing animal by-product protein sources in young adult Korean rockfish diets. Complete replacement of fish meal by animal by-products in fish diets was not accomplished without decrease in fish performance. However, Further research are required to modify BAIFA-M replacing WFM up to 100%

Table 5. Proximate analysis of whole-body (crude) composition (% of dry matter basis)[1]

	Diet				Pooled SEM[2]
	M_0	M_{30}	M_{45}	M_{60}	
Moisture	64.0	66.0	65.8	65.8	0.50
Protein	53.0	52.3	53.3	54.1	0.26
Lipid	30.4	30.1	30.5	30.6	0.16
Ash	14.7	14.3	14.5	14.4	0.10

[1]Values are means from triplicate groups of fish where the means in each row with a different superscript are significantly different (P<0.05)

[2]Pooled standard error of mean : $SD/\sqrt{n}$

with maximum growth rates in young adult Korean rockfish.

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