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The Use of Meat Meal as a Dietary Protein Source Replacing Fish Meal in Juvenile Rockfish *Sebastes schlegeli*

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This study examined the partial replacement of the fish meal with meat meal in practical diets for juvenile rockfish. Five isonitrogenous (48% CP) diets were prepared to contain meat meal at 0% (control), 10%, 20%, 30% and 40% with substituting the mackerel meal in the control diet. Three replicate groups of fish (initial average weight, 4.1 g) were hand-fed to visual satiety two times daily for 8 weeks. Survival (>93%) and daily feed intake were not significantly different (P>0.05) among treatments. The best weight gain, feed efficiency and protein efficiency ratio were obtained from fish fed the diets containing 0% and 10% meat meal, and were not significantly different (P>0.05) to those of fish fed diet containing 20% meat meal. Condition factor, visceralsomatic index and hepatosomatic index were not influenced by dietary meat meal levels. The contents of crude protein and ash of whole body were not significantly affected (P>0.05) by dietary meat meal levels, whereas crude lipid content of fish fed the diets containing 30% and 40% was lower than that of fish fed the control diet. Proximate composition of liver was not influenced by dietary meat meal level (P>0.05). The data obtained in this study indicate that a diet containing 10~20% meat meal could be used for least-cost formulation in juvenile rockfish diet.

Keywords: *Sebastes schlegeli,* Meat meal, Protein source

Introduction

Rockfish *Sebastes schlegeli* is one of the fastest growing aquaculture species in Korea. It has several desirable characteristics for aquaculture such as high tolerance to low water temperature and easiness of seedling production due to the viviparous reproduction style. Farming technique for this species has been rapidly developed since late 1980s, and its aquaculture production is next to flounder *Paralichthys olivaceus* which is the highest among marine fish aquaculture production in Korea. In culturing rockfish, raw fish-based moist feeds have been intensively used. However, the use of the feeds not only cause deterioration of water quality but also increase labor cost and freeze space. Therefore, it is needed to employ practical dry feeds that would be able to support reasonable growth of rockfish.

Dietary protein is the most important factor affecting growth performance of fish and feed cost. High levels of good quality protein in diets generally result in rapid growth rate, especially in carnivorous fish. Rockfish exhibit carnivorous feeding behavior with relatively high protein requirement like other carnivorous marine fishes. Lee et al. (2002) suggested that digestible protein level of 42% is recommended for optimum growth and efficient protein utilization of growing rockfish between 22 to 63 g.

Although the global production of fish meal has been static or decreasing, the demand of high quality fish meal for fish feeds has been steadily increasing. Therefore, suitable alternatives to fish meal should be identified. Several attempts have been made to partially replace fish meal with less expensive materials having stable supply and palatabilities for the fish species. For carnivorous fish, poultry by-product meal appears to have good potential as a cost effective substitute for fish meal (Sato and Kikuchi, 1997). Previous studies with meat meal products have generally shown positive results with respect to fish meal replacement (Lovell, 1992; Shimeno et al., 1993; Tacon, 1994). Some studies were also conducted on the use of animal by-products as a fish meal replacer in rockfish diets (Lee and Lee, 1996; Lee et al, 1996a; Lee et al., 1996b). However, optimum level of meat meal in diet was not investigated for juvenile rockfish. Therefore, this study was conducted to investigate the optimum inclusion level of meat meal in diet for growth of juvenile rockfish.

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Materials and methods

Experimental diet and feeding trial

Proximate analysis and essential amino acids composition of the ingredients used in the experimental diets are shown in Table 1. Five experimental diets with isonitrogenous, isolipidic and isocaloric were prepared to contain meat meal at 0% (control), 10%, 20%, 30% and 40% with substituting the mackerel meal in the control diet (Table 2). Ingredients of the experimental diets were mechanically mixed with water, pressure-pelleted, and dried at room temperature overnight. All diets were stored in a freezer at -25°C until use.

Juvenile rockfish were obtained from private hatchery (Gangneung, Korea). They were acclimated to the experimental conditions for 2 weeks before the beginning of feeding trial. Fish (4.1 g/fish) were randomly selected and distributed into fifteen 300 L fiberglass reinforced plastic tanks with 30 fish to each tank for feeding trial. Three replicate groups of fish were hand-fed to visual satiety two times daily at 09:00 h and 17:00 h for 8 weeks (for 6 days per week). Satiation feeding was determined by the point of cessation of voluntary feeding activity by fish. Fish were carefully fed by the same person during the feeding trial. Pellet size was adjusted and appropriate size pellet was fed as the fish grow. Filtrated seawater (33±0.6‰) was supplied at a flow rate of 5 L/min and air was supplied to each tank. Average water temperature was 17.8±2.3°C, and photoperiod followed natural conditions during the experimental period. All fish in each tank were collectively weighed at the beginning and the end of feeding trial after being fasted for 24 h and anesthetized with MS222 (Tricaine methanesulfonate, Sigma, USA) at a concentration of 100 mg/L. Records were kept for daily feed consumption, mortalities and feeding behavior.

Sample collections and chemical analysis

Fifty fish at the beginning and all the survived fish at the end of feeding trial were stored at -75°C in freezer for subsequent proximate analysis of liver and whole body. At the end of the feeding trial, blood samples were obtained from the

Table 1. Proximate analysis and essential amino acid composition of the dietary ingredients

	Protein sources			
	Mackerel meal[1]	Meat meal[2]	Brewer's yeast	Wheat flour
Proximate composition (% of dry matter basis)				
Moisture	8.7	5.0	3.6	10.4
Crude protein	75.3	74.4	48.7	11.7
Crude lipid	7.9	10.8	2.0	1.0
Essential amino acids (% in protein)				
Arg	6.7	5.7	6.2	6.5
His	4.0	3.2	3.6	4.1
Ile	4.0	4.0	4.0	3.9
Leu	8.2	10.1	9.2	8.6
Lys	8.4	6.1	7.4	7.1
Met +Cys	3.0	1.8	1.6	1.6
Phe +Tyr	7.3	7.6	7.9	7.6
Thr	5.1	4.7	4.9	4.7
Val	5.6	5.7	5.5	5.6
Total	52.3	48.9	50.3	49.7

[1]Produced by steam dry method, imported from Chile.
[2]Provided by SCF. Co. Ltd, Incheon, Korea.

Table 2. Ingredients and nutrients composition of the experimental diets

	Meat meal levels (%)				
	0	10	20	30	40
Ingredients (% as air-dried)					
Mackerel meal	55.0	47.0	39.0	31.0	23.0
Meat meal	-	10.0	20.0	30.0	40.0
Wheat flour	26.0	24.0	22.0	20.0	18.0
Alpha-starch	5.0	5.0	5.0	5.0	5.0
Brewer's yeast	2.0	2.0	2.0	2.0	2.0
Squid liver oil	6.0	6.0	6.0	6.0	6.0
Vitamin premix[1]	2.0	2.0	2.0	2.0	2.0
Mineral premix[1]	2.0	2.0	2.0	2.0	2.0
Sargassum meal	1.5	1.5	1.5	1.5	1.5
Choline salt	0.5	0.5	0.5	0.5	0.5
Proximate analysis (%, dry basis)					
Dry matter	73.1	72.4	72.1	75.6	79.4
Crude protein	48.0	47.8	47.1	48.4	48.6
Crude lipid	11.0	11.2	11.7	12.1	13.8
Ash	8.6	9.6	10.7	11.4	12.1
n-3HUFA[2]	2.2	2.1	1.9	1.8	1.7
Gross energy (Kcal/g)	5.0	5.1	4.9	5.0	5.1
Essential amino acids (% in protein)					
Arg	6.6	6.8	7.1	7.2	7.5
His	4.7	4.3	4.1	3.9	3.6
Ile	4.0	3.9	3.8	3.6	3.4
Leu	8.0	7.8	7.7	7.6	7.4
Lys	8.5	8.1	7.8	7.4	7.1
Met +Cys	3.4	2.5	2.3	2.2	2.0
Phe +Tyr	7.3	7.2	7.1	6.9	6.8
Threonine	5.2	5.1	4.9	4.9	4.6
Val	5.7	5.3	5.8	5.7	5.5

[1]Same as Lee et al. (2000).
[2]Highly unsaturated fatty acid (C≥20).

caudal vein of six fish from each tank by using heparinized syringes after they were starved for 24 h and anesthetized with MS222. Blood plasma was collected after centrifugation (3500×g for 5 min) and stored at -75°C as separate aliquots for analysis of total protein and glucose. Chemical composition of the experimental diets and fish carcasses were determined using the following AOAC (1990) procedures: dry matter by drying in an oven at 105°C for 24 h; crude protein (N×6.25) by the Kjeldahl method after an acid digestion method using an Auto Kjeldahl System (Buchi, Flawil, Switzerland); crude lipid by ether extraction after acid hydrolysis; ash by incineration in a muffle furnace at 550°C for 24 h and crude fiber by Fibertec automatic analyzer (Tecator, Hoganas, Sweden). Gross energy contents were analyzed using an adiabatic bomb calorimeter (Parr, Moline, IL, USA). Amino acids were analyzed using an automatic amino acid analyzer (Hitachi L-8800, Japan). Fatty acid methyl esters were analyzed by using a gas chromatography (HP-5890 II; Hewlett-Packard, Palo Alto, USA) with a flame ionization detector, equipped with capillary column (HP-INNOWax; 30 m×0.32 mm×0.5 μm, USA). The plasma glucose and protein contents were measured using commercial clinical investigation kits (Wako Pure Chemical Industries, Ltd., Japan).

Statistical analysis

The data were subjected to one-way ANOVA. If significant ($P<0.05$) differences were found, Duncan's multiple range test (Duncan, 1955) was used to rank the group. The data are presented as mean±SE of three replicate groups. All statistical analyses were carried out using the SPSS Version 10 (SPSS, Michigan Avenue, Chicago, IL, USA).

Results

Growth performance

The growth performance of juvenile rockfish fed the experimental diets containing different meat meal levels for 8 weeks are presented in Table 3. Survival, daily feed intake and daily protein intake were not affected by dietary meat meal levels ($P>0.05$). Survival of each group was over 93% and was not different among treatments ($P>0.05$).

Weight gain, feed efficiency and protein efficiency ratio (PER) of juvenile rockfish were significantly affected by dietary meat meal levels ($P<0.05$). Weight gain, feed efficiency and PER of fish fed the diets containing 10% meat meal and the control diet were significantly higher than those of fish fed the diets containing 30% and 40% meat meal ($P<0.05$). These values of fish fed the diets containing 20% meat meal were not different to those of fish fed the control diet ($P>0.05$). Condition factor, hepatosomatic index and visceralsomatic index of fish were not significantly affected by the dietary meat meal levels.

Table 3. Growth performance of juvenile rockfish fed diets containing various meat meal levels for 8 weeks

	Meat meal levels (%)				
	0	10	20	30	40
IMW[1] (g/fish)	4.1±0.10	4.1±0.06	4.0±0.11	4.2±0.17	4.0±0.04
Survival (%)	97±0.0	99±1.0	100±0.0	93±5.2	100±0.0
WG[2] (%)	388±9.3^{c}	378±9.6^{c}	364±8.5^{bc}	341±8.2^{b}	297±10.5^{a}
FE[3] (%)	87±2.0^{b}	87±3.8^{b}	82±1.2^{ab}	79±0.6^{a}	77±1.9^{a}
DFI[4] (%)	2.56+0.037	2.59+0.118	2.71+0.061	2.68+0.030	2.67+0.103
PER[5]	1.81±0.016^{c}	1.82±0.079^{c}	1.74±0.025^{bc}	1.63±0.012^{ab}	1.59±0.040^{a}
CF[6]	1.9±0.30	1.9±0.06	1.9±0.33	2.0±0.03	1.9±0.06
VSI[7]	4.5+0.14	4.2+0.17	4.6+0.28	4.3+0.22	4.5+0.40
HSI[8]	3.9±0.19	4.0±0.12	4.1±0.08	4.2±0.15	3.7±0.10

Values (mean±SE of three replications) in each row with a different letter are significantly different ($p<0.05$).
[1]Initial mean weight.
[2]Weight gain=weight gain of fish×100/initial fish weight.
[3]Feed efficiency=fish weight gain×100/feed intake (dry matter).
[4]Daily feed intake=[feed (protein) intake (dry matter)×100]/[(initial fish weight + final fish weight + dead fish weight)/2×days].
[5]Protein efficiency ratio=fish wet weight gain/protein intake.
[6]Condition factor=body weight×100/total body length $(cm)^3$.
[7]Visceralsomatic index=viscera weight×100/body weight.
[8]Hepatosomatic index=liver weight×100/body weight.

Proximate composition of fish

Proximate composition of whole body and liver of juvenile rockfish fed the experimental diets are shown in Table 4. Crude protein and ash contents of whole body were not affected by dietary meat meal levels (P>0.05). Crude lipid content of whole body fed the diets containing 30% and 40% meat meal was significantly lower than that of fish fed the diets containing 0~20% meat meal (P<0.05). The trend of moisture content of whole body was inversely related to that of crude lipid content. Proximate composition of liver, plasma total protein and glucose concentrations of fish were not affected by the dietary meat meal levels (Table 5).

Discussion

Fish meal has commonly been the major protein source for fish diets due to its high nutritive values such as high protein content, balanced essential amino acids and fatty acids. Lee et al. (1993; 2002) used fish meal as a main protein source to control the protein level in rockfish diets. However, fish meal is the most expensive protein source in fish feeds and the supply of high quality fish meal for aquaculture feeds has been gradually decreased during the last decade, leading to an increased price of the product. Therefore, cost effective non-marine alternative sources of protein to fish meal for use in aquaculture feeds must be investigated. Inclusions of plant and animal protein sources for partial replacement of fish meal for rockfish diets have been studied in many previous studies (Lee and Lee, 1996; Lee et al 1996a; Lee et al., 1996b). They have suggested that soybean meal, corn gluten meal, blood meal, feather meal, and meat meal could substitute for fish meal up to about 10% in practical diets for growth of rockfish. However, previous studies compared growth performance of rockfish by feeding diets containing 0~10% meat meal. In the present study, growth and feed utilization of rockfish fed diets containing 10~20% meat meal were not different to those of the fish fed the control diet. The data obtained from this study indicate that a diet containing up to 20% meat meal could be used for least-cost formulation in juvenile rockfish diet.

A number of investigations have been directed toward the evaluation of different animal by-product meal in diets for several fish species. Studies showed that inclusions (30~70 %) of meat meal as a substitute for fish meal were readily accepted by both omnivorous and carnivorous species such as tilapia, gilthead sea bream, rainbow trout, and yellowtail (Davies et al., 1989; Watanabe and Pongmaneerat, 1991; Davies et al., 1993; Shimeno et al., 1993). Sato and Kikuchi (1997) reported that a diet containing 38% meat meal, corresponding to replacement of about 60% white fish meal protein, showed comparable growth and protein efficiency ratio to the control diet in flounder. The maximum level (20%) of

Table 4. Proximate composition (%) of the whole body and liver in juvenile rockfish fed the experimental diets for 8 weeks

	Initial	Meat meal levels (%)				
		0	10	20	30	40
			Whole body			
Moisture	73.1	69.2±0.18[a]	69.2±0.38[a]	69.2±0.15[a]	69.7±0.12[ab]	70.0±0.27[b]
Crude protein	15.4	16.4±0.40	16.6±0.12	16.3±0.11	16.3±0.07	16.2±0.11
Crude lipid	3.4	9.03±0.37[b]	9.3±0.13[b]	9.0±0.14[b]	8.0±0.39[a]	7.5±0.26[a]
Ash	5.3	4.0±0.09[a]	3.9±0.22[a]	4.1±0.09[ab]	4.1±0.05[ab]	4.5±0.09[b]
			Liver			
Moisture	62.4	58.1±0.42	57.8±0.20	56.4±0.82	56.1±0.57	58.1±1.25
Crude protein	12.7	10.0±0.08	9.7±0.42	9.3±0.04	9.3±0.28	9.7±0.20
Crude lipid	14.8	12.3±0.93	13.7±1.60	13.5±0.56	14.6±2.31	17.1±2.07

Values (mean±SE of three replications) in each row with a different letter are significantly different (p<0.05).

Table 5. Blood chemistry of juvenile rockfish fed the experimental diets for 8 weeks

	Meat meal levels (%)				
	0	10	20	30	40
Total protein (g/100 mL)	3.63±0.186	3.77±0.186	3.90±0.100	3.97±0.067	3.97±0.260
Glucose (mg/100 mL)	42.3±7.78	38.7±8.59	40.5±2.93	42.8±8.87	49.9±12.47

Values are mean±SE of three replications.

meat meal for juvenile rockfish diet recommended from this study was generally lower than that obtained from other studies as described above. It has been suggested that high inclusion of meat meal could increase feed intake mainly due to its good palatability to fish (Williams et al., 2003; Yang et al., 2004). However, feed intake in the present study was not affected by dietary meat meal levels. The difference in feed intake between the previous and the present studies seems to be related to the differences in the fish species or dietary energy levels. It has been suggested that feed intake is regulated by dietary available energy (Lee and Putnam, 1973; Jobling and Wandsvik, 1983; Lee et al., 2002).

High inclusions of 30~40% meat meal in the diets resulted in a significant growth depression and correspondingly lower feed efficiency in this study. Generally, a higher level of alternative protein sources than an optimum use in fish diets resulted in poor growth and feed utilization (Cowey et al., 1971; Reigh and Ellis, 1992; Shimeno et al., 1993; Millamena, 2002; Yang et al., 2004). Flounder and rainbow trout fed diets containing meat meal as a main protein source also showed lower growth than those fed fish meal based diets (Watanabe and Pongmaneerat, 1991; Sato and Kikuchi, 1997). They attributed poor growth of the fish to limiting essential amino acids (EAA) profile of meat meal. Although dietary essential amino acids requirements of juvenile rockfish have not been verified, possible reasons for the lower growth of rockfish fed the diets containing high levels of meat meal may be due to deficiencies in EAA such as methionine in meat meal compared to that in fish meal shown in Table 1. It has also been reported that the first limiting EAA in poultry by-products is methionine (Nengas et el., 1999).

Previous studies were conducted to investigate the effect of supplementation of EAA mixture in practical diets on growth of juvenile rockfish (Lee and Jeon, 1996; Lee and Lee, 1996; Lee et al., 1996a). They concluded that the supplementation of EAA mixture in diets containing animal and plant protein sources as partial substitutes for fish meal has no beneficial effects on growth and feed utilization of rockfish. Sato and Kikuchi (1997) also suggested that there was no need to supply additional EAA to meat meal based diets when they are included up to 40% in the diet for flounder. Murai et al. (1984; 1989) reported that supplemented crystalline amino acids in diet for carp were poorly utilized. This was probably due to rapid absorption of free amino acid in the gut, and that the absorbed unbalanced amino acids might be excreted before body cells utilize it for growth. Considering informations obtained from previous studies, we prepared experimental diets without supplemental EAA in the meat meal based diets.

Another possible explanation for the low growth responses of the rockfish fed the diets containing high levels of meat meal in the present study is low content of highly unsaturated fatty acid (n-3HUFA) such as EPA and DHA in the lipids in the meat meal. Lee (2001) reported that EPA and DHA were essential fatty acids for rockfish and the n-3HUFA requirement was 0.9% in the diet. In the present study, the contents of n-3HUFA in the experimental diets ranged from 1.7% to 2.2% which was above the requirement levels for rockfish. Lee (2001) also reported that the rockfish exhibited optimal growth and feed efficiency when n-3HUFA levels are 0.9~4.0% in diets and that no side effect of soybean oil substituted for dietary squid liver oil was observed when n-3HUFA requirement for the growth of rockfish was met. In considering these results, low growth of rockfish fed the diets containing high levels of meat meal in the present study does not seem to be due to the essential fatty acids levels in the diets.

The results from this study indicate that meat meal is an acceptable feed ingredient for the partial replacement of fish meal protein in practical diets for juvenile rockfish. It is concluded that a diet containing 10~20% meat meal could be used for least-cost formulation in juvenile rockfish diet.

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