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Effect of Partial Replacement of Fish Meal with Squid Liver Meal™ in the Diet on Growth and Body Composition of Juvenile Olive Flounder (*Paralichthys olivaceus*) during Winter Season

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We evaluated the effects of the partial dietary replacement of fish meal with squid liver meal™ on the growth and body composition of juvenile olive flounder *Paralichthys olivaceus* during the winter season. Twenty-five fish with an initial body weight of 23 g per tank were distributed among 12,250 L flow-through tanks. The experimental diets, which were designated SLM5, SLM10, and SLM15 diets, were prepared in triplicate along with control diet by replacing 5, 10, and 15% of mackerel fish meal with squid liver meal™, respectively. The weight gain and specific growth rate of flounder that were fed the control and SLM5 diets did not differ from those of the fish fed the SLM10 diet, but they were significantly (P<0.05) higher than those of the fish fed the SLM15 diet. The feed efficiency ratios for the flounder that were fed the control, SLM5 and SLM10 diets were significantly (P<0.05) higher than for the fish that were fed the SLM15 diet. However, the protein efficiency ratio for the flounder was not significantly affected by the experimental diets. The crude protein, crude lipid and ash content, and blood chemistry of the flounder were not significantly affected by the experimental diets. Therefore, the replacement of up to 10% of dietary fish meal with squid liver meal™ can be made without a reduction in growth or a deterioration of the feed efficiency of juvenile olive flounder during the winter season.

Key words: Olive flounder, *Paralichthys olivaceus*, Replacement of fish meal, Squid liver meal™

Introduction

The olive flounder *Paralichthys olivaceus* has been the most commercially important marine fish species for aquaculture over the last two decades in Korea. Many feeding trials on the substitution effects of alternative protein sources in dietary fish meal have been performed to promote cost effective flounder production. It reported that 40% fish meal could be replaced by feather meal Kikuchi et al. (1994a), 20% by meat and bone meal Kikuchi et al. (1997), 60% by meat meal (Sato and Kikuchi, 1997), 50% by defatted soybean meal (1994b), 40% by corn gluten meal (Kikuchi, 1999a) and 46% by defatted soybean meal in combinations with blood meal, corn gluten meal and blue mussel meat (Kikuchi, 1999b). However, meat meal and defatted soybean meal with the supplementation of deficient amino acids in each meal did not show a positive effect on the growth of flounder (Kikuchi et al., 1994b; Sato and Kikuchi, 1997).

Squid meal has a high dietary value due to its fat-insoluble fraction (Watanabe et al., 1984a). The supplementation of squid meal into feed is effective at improving the broodstock egg quality of the red seabream *Pagrus major* (Watanabe et al., 1984a, 1984b), and the striped jack *Pseudocaranx dentex*

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(Vassallo-Aguis et al., 2001) due to the high requirements of the essential fatty acids for breeding. And Fernandez-Palacios et al. (1997) highlighted the value of squid meal as a good protein and lipid source in the diet of the gilthead seabream *Sparus aurata*. Therefore, a better understanding of the dietary effect of squid or squid by-product meal for fish production is needed.

Squid liver meal™, which is a by-product of squid processing is a commercially available product (Daesung Industrial Co., Uljin, Korea) that is composed of 60% squid liver meal and 40% soybean meal, costs 15,000 Won/ 20 kg and is high in nutrients (45.1% crude protein, 17.4% crude fat and 6.4% ash). However, no study of the dietary effect of squid liver meal on the performance of flounder has been conducted. We, therefore, evaluated the effect of partial dietary replacement of fish meal with squid liver meal™ on the growth and body composition of juvenile flounder during the winter season.

Materials and Methods

Experimental fish

Juvenile olive flounder were purchased from a private flounder hatchery (Kyungbook, Korea) and transported to the lab. Twenty-five fish with an initial body weight of 23 g per tank were distributed to 12 of 250 L flow-through tanks (water volume: 150 L) and acclimated to the experimental conditions for 1 week. During the acclimation period, the fish were given a commercial flounder feed containing 52% crude protein and 8% crude lipid (Jeil Feed Co., Korea) twice daily.

Preparation of the experimental diets

Four experimental diets were prepared in triplicate for this study: control, SLM5, SLM10, and SLM15 diets. A 60% proportion of mackerel meal was used as the primary protein source in the control diet (Table 1). Squid liver meal™ was used to replace 5, 10, and 15% of mackerel meal in the SLM5, SLM10, and SLM15 diets, respectively. The amino acid compositions of the ingredients used as the primary protein sources in the experimental diets are given in Table 2. The content of lysine (Lys) and methionine +cystine (Met+Cys) in the squid liver meal™ was relatively low compared to the mackerel meal, but supplementation of these amino acids was not made for practical use. Wheat flour and α-starch (Sigma Chemical, St. Louis, USA), and squid liver oil were used as the primary carbohydrate and lipid sources in the experimental diets, respectively. The ingredients in the experimental diets were well mixed with water at a ratio of 7:3 and formed into pellets by a pellet-extruder in the lab. The experimental diets were dried overnight and stored at -20 until use.

Table 1. Ingredients (%) of the experimental diets

	Diets			
	Control	SLM5	SLM10	SLM15
Ingredients				
Mackerel meal (Chile)	60	57	54	51
Squid liver meal™		5	10	15
Wheat flour	26.8	25.2	23.6	22.0
α-starch	5	5	5	5
Brewer's yeast	2	2	2	2
Squid liver oil	2	1.6	1.2	0.8
Vitamin premix[1]	2	2	2	2
Mineral premix[1]	2	2	2	2
Choline (50%)	0.2	0.2	0.2	0.2
Nutrients (%)				
Crude protein	48.7	48.6	47.0	45.4
Crude lipid	7.5	6.9	6.9	6.9
Ash	9.2	8.7	8.8	8.9

[1]Same as Lee et al. (2000).

Table 2. Essential amino acids compositions of the ingredients of the experimental diets

	Protein sources			
	Mackerel meal	Squid liver meal™	Wheat flour	Brewer's yeast
Proximate composition (% of DM basis)				
Moisture	8.7	0.1	10.5	3.6
Crude protein	75.3	44.9	11.7	48.7
Crude lipid	7.9	14.4	1.0	2.0
Ash	15.7	7.4	1.0	9.1
Essential amino acids (% in protein)				
Arg	6.7	6.5	6.5	6.2
His	4.0	3.7	4.1	3.6
Ile	4.0	4.1	3.9	4.0
Leu	8.2	8.9	8.6	9.2
Lys	8.4	7.9	7.1	7.4
Met + Cys	3.0	1.7	1.6	1.6
Phe + Tyr	7.3	7.7	7.6	7.9
Thr	5.1	4.9	4.7	4.9
Val	5.6	5.5	5.6	5.5
Total	52.3	50.8	49.7	50.3

Experimental conditions

Sand-filtered seawater was supplied into each tank at a flow rate of 6.6 L/min. The fish were hand-fed to apparent satiation twice daily (09:00 and 17:00). The water temperature ranged 9.5-16.0℃ (Mean±SD: 12.3±1.75℃) during the feeding trial and the photoperiod was left natural. The feeding trial lasted for 10 weeks.

Analysis of the chemical composition and blood chemistry of the fish

Ten fish at the beginning and termination of the feeding trial were sampled and killed for proximate analysis. The crude protein content was determined by the Kjeldahl method using the Auto Kjeldahl System (Buchi B-324/435/412, Switzerland), the lipid content was determined by ether-extraction method, the moisture content was measured after oven-drying at 105 for 24 h, fiber content was measured by an automatic analyzer (Fibertec, Tecator, Sweden) and ash content was estimated using a furnace muffle (550 for 4 h) according to standardized methods (AOAC, 1990). Blood samples were obtained from the caudal vein of five fish from each tank using a heparinized syringe after the fish were starved for 24 h and anesthetized with 100 ppm tricaine methanesulfonate (MS-222) at the end of the feeding trial. Plasma was collected after centrifugation (3,000 rpm for 10 min) and stored at -70 as separate aliquots for the analysis of protein, glucose and glutamic oxaloacetic transaminase (GOT).

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 groups. All statistical analyses were carried out using the SAS software (Version 9.1, North Carolina, USA).

Results and Discussion

The survival, weight gain (g/fish) and specific growth rate (SGR) of olive flounder fed the experimental diets for 10 weeks during the winter season are given in Table 3. Survival was not significantly ($P>0.05$) affected by the experimental diets and the partial substitution of squid liver meal™ in the diets did not improve the growth of olive flounder compared to the control fish. The weight gain and SGR of the flounder fed the control and SLM5 diets did not differ from those of flounder fed the SLM10 diet, but were significantly ($P<0.05$) higher than in fish that were fed the SLM15 diet. This could have been caused by the poor protein quality of squid liver meal™, which had deficient amount of amino acids Lys and Met+Cys, or by the existence of some antinutritional factors in squid liver meal™ that affected flounder growth. The quality of a protein source depends on its amino acids profile and digestibility for each particular fish species. Fernandez-Palacios et al. (1997) indicated that squid meal, with its high fat-insoluble fraction, is a good protein source for gilthead seabream. Also Vassallo-Aguis et al. (2001) showed that when broodstock of striped jack were fed by diets substituted with 50% squid meal or a 50% com-bination of squid meal and krill meal substituted for fish meal, the 50% squid meal diet did not improve either egg quality, or production. Therefore, the supplementation of squid liver meal™ into the diet could be expected to improve egg quality rather than growth.

The growth of olive flounder and yellowtail *Seriola quinqueradiata* that were fed diets substituting meat meal for 40 and 30 % of the fish meal, respectively was equal to that of fish given fishmeal-based diets, however, the deficient amino acids, such as Met and Lys in the meat meal were not supplemented into the diets (Shimeno et al., 1993). Similarly, the replacement with 60% meat meal and 50% defatted soybean meal for fish meal in the diets did not produce a desirable effect on the growth of flounder even with the supplementation of the deficient crystalline amino acids in each meal (Kikuchi et al., 1994b; Sato and Kikuchi, 1997). The effectiveness of the supplementation of these amino acids in the diets of fish should be carefully considered because the availability of the supplemented crystalline amino acids in the fishmeal-based diets is relatively poor.

The feed intake (g/fish), feed efficiency ratio (FER) and protein efficiency ratio (PER) for olive flounder fed the experimental diets substituting squid liver meal™ for fish meal for 10 weeks during the winter season are presented in Table 4. The feed intake was not significantly ($P>0.05$) affected by the experimental diets. However, the FER for flounder fed the control, SLM5, and SLM10 diets was signifi cantly

Table 3. Survival (%), weight gain (g/fish) and specific growth rate (SGR) of juvenile olive flounder fed the experimental diets substituting squid liver meal™ for fish meal for 10 weeks during the winter season. Different superscripts within columns denote significant differences ($P < 0.05$). SGR = (Ln final weight - Ln initial weight) 100/days.

Diets	Initial weight (g/fish)	Final weight (g/fish)	Survival (%)	Weight gain (g/fish)	SGR
Control	23.1	56.1^{a}	$96.0^{n.s.}$	33.0^{a}	1.25^{a}
SLM5	23.0	54.7^{a}	94.7	31.6^{a}	1.22^{a}
SLM10	23.0	51.0^{ab}	96.0	28.0^{ab}	1.12^{ab}
SLM15	22.9	45.1^{b}	97.3	22.2^{b}	0.95^{b}
Pooled SEM		1.01	0.89	1.00	0.013

Table 4. Feed intake (g/fish), feed efficiency ratio (FER) and protein efficiency ratio (PER) of juvenile olive flounder fed the experimental diets substituting squid liver meal™ for fish meal for 10 weeks during the winter season. Different superscripts within columns denote signifycant differences (P<0.05). FER=Weight gain/dry feed intake. PER=Weight gain/protein fed.

Diets	Feed intake	FER	PER
Control	36.2$^{n.s.}$	0.93^{a}	1.91$^{n.s.}$
SLM5	35.0	0.90^{a}	1.86
SLM10	33.6	0.85^{a}	1.80
SLM15	31.5	0.72^{b}	1.57
Pooled SEM	0.79	0.007	0.037

(P<0.05) higher than in the fish fed the SLM15 diet. Alternative protein source for fish meal beyond maximum level resulted in poor fish production and feed efficiency (Kikuchi et al., 1994a; Sato and Kikuchi, 1997; Kikuchi et al., 1997; Kikuchi, 1999a, 1999b). We observed that the FER for flounder fed the diets substituting meat meal for up to 40% of the fish meal was similar to fish fed the fishmeal-based diet during the winter season. The PER for flounder was not significantly (P>0.05) affected by the experimental diets, but there was a trend toward a decreased PER with in an in-crease in squid liver meal™ in this study. According to the growth performance and feed efficiency of the olive flounder in this study, a 10% substitution of squid liver meal™ for fish meal can be made without a suppression of growth during the winter season. However, because water temperature largely affects the availability and digestibility of nutrients by fish, the effect of squid liver meal™ substitution on fish performance could differ during the summer season when fish grow relatively fast.

The crude protein, crude lipid and ash of the whole flounder ranged from 17.6 to 18.4, 2.7 to 3.9 and 2.6 to 3.4%, respectively, and it was not significantly (P>0.05) affected by the experimental diets. However, other researchers have observed that the body composition of fish is related to the substitution levels of alternative protein sources for fish meal (Kikuchi et al., 1994a, 1994b; Sato and Kikuchi, 1997; Kikuchi et al., 1997; Kikuchi, 1999a). Plasma protein, glucose and glutamic oxaloacetic transaminase of flounder ranged from 3.7 to 4.6 g/dL, 18.0 to 19.4 mg/dL and 12.7 to 24.4 IU/L, respectively, and was not signifycantly (P> 0.05) affected by the experimental diets. In contrast to our study, other researchers have observed that the blood characteristics of fish are affected by the substitution of alternative protein sources for fish meal (Shimeno et al., 1993; Kikuchi et al., 1994a). Although we did not observe a positive effect of the substitution of squid liver meal™ on the performance of flounder, further studies on supplementation of squid liver meal™ into feed for the early period of marine and coldwater fish that require high indispensable fatty acid contents or shrimp that require high fat content feed are needed in the future.

The replacement of up to 10% of dietary fish meal with squid liver meal™ can be made without a reduction in growth or a deterioration of the feed efficiency of juvenile olive flounder during the winter season.

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