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Partial Replacement of Fish Meal by Fermented Skipjack Tuna Viscera in Juvenile Olive Flounder (*Paralichthys olivaceus*) Diets

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This study was carried out to evaluate the use of fermented skipjack tuna viscera (FSTV) as an alternative for fish meal in juvenile olive flounder (*Paralichthys olivaceus*) diets. *Lactobacillus bulgaricus* was used as a starter for fermentation of skipjack tuna viscera. Four isonitrogenous (49% crude protein) and isocaloric (4 kcal/g DM) diets were formulated to contain graded levels (0, 5, 10, and 15%) of FSTV. Each experimental diet was fed three replicate groups (40 fish per tank) of juvenile flounder (average weight, 3.3±0.2 g) for 5 weeks. At the end of feeding experiment, inclusion of FSTV up to 15% in diets did not affect survival rate (%) and weight gain of fish. Feed efficiency, protein efficiency ratio, protein and lipid retentions of fish fed the diet containing 10% FSTV were higher than those of fish fed the control diet ($P<0.05$). The values of fish fed the diet containing 15% FSTV were not different from those of fish fed other diets. Whole body lipid content of fish fed the diet containing 10% FSTV was higher than that of fish fed the diet containing 15% FSTV and control diet. The present results indicate that fermented skipjack tuna viscera could partially replace fish meal in juvenile flounder feed, and the inclusion of 10% FSTV may be efficient in improving the feed utilization of fish.

Key words: Olive flounder, *Paralichthys olivaceus,* Alternative protein source, Fermented Skipjack Tuna Viscera, Growth

Introduction

Olive flounder *(Paralichthys olivaceus)* requires high protein level in feeds for their optimal growth performance (Lee et al., 2002). Fish meal is a conventional main protein source in flounder feeds (Jang et al., 2005). However, an eruption in fish meal demands due to a rapidly expansion of food animal producing industries including aquaculture (Watanabe, 2002) and the decline of fish meal production has resulted in a soar of its price. Thus, an available and cheap protein source which can replace fish meal is urgently needed for a sustainable and efficient aquaculture industry. Recently, several attempts have been being made to replace fish meal by less expensive protein sources including soybean meal (Kikuchi, 1999; Saitoh et al., 2003), soy protein concentrate (Deng et al., 2006), cottonseed and soybean meal (Pham et al., 2007, 2008; Lim and Lee, 2008), tuna muscle by-product powder (Uyan et al., 2006) for juvenile flounder. The results of the studies suggested that growth and feed utilization of fish were affected by protein sources and inclusion levels. For instance, Kikuchi (1999) reported that soybean meal in combination with other animal protein sources replaced for 45% fish meal in flounder diets. Whereas Pham et al. (2007) revealed that up to 20% fish meal in diets for this fish species could be safely substituted by a mixture of cottonseed and soybean meal. Particularly, Uyan et al. (2006) reported that replacement of 50% fish meal in diets by tuna muscle by-product powder could reduce 50% effluent phosphorus in flounder aquaculture.

Industrial processing of fish for human consumption yielded around 60% as by-product (Raa and Gildberg, 1982). Fish and fish by-product silages have been reported to be potential alternatives for fish meal in finfish and shellfish diets (Fagbenro and Jauncey, 1994, 1995, 1998; Gonçalves et al., 1989; Hardy et al., 1984; Jackson et al., 1984a, b; Lee et al.,

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2004; Vidotti et al., 2003; Vijayan et al., 2009). Two fermentation methods being employed in production of fish silages are direct addition of acid and biological fermentation with lactic acid bacteria, where the biological fermentation was reported to yield higher nutritional quality fish silage compared to the former one (Kompiang et al., 1980). Gonçalves et al. (1989) reported that eel fingerlings fed the diets containing fish silage had higher specific growth rate, food conversion and protein efficiency ratios compared to those of eels fed the diets containing fish and meat meal. Skipjack tuna canning industry is developing rapidly in Korea. Annually, large amount of skipjack tuna viscera, a skipjack tuna processing by-products, is released. Fermented skipjack tuna viscera may be a good protein source for flounder and reduce dependence on expensive fish meal. But, to date no information on the use of fermented skipjack tuna viscera (FVTS) in flounder diets is available. This study was conducted to evaluate the effects of FSTV as a dietary fish meal replacer on growth and body composition of juvenile flounder.

Materials and Methods

Fermentation of skipjack tuna viscera

Viscera of skipjack tuna (*Katsuwonus pelamis*) obtained from Dongwon Industry Co. (Changwon, Korea) was ground and mixed thoroughly with sugar beet molasses at a concentration of 100 g/kg. *Lactobacillus bulgaricus* KCTC 3188 provided by Korean Collection for Type Cultures (Biological Resource Center, Korea) were inoculated as a starter in the mixture and incubated at 35°C for 10 days in sealed 20 L plastic buckets. After this fermentation period, the mixture was filtered by gauzes and the filtrate was incorporated in experimental diets based on nutritional composition in dry basis.

Experimental diets

Four iso-nitrogenous (49% crude protein) and isocaloric (4 kcal/g) diets were formulated to contain 0%, 5%, 10%, and 15% of fermented skipjack tuna viscera (FSTV). Dietary ingredients and proximate composition are presented in Table 1. Proximate composition, volatile basic nitrogen (VBN) content, total and free amino acids, and fatty acid composition of raw skipjack tuna viscera (RSTV) and FSTV are presented in Tables 2, 3 and 4, respectively. The experimental diets were pelleted, dried at room temperature for 48 h and stored at -25°C until use.

Table 1. Ingredients and proximate composition of the experimental diets

	Dietary FSTV levels (%)			
	0	5	10	15
Ingredients (%)				
White fish meal[1]	62.0	59.0	56.0	53.0
Wheat flour	20.0	18.6	17.2	15.8
FSTV[2]	-	5.0	10.0	15.0
Brewer's yeast	2.0	2.0	2.0	2.0
Squid liver oil[3]	8.0	7.4	6.8	6.2
Mineral premix[4]	3.0	3.0	3.0	3.0
Vitamin premix[5]	2.2	2.2	2.2	2.2
Carboxymethyl cellulose[6]	2.0	2.0	2.0	2.0
Choline chloride (50%)[6]	0.8	0.8	0.8	0.8
Proximate composition (%, dry matter basis)				
Crude protein	47.8	48.3	48.3	50.0
Crude lipid	13.1	12.6	12.3	16.6
Ash	16.1	15.8	14.8	15.2
Carbohydrate[7]	23.0	23.3	24.6	18.2
Energy (kcal/100 g)	396	395	398	418

[1]Imported from Russia.
[2]Fermented skipjack tuna viscera.
[3]Provided by E-wha Oil & Fat Ind. Co., Busan, Korea.
[4,5]Same as Lee et al. (2003).
[6]Sigma, St. Louis, MO, USA.
[7]Calculated, 100 - (crude protein + crude lipid + ash).

Table 2. Proximate composition and volatile basic nitrogen (VBN) content of the raw skipjack tuna viscera (RSTV) and fermented skipjack tuna viscera (FSTV)

Composition	RSTV	FSTV
Moisture (%)	73.6	69.7
Crude protein (%)	18.0	14.8
Crude lipid (%)	4.7	2.6
Carbohydrate[1] (%)	0.7	9.7
Ash (%)	3.0	3.2
VBN (mg/100 g)	47	87

[1]Calculated, 100 - (moisture + crude protein + crude lipid + ash).

Fish and feeding trial

Juvenile flounder were transported from a hatchery to Marine Biology Center for Research and Education, Gangneung-Wonju National University and acclimated to a laboratory condition for 2 weeks. After this conditioning period, juvenile (average weight, 3.3±0.2 g) were randomly distributed into twelve 250 L fiberglass reinforced tanks in a flow-through tank system at a density of 40 fish per tank. Filtered sea water was supplied at a flow rate of 5 L/min in each tank. Photoperiod was left at the natural condition. Three replicate groups of fish were hand-fed each experimental diet to visual satiation, twice a day (07:00 and 17:00 h) for 5 weeks.

Table 3. Amino acid composition (g/kg) of the raw skipjack tuna viscera (RSTV) and fermented skipjack tuna viscera (FSTV)

Amino acids	Total amino acids		Free amino acids	
	RSTV	FSTV	RSTV	FSTV
Ala	10.08	10.70	2.62	6.40
Arg	7.79	6.65	1.94	5.07
Asp	14.03	12.65	0.39	11.30
Cys	1.08	1.30	0.32	0.69
Glu	21.65	19.58	6.49	14.94
Gly	9.82	11.24	2.75	8.34
His	3.66	3.06	1.02	2.24
Ile	8.86	8.32	2.65	8.21
Leu	14.66	14.37	3.66	14.33
Lys	3.01	2.47	1.67	2.41
Met	4.31	2.65	1.16	2.28
Phe	7.00	6.35	1.89	6.06
Pro	7.15	7.14	2.14	5.40
Ser	5.37	5.83	1.45	4.59
Tau	4.82	3.51	1.39	2.59
Trp	1.96	1.94	0.48	1.82
Thr	6.80	8.55	1.97	8.22
Tyr	2.89	1.58	0.69	1.26
Val	8.92	8.72	2.49	8.37
Total	143.86	136.61	37.2	114.52

Table 4. Major fatty acid composition (% of total fatty acids) of the raw skipjack tuna viscera (RSTV) and fermented skipjack tuna viscera (FSTV)

Fatty acids	RSTV	FSTV
16:0	30.1	23.9
16:1n7	4.2	7.5
18:0	10.1	7.1
18:1n9	18.3	14.7
18:2n6	0.3	3.9
18:3n3	0.3	1.0
20:0	0.0	0.2
20:1n9	0.6	0.6
20:2n6	0.0	2.0
20:4n6	2.9	0.6
20:5n3	6.6	9.4
22:2n6	0.0	1.7
22:3n6	0.0	0.6
22:6n3	24.2	22.1

Seawater temperature and salinity during the feeding period were 21.0±1.1°C and 34±0.1 ppt, respectively. At the end of the feeding trial, fish in each tank were collectively weighed and counted for weight gain, survival and feed utilization calculations after being fasted for 24 h.

Sample collection and chemical analyses

Fifty fish at the initiation and all surviving fish at the end of the feeding experiment were sampled and stored at -40°C for chemical analysis. Proximate composition of experimental diets and fish samples were analyzed by standard methods (AOAC, 1990). Crude protein was analyzed using an Auto Kjeldahl System (Buchi, Flawil, Switzerland). Crude lipid was analyzed by ether extraction in a soxhlet extractor (SER 148, VELP Scientifica, Milano, Italy), and moisture was determined by oven drying at 105°C for 24 h. Ash content was determined after combustion at 550°C for 4 h in a muffle furnace. Amino acid composition was measured using an automatic amino acid analyzer (Hitachi, Tokyo, Japan). Tryptophan was determined according to method of Hugli and Moore (1972). Volatile basic nitrogen (VBN) was measured by Conway's micro-diffusion method (Conway, 1950). Lipid for fatty acid analysis was extracted by a mixture of chloroform and methanol (2:1, v/v) as the method described by Folch et al. (1957). Fatty acid methyl esters prepared by transesterification with 14% BF_3-MeOH were determined by a gas chromatography (HP-5890 II, Hewlett-Packard, Palo Alto, USA) with flame ionization detector, equipped with HP-INNOWax capillary column (30 m×0.32 mm, i.d., film thickness 0.5 μm). Injector and detector temperatures were 250 and 270°C, respectively. The column temperature was programmed from 170 to 225°C at a rate of 1°C/min. Helium was used as the carrier gas. Concentration of fatty acids were determined by comparison with the known standards consisting of 12:0, 13:0, 14:0, 14:1, 16:0, 16:1, 17:0, 17:1, 18:0, 18:1, 18:2n-6, 18:3n-6, 18:3n-3, 18:4n-3, 18:4n-6, 20:0, 20:1, 20:2n-6, 20:3n-6, 20:4n-3, 20:5n-3, 22:0, 22:1, 22:4n-3, 22:5n-3, 22:6n-3, and 24:1 (Sigma, St Louis, MO, USA).

Statistical analysis

Data were subjected to one-way ANOVA to evaluate the effects of fermented skipjack tuna viscera on growth and body composition of flounder. Where significant difference ($P<0.05$) was found, Duncan's multiple range test (Duncan, 1955) was used to rank groups. All statistical analyses were carried out using SPSS program (SPSS Michigan Avenue, Chicago, IL, USA). The data presented are mean ± SE of three replications.

Results and Discussion

Growth and feed utilization of juvenile flounder fed the experimental diets are presented in Table 5. Weight gain of fish tended to increase with the increase of FSTV inclusion level up to 10% in diets. Daily feed intake of fish negatively related to dietary FSTV inclusion levels. Feed efficiency, protein efficiency ratio, protein retention, and lipid retention

Table 5. Growth and feed utilization of juvenile flounder fed the experimental diets containing different fermented skipjack tuna viscera (FSTV) levels for 5 weeks[1]

	Dietary FSTV levels (%)			
	0	5	10	15
Initial weight (g/fish)	3.4 ± 0.02	3.3 ± 0.19	3.3 ± 0.03	3.3 ± 0.02
Weight gain (%)[2]	290 ± 7.8	329 ± 22.7	369 ± 12.7	283 ± 23.2
Feed efficiency (%)[3]	74 ± 1.4[a]	88 ± 7.9[ab]	111 ± 8.5[b]	96 ± 0.8[ab]
Daily feed intake (%)[4]	4.8 ± 0.21[b]	4.3 ± 0.27[ab]	3.5 ± 0.34[a]	3.3 ± 0.26[a]
Protein efficiency ratio[5]	1.6 ± 0.03[a]	1.8 ± 0.17[ab]	2.3 ± 0.18[b]	2.0 ± 0.02[ab]
Protein retention (%)[6]	24.9 ± 0.9[a]	30.8 ± 3.1[ab]	38.4 ± 3.1[b]	31.3 ± 0.3[ab]
Lipid retention (%)[6]	14.5 ± 2.6[a]	21.1 ± 2.1[a]	34.6 ± 3.1[b]	24.9 ± 2.1[ab]
Survival (%)	71.0 ± 11.1	66.0 ± 7.3	62.0 ± 3.0	52.0 ± 13.1

[1]Values (mean±SE of three replications) in each row having different letters are significantly different ($P<0.05$).
[2](Final body weight - initial body weight) × 100/initial body weight.
[3](Final fish wt + dead fish wt. – initial fish wt.) × 100/feed intake (dry matter).
[4]Feed intake × 100/[(initial fish wt. + final fish wt. + dead fish wt.)/2 × days reared].
[5](Final fish wt + dead fish wt. – initial fish wt.) /protein intake.
[6]Fish protein (or lipid) deposited × 100 /protein (or lipid) intake.

in fish fed the diet containing 10% FSTV were higher than those of fish fed the control diet, but were comparable to those of fish fed the diet containing 15% FSTV. Survival was not affected by the dietary treatments. Studies (Fagbenro and Jauncey, 1995; Gonçalves et al., 1989; Jackson et al., 1984a, b; Lie et al., 1988) have demonstrated that fish silage could be a promising alternative for fish meal in fish feeds. Jackson et al. (1984b) reported that the final weight of salmon fed the diet containing blends comprising of sprat silage with a binder meal (1:1) were similar to that of fish fed a commercial feed. And the most rancid silage appeared to be more palatable and produced the fastest growth during the early phase of the feeding trial. Gonçalves et al. (1989) elucidated that higher specific growth rate, food conversion and protein efficiency ratios in eel fingerlings when fed a diets containing fish silage as a replacer for fish and meat meal were likely due to higher concentration of free fatty acids. Fagbenro and Jauncey (1995) reported that blends of fermented fish silage and other feedstuffs could provide up to 50% dietary protein for juvenile catfish. The growth and feed utilization results of the present study suggest that FSTV may replace up to 15% fish meal in juvenile flounder diets, and relatively high protein efficiency ratio, protein and lipid retentions is likely due to higher content of free amino acids and poly-unsaturated fatty acids in the FSTV.

Whole body composition of flounder fed the experimental diets is shown in Table 6. Inclusion of the FSTV in the diets did not alter body moisture, crude protein, and ash contents in flounder during the feeding experiment. Crude lipid content of fish fed the diet containing 10% FSTV was higher than that

Table 6. Proximate composition (%) of whole body of juvenile flounder fed the experimental diets for 5 weeks[1]

	Dietary FSTV levels (%)			
	0	5	10	15
Moisture	78.4 ± 2.3	77.4 ± 2.7	76.9 ± 3.3	77.4 ± 10.3
Crude protein	15.7 ± 2.0	16.3 ± 4.2	16.1 ± 3.9	15.4 ± 1.7
Crude lipid	1.7 ± 0.3[a]	2.2 ± 0.2[ab]	2.8 ± 0.1[b]	2.0 ± 0.2[a]
Ash	3.6 ± 0.2	3.9 ± 0.2	3.9 ± 0.4	3.6 ± 0.2

[1]Values (mean±SE of three replications) in each row having different letters are significantly different ($P<0.05$).

of fish fed the diet containing 15% FSTV and control diet, but not different from that of fish fed the diet containing 5% FSTV. The present body composition results suggest that lipid content in juvenile flounder was altered by dietary inclusion of the fermented skipjack tuna viscera. Lie et al. (1988) reported that lipid retention in fish body linearly related to lipid intake. A similar trend was also observed in body lipid content of eels when fed a fish silage diet (Gonçalves et al., 1989). In the contradictory, no significant difference was observed in whole body composition of catfish fed the diets containing mixture of fish silage and soybean meal as fish meal replacement (Fagbenro and Jauncey, 1995). In a previous study (Lee et al., 2004), no differences in lipid content in abalone fed the diets containing the same fermented skipjack tuna viscera, but the values seemed to be higher when dietary soybean meal was substituted by the FSTV. The discrepancies between the studies are likely due to the differences in feed composition and fish species.

Based on the present findings, it is suggested that fermented skipjack tuna viscera could partially replace for fish meal in juvenile flounder feed, and the inclusion of 10% FSTV may be efficient in improving the feed utilization of fish.

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