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Evaluation of fermented soybean meal to replace fish meal for juvenile olive flounder, *Paralichthys olivaceus*: growth performance, amino acid profile and biochemical parameters

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ABSTRACT

This study was carried out to evaluate the effect of replacing fish meal protein with fermented soybean meal (FSM) on growth performance, feed utilization, morphological parameters, amino acid profile, body composition, activity of antioxidant and digestive enzymes and blood chemistry in diet of juvenile olive flounder (Paralichthys olivaceus). Five isonitrogenic and isolipidic diets were prepared with using 0 (control), 80, 160, 240 and 320 g kg^{-1} FSM in diets. Juvenile olive flounder with initial average weight of 11.9±0.19 g were randomly distributed in fifteen 300 L tanks. Triplicate groups (30 fish for each tank) of juvenile fed each diet to visual satiation at two meals per day for 8 weeks. The fish fed diets containing up to 240 g kg^{-1} FSM had no significant differences in the survival, growth, feed efficiency and protein efficiency ratio compared with control group. Fish fed diet containing 320 g kg^{-1} FSM had significantly lower survival, growth, feed efficiency and protein efficiency ratio compared with control group. Hepatosomatic index of fish fed diets containing 240 and 320 g kg^{-1} FSM was higher than control group. Triglyceride and total protein of plasma in fish fed the diets containing 80-320 g kg^{-1} FSM were higher than control group. Proximate composition of whole body, amino acid profile of dorsal muscle, activity of digestive enzymes in intestine and also the activity of antioxidant enzymes in fish liver and plasma were not affected by different levels of FSM compared with control group. This study showed that FSM has potential to replace fish meal up to 240 g kg^{-1} in diet of juvenile olive flounder.

Key Words: fermented soybean meal, olive flounder, growth

INTRODUCTION

One of the important economic factors in success of aquaculture production is feed. Carnivorous species need a high fishmeal, the inclusion level of which is above 50% in commercial diets [18]. On the other hand, the cost of fish meal has increased during the last few years. Therefore, it is necessary to find alternative protein sources that can have a positive effect on the price of fish diet [31].

Large and growing bodies of literatures try to find out alternative protein sources, both from plant and animal origin. Previous studies reported the reduction in growth and feed utilization by soybean meal protein through imbalanced dietary amino acid, reduced mineral content, increased fiber, reduced palatability and presence of anti-nutrients factors [18]. Fibers and anti-nutrients are related to reduce digestibility in fish [9]. In salmonids, reduced nutrient digestibility with soy non starch carbohydrates or heat-stable anti-nutrients has been reported as important factors responsible for decreased growth performance [11]. Also several researchers were reported about the effect of

soybean meal as an alternative protein source instead of fish meal in diet of flounder but the result showed a relatively low substitution of fish meal with soybean meal in diet of flounder [34]. It has been reported fermentation is a suitable technique for drying wet products with minimal nutrient loss [17; 33]. Fermentation is a process allows microorganisms as *Bacillus subtilis* degrades protein macromolecules to a large extent water-soluble low molecular weight compounds [14; 29] It was found the beneficial effect of fermentation plant protein on removal or inactivation of anti-nutrients [27], improvement of nutritional quality [5], increasing digestibility [14] and shelf life of the processed food [30]. It was reported that growth performance was significantly improved by fermented sesame, black gram and duckweed leaf meal in Indian major carp, *Labeo rohita* compared to non-fermented meal [3; 22 ;26] . Fermented fish silage and soybean meal was found as a suitable protein source in the diets of catfish, *Clarias gariepinus* and nile tilapia *Oreochromis niloticus* [8]. So far, limited information is available on the effects of FSM by *Bacillus sabtilis* on the growth performance and feed utilization of olive flounder. Also less attention has been paid to the relationship between dietary plant protein inclusion and fish physiological status [23]. So, this study was conducted to determine the effects of partial substitution of dietary fish meal by FSM on growth performance, amino acid profile and some biochemical parameters of juvenile olive flounder in an attempt to understand the mechanisms and appropriated level of FSM inclusion in diet of olive flounder.

MATERIALS AND METHODS

Diet preparation

The FSM used in this study was supplied by CJ CheilJedang Co. (Seoul, Korea). The FSBM was fermented using *Bacillus subtilis* by a process modified to method as mentioned in the Lim et al (2010) [19].Briefly, soybean meal was steam cooked in an autoclave at 100 ˚C for 20 min (pH 5-6). After cooling, the steamed soybean meal was inoculated by evenly spraying spore suspension of *B. subtilis*. After a thorough mixing, the inoculated soybean meal substrate was incubated at 37℃ for 24 h (pH 8.35). The products of fermentation were dried in a vacuum drying oven at below 60℃ for 15 h (pH 7-8). Finally, FSM was ground to be below 400 um mesh size.

Table 1 Ingredient and proximate composition of experimental diets

Ingredients (g kg $^{-1}$)	Diets				
	CON	FSM8	FSM16	FSM24	FSM32
Pollack fish meal	600	540	480	420	360
Fermented soybean meal	0	80	160	240	320
Wheat flour	238	211.9	185.8	159.7	133.6
Corn gluten meal	50	50	50	50	50
α-potato-starch	50	50	50	50	50
Fish oil	20	25	30	35	40
Soybean oil	4	3	2	1	0
Vitamin premix[1]	10	10	10	10	10
Mineral premix[2]	10	10	10	10	10
Stay-C (50%)	5	5	5	5	5
Choline salt (50%)	3	3	3	3	3
DL-Methione	0	1	2	3	4
L-Lysine	0	0.6	1.2	1.8	2.4
Taurine	10	10.5	11	11.5	12
Proximate analysis (g kg^{-1}, dry matter basis)					
Crude protein	516	500	504	515	515
Crude lipid	73	72	72	72	72
Ash	140	128	126	120	110
Essential amino acid (g 100 g^{-1} protein)					
Arg	6.4	6.4	6.4	6.4	6.3
His	2.3	2.4	2.4	2.3	2.3
Ile	4.1	4.1	4.1	4.1	4
Leu	8.6	8.5	8.5	8.5	8.4
Lys	8.3	8.1	8.6	7.5	7.8
Met + Cys	3.2	3.5	3	3.6	3.1
Phe + Tyr	6.8	7.1	6.9	7.6	7.2
Thr	4.6	5.1	5.2	4.7	4.4
Val	4.6	4.6	4.6	4.5	4.3

[1]*Vitamin premix contained the following amount which were diluted in cellulose (g/kg premix): L-ascorbic acid, 121.2 ; DL-(tocopheryl acetate, 18.8; thiamin hydrochloride, 2.7; riboflavin, 9.1; pyridoxine hydrochloride, 1.8; niacin, 36.4; Ca-D-pantothenate, 12.7; myo-inositol, 18.8; D-biotin, 0.27; folic acid, 0.68; p-aminobenzoic acid, 18.2; menadione, 1.8; retinyl acetate, 0.73; cholecalciferol, 0.003; cyanocobalamin, 0.003.*

[2]*Mineral premixcontained the following ingredients (g/kg premix); $MgSO_4.7\ H_2O$, 80.0; $NaH_2PO_4.2H_2O$, 370.0; KCl, 130.0; Ferric citrate, 40.0; $ZnSO_4.7H_2O$, 20.0; Ca-lactate, 365.5; CuCl, 0.2; $AlCl_3.6H2O$, 0.15;KI, 0.15; $Na_2Se_2O_3$, 0.01; $MnSO_4.H_2O$, 2.0; $CoCl_2.6H_2O$, 1.0.*

Five isonitrogenus and isolipidic diets were formulated containing 0, 80, 160, 240 and 320 g kg^{-1} FSM produced by *Bacillus sabtilis* as a control, FSM8, FSM16, FSM24 and FSM32. Ingredients and nutrient contents of the experimental diets are presented in Table 1. Pollack fish meal was used as the primary protein source. Cod liver oil and soybean oil were used as lipid sources. All ingredients were thoroughly mixed with 300 g kg^{-1} distilled water, and pellets were prepared using a pellet machine. The pellets were dried at room temperature for 24 h and ground into desirable particle sizes. All diets were stored at -30℃ until used.

Fish rearing

Juvenile flounder were obtained from a local farm (Namhae, Korea). The fish were acclimated to laboratory condition for 2 weeks before starting the feeding trial. Juvenile fish (initial mean weight, 11.9±0.19 g) were allocated randomly into 300 L cylindrical plastic tanks with 30 fish per each tank for the feeding trial after being collectively weighed. Three replicate groups of fish were hand-fed to apparent satiation two times a day (9:00 and 17:00) for 8 weeks. Filtrated sea water was supplied at a flow rate of 4 $Lmin^{-1}$ in each tank and the mean water temperature was 16.8±0.39℃. The photoperiod was left under natural conditions during the feeding trail. At the end of experiment juvenile flounder in each tank were collectively weighed after anesthetizing with tricaine methanesulfonate (MS222, Sigma, St. Louis, MO, USA) at a concentration of 100 mg L^{-1} after starvation for 24 h.

Fish sampling and chemical analysis

Fifty fish at the beginning of the feeding trial and five fish from each tank at the end of experiment were randomly sampled and stored at -25℃ in freezer for proximate composition and amino acid analyses. At the end of the feeding experiment, blood was drawn from caudal vessel with 1 ml heparinized syringes from three fish in each tank and transferred to micro centrifuge tubes. Also five fish from each tank were sampled and stored at liquid nitrogen for digestive and antioxidant enzymes analyses.

Proximate analysis of the diets and dorsal muscle of fish were determined according to the method of AOAC (1995) [1]. Crude protein content was determined using the Kjeldahl method using an Auto Kjeldahl System (Buchi, Flawil, Switzerland). Crude lipid was analyzed by ether extraction, moisture content by a dry oven drying at 105℃ for 6 h and ash by a furnace muffler (550℃ for 4 hours). Amino acid composition in the experimental diets and dorsal muscle of fish was performed with acid hydrolysis with 6 N HCL (reflux for 23 h at 110℃) followed by using an automatic amino acids analyzer (Hitachi, Tokyo, Japan). The collected blood was centrifuged at 7500 g for 10 min and the plasma was separated and stored in –80℃ freezer. Plasma glucose, total protein, triglyceride and cholesterol concentration were determined using a clinical investigation commercial kit (Asan Pharmaceutical Co., Seoul, Korea).

Digestive enzymes activity

At the end of feeding trial (after one day starvation), samples (5 fish per tank) washed in cold distilled water and stored in liquid nitrogen were dissected on a glass maintained on ice. Samples of intestine were homogenized immediately in 50 mM Tris-HCl containing 20 mM $CaCl_2$ and 50 mM KCl (pH 7.5) by a homogenizer (Wiggenhauser, Berlin, Germany), followed by centrifugation (15000 g for 40 min at 4℃). We used 100 mg tissue mL^{-1} buffer for homogenization and then homogenates were kept frozen in -80℃ to determine biochemical analysis. All the assay techniques were based on photometric procedures in which disappearance of the substrate for product formation was measured.

The activity of α-amylase, lipase and trypsin was assayed according to the methods described below. Enzyme activity expressed as specific activity of U mg^{-1} protein. The specific activity of α-amylase was performed by the enzymatic photometric method using amylase kit (Abcam, Cambridge, UK). The formation rate of p-nitrophenyl (pNP) is proportional to the α-amylase activity present of the sample which is measured by the increase of the wavelength absorbance at 405 nm (405-420 nm). One unit amylase is defined as the amount of amylase which hydrolyses Ethylidene-pNP-G7 to generate 1 µmol of p-nitrophenol per minute at 25℃. Briefly, in this method, 50 µl assay buffer and 50 µl substrate were mixed to 50 µl sample and after completing 15 min incubation at room temperature; the mixture read at the wavelength of 405 nm by Tecan Sunrise (Mannedorf, Zurich, Switzerland) Elisa reader. The Specific activity of lipase was measured by the enzymatic photometric method using lipase kit (Abcam, Cambridge, UK). Lipase hydrolyses specific substrate to generate TNB which is reacted with 5, 5- dithiobis (2-nitro benzoic acid) DTNB prob to generate color at the wavelength of 405 nm. Therefore, one unit lipase is defined as the amount of lipase which hydrolyses the substrate and generated 1 µmol of TNB per minute at 37℃. Briefly, in this method, 100 µl reaction mixture containing assay buffer, DTNB probe and lipase substrate was added to 50 µl sample and OD_1 read at the wavelength of 412 nm. After completing 3 min incubation in 37℃, OD_2 read at the wavelength of 412 nm by Tecan Sunrise (Mannedorf, Zurich, Switzerland) Elisa reader. Specific activity of trypsin

was measured by the enzymatic photometric method using tripsin kit (Abcam, Cambridge, UK). It is based on cleaves a substrate to generate p-nitroaniline (p-NA) which is detected at the wavelength of 405 nm. One unit is defined as an amount of trypsin cleaves the substrate, yielding 1 µmol of p-NA per minute at 25℃. In this method briefly, 50 µl reaction mixture containing assay buffer and trypsin substrate was added to 50 µl sample and OD_1 read at the wavelength of 405 nm. After completing 5 min incubation in 37℃, OD_2 read at the wavelength of 405 nm by Tecan Sunrise (Mannedorf, Zurich, Switzerland) Elisa reader. Also protein content in the supernatants was measured using the Bradford (1976) method [4].

Antioxidant enzymes activity

To determine the activity of hepatic superoxide dismutase (SOD) and glutathione peroxides (GPx), 0.1 g of fish liver were homogenized in 9 volumes of 5 mM Tris and 35 mM glycine (pH 7.6). The homogenates were centrifuged at 10000 g for 30 min to remove debris. The resultant supernatants were used for SOD and GPx assay. The activity of SOD in liver was assayed using kit manufactured by (Sigma Aldrich Inc.Saint-Louis, Switzerland). The rate of the reduction with O_2 was linearly related to the xanthine oxidase (XO) activity, and was inhibited by SOD. Therefore, the IC50 (50% inhibition activity of SOD or SOD-like materials) can be determined by a colorimetric method. In this method 20 µl sample was added to 220 µl substrate solution and then Inhibition activity determined by a kinetic method up to 20 min at the wavelength of 410 nm by Tecan Sunrise (Mannedorf, Zurich, Switzerland) Elisa reader. Also the activity of SOD in plasma of juvenile fish was assayed in a similar approach with measurement of SOD activity in liver. Activity of GPx in fish liver was assayed using kit manufactured by (BioVision, Inc. California, USA). In this assay, cumene hydroperoxide as a peroxide substrate (ROOH), glutathione reductase (GSSG-R) and NADPH (b−Nicotinamide Adenine Dinucleotide Phosphate, Reduced) were mixed by each other. Then the changing in the wavelength of 340 nm due to NADPH oxidation was monitored as an indicative of GPx activity. Briefly, 50 µl sample was added to 40 µl reaction mixture and incubated for 15 minutes. Then 10 µl Cumene Hydroperoxide was added and OD_1 read at the wavelength of 340 nm. After completing 5 min incubation OD_2 read in 340 nm by Tecan Sunrise (Mannedorf, Zurich, Switzerland) Elisa reader. Activity of glutathione peroxidase was calculated as a U mg^{-1} protein.

Statistical analysis

The data were subjected to one-way analysis of variance (ANOVA) and if significant ($P<0.05$) differences were found, Duncan's multiple range test was used to rank the groups. The data are presented as mean ± SE of three replicate groups. All statically analyses were carried out using the SPSS version 19 (SPSS Inc., Michigan Avenue, Chicago, Illinois, USA).

RESULTS

The results of growth performance and feed utilization were shown in Table 2. The fish fed diets containing up to 240 g kg^{-1} FSM had no significant difference of survival, growth, feed efficiency and protein efficiency ratio compared with control group ($P > 0.05$). Survival, growth, feed efficiency and protein efficiency ratio of fish fed the diet containing 320 g kg^{-1} FSM were significantly lower than control group ($P < 0.05$). Daily feed intake and daily protein intake were not affected by dietary levels of FSM compared with control group ($P > 0.05$). Morphological parameters of fish were shown in Table 3. Hepatosomatic index of fish fed diets containing 80 and 160 g kg^{-1} FSM had not significant difference compared with control group ($P > 0.05$). It was significantly higher for fish fed diets containing 240 and 320 g kg^{-1} FSM compared with control group ($P < 0.05$). Condition factor and visceral somatic index were not affected by dietary levels of FSM compared with control group ($P > 0.05$).

Body composition and amino acid profile of fish dorsal muscle were shown in Tables 4 and 5. Proximate composition of whole body and amino acid profile of fish dorsal muscle were not affected by different levels of FSM compared with control group ($P > 0.05$). The results of hematological parameters of plasma in juvenile flounder were shown in Table 6. Triglyceride and total protein of plasma in fish fed the diets containing 80-320 g kg^{-1} FSM were higher than control group ($P < 0.05$). Cholesterol concentration of plasma in fish fed 320 g kg^{-1} FSM diet was lower compared with other groups ($P < 0.05$). Total protein concentration of plasma in fish fed control diet was the lowest compared with other group ($P < 0.05$). The activity of digestive and antioxidant enzymes was presented in Table 7. The activity of α-amylase, lipase and trypsin in intestine of fish were not affected by different levels of FSM compared with control group ($P > 0.05$). The activity of glutathione peroxidase and superoxide dismutase in liver and also superoxide dismutase in plasma was not affected by different levels of FSM compared with control group ($P > 0.05$).

Table 2 Growth performances and feed utilization of juvenile olive flounder fed the experimental diets for 8 weeks

	Diets				
	Con	FSM8	FSM16	FSM24	FSM32
Initial average weight (gfish^{-1})	12±0.11^{ns}	11.83±0.17	11.80±0.05	11.96±0.03	12.03±0.12
Final average weight (g fish $^{-1}$)	34.9±0.58^{bc}	36.5±1.27^{c}	32.7±1.82^{ab}	31.6±0.83^{ab}	29.8±0.09^{a}
Specific growth rate ^{2}(%)	1.9±0.03^{bc}	2.0±0.01^{c}	1.8±0.09^{b}	1.7±0.05^{ab}	1.6±0.01^{a}
Survival	81±1.1 ^{b}	71±5.5^{ab}	69±5.8^{ab}	67±10.0^{ab}	63±4.8^{a}
Feed efficiency^{3} (%)	118±5.7^{b}	107±5.6^{b}	104±3.9^{b}	104±3.7 ^{b}	84±0.5 ^{a}
Daily feed intake ^{4}(%)	1.2±0.06^{ns}	1.2±0.06	1. 3±0.07	1.3±0.06	1.4±0.12
Daily protein intake ^{5} (%)	0.6±0.03^{ns}	0.7±0.03	0.7±0.03	0.6±0.03	0.6±0.06
Protein efficiency ratio^{6}	2.3±0.10^{b}	2.1±0.13^{b}	2.0±0.57^{b}	2.0±0.10^{b}	1.6±0.00 ^{a}

Values (mean ±SE of three replication) in the same row not sharing a common superscript are significantly different (P<0.05).ns= values are not significant (P>0.05).

^{21}Specific growth rate (%) = [ln (final fish wt.) - ln (initial fish wt.)] × 100/days of feeding.

^{2}Feed efficiency = wet weight gain × 100/feed intake.

^{3}Daily feed intake = feed intake × 100/ [(initial fish wt. + final fish wt. + dead fish wt.) × days reared/2].

^{4}Daily protein intake = protein intake × 100/ [(initial fish wt. + final fish wt. + dead fish wt.) × days reared/2].

^{45}Protein efficiency ratio = (wet weight gain / protein intake) × 100.

Table 3 Morphological parameters of juvenile olive flounder fed the experimental diets for 8 weeks

	Diets				
	Con	FSM8	FSM16	FSM24	FSM32
Condition factor^{1}	1.1±0.06^{ns}	1.1±0.12	1.2±0.00	1.1±0.01	1.2±0.04
Hepatosomatic index ^{2}	1.5±0.26^{ab}	1.3±0.07^{a}	2.0±0.11^{bc}	2.3±0.13^{c}	2.2±0.17^{c}
Visceral somatic index^{3}	2.6±0.35^{ns}	2.0±0.10	2.4±0.09	2.6±0.04	2.5±0.00

Values (mean ±SE of three replication) in the same row not sharing a common superscript are significantly different (P < 0.05).ns= values are not significant (P > 0.05).

^{1} Condition factor = [fish weight (g)/fish length (cm)^{3}] × 100.

^{2} Hepatosomatic index = (liver weight/body weight) × 100.

^{3}Visceral somatic index = (viscera weight/body weight) × 100.

Table 4 Proximate composition (%) of the whole body of juvenile olive flounder fed the experimental diet for 8 weeks

	Diets				
	Con	FSM8	FSM16	FSM24	FSM32
Crude protein	17.4±0.32^{ns}	16.8±0.34	17.9±0.81	16.9±0.35	17.8±0.09
Crude lipid	1.7±0.17^{ns}	1.9±0.13	1.9±0.08	1.7±0.16	1.5±0.21
Moisture	74.8±0.32^{ns}	74.8±0.33	74.7±0.70	75.6±0.59	74.5±0.11
Ash	3.8±0.14^{ns}	3.9±0.13	4.0±0.19	3.9±0.19	4.0±0.09

Values are mean ±SE of three replication.

ns= values are not significant (P>0.05).

Table5 Amino acids composition (% of protein) of the dorsal muscle in juvenile olive flounder fed the experimental diets for 8 weeks

	Diets				
	CON	FSM8	FSM16	FSM24	FSM32
Arg	6.4±0.03^{ns}	6.4±0.03	6.4±0.09	6.5±0.06	6.6±0.09
His	2.4±0.00^{ns}	2.4±0.00	2.4±0.03	2.3±0.03	2.3±0.03
Isl	4.2±0.18^{ns}	4.1±0.07	3.9±0.23	3.8±0.13	3.7±0.29
Leu	8.7±0.37^{ns}	9.0±0.03	9.1±0.03	9.1±0.07	9.1±0.03
Lys	9.8±0.06^{ns}	9.7±0.03	9.7±0.06	9.6±0.03	9.5±0.12
Met + Cys	4.2±0.06^{ns}	4.2±0.06	4.2±0.00	4.2±0.03	4.20±0.01
Phe + Tyr	7.9±0.03^{ns}	7.9±0.03	7.9±0.03	7.8±0.03	7.9±0.03
Thr	4.8±0.07^{ns}	4.8±0.03	4.8±0.03	4.8±0.00	4.8±0.06
Val	10.0±0.15^{ns}	10.0±0.09	10.1±0.07	10.1±0.06	10.1±0.15

Values are mean ±SE of three replication.

ns= values are not significant (P>0.05).

Table 6 Hematological values of the plasma in juvenile olive flounder fed the experimental diets for 8 weeks

	Diets				
	Con	FSM8	FSM16	FSM24	FSM32
Total protein (g dl^{-1})	3.4±0.47^{a}	6.2±0.15^{b}	7.0±0.10^{c}	7.2±0.11^{c}	7.0±0.05^{c}
Glucose (mg dl^{-1})	20.1±3.50^{ns}	21.5±1.00	21.9±0.50	20.7±2.00	19.2±2.10
Cholesterol (mg dl $^{-1}$)	35.5±1.10^{c}	31.7±2.10^{b}	65.7±0.10^{d}	38.5±0.10^{c}	23.9±1.30^{a}
Triglyceride (mg dl $^{-1}$)	168.7±12.90^{a}	341.3±33.50^{b}	430.7±34.80^{b}	699.4±76.70^{c}	750.9±41.10^{c}

Values (mean ±SE of three replication) in the same row not sharing a common superscript are significantly different (P <0.05).

ns= values are not significant (P>0.05).

Table 7 Digestive and antioxidant enzyme activities in juvenile olive flounder fed the experimental diets for 8 weeks

	Diets				
	Con	FSM8	FSM16	FSM24	FSM32
Amylase (U mg $^{-1}$ protein)	0.6±0.20^{ns}	0.6±0.19	0.7±0.14	0.7±0.06	0.6±0.05
Lipase (U mg $^{-1}$ protein)	4.9±1.84^{ns}	2.3±0.61	2.7±0.49	4.9±1.85	3.9±0.84
Trypsin (U mg $^{-1}$ protein)	56.9±0.32^{ns}	57.4±2.28	61.5±7.46	59.26±7.43	57.2±9.93
Glutathione peroxidase of liver (U mg $^{-1}$ protein)	8.2±0.66^{ns}	7.1±0.91	7.5±0.56	6.4±0.21	6.5±0.70
Superoxide dismutase of liver (%)	76.6±2.73^{ns}	74.6±3.27	79.5±3.54	71.7±5.52	75.9±3.03
Super oxide dismutase of plasma (%)	67.4±0.33^{ns}	62.0±5.32	57.5±4.87	60.6±2.46	61.8±3.35

Values are mean ±SE of three replication.
ns= values are not significant (P>0.05).

DISCUSSION

Generally poor growth performance found in fish fed plant protein is related to palatability, amino acid imbalance, phosphorus availability, anti-nutritional factors [10] and disorder in lipid metabolism [34]. Ye reported [34] that replacement of fish meal protein above 240 g kg^{-1} could adversely affect the growth, protein and lipid metabolism of olive flounder. Similarly it was observed that growth of turbot, *Scophthalmus masimus* was gradually decreased by increasing levels of soybean protein concentrate, even at the minimum level of 250 g kg^{-1} substitution [6]. So, the improved growth performance of fish is attributed to a number of factors including improved palatability, digestibility and reduced exposure to anti-nutritional factors. Therefore, several practical ways have been suggested to improve utilization of plant proteins including; blending [12], feeding stimulants [7] and fermentation [13; 17]. It has been reported that soybean meal could be used as a partial substitute for fish meal up to 100 -159 g kg^{-1} of diet for juvenile founder *Paralichthys olivaceus* [7; 15]. In the present study, survival, growth, feed efficiency and protein efficiency ratio of fish fed diets containing up to 240 g kg^{-1} FSM were not different compared with control group. The different results among the studies may be due to improvement of digestibility and removing anti-nutritional factor of soybean meal such as protease inhibitor by fermentation. The process of fermentation by microorganisms might help towards higher replacement of fish meal protein with alternative plant proteins by decreasing amount of anti-nutritional factors and increasing nutrient availability [13]. It has been reported that fish meal protein could be replaced up to 500 g kg^{-1} by fermented fish offal, mustard oil cake and rice bran mixture in the diet for Indian major carp and catfish, *Heteropneustes fossilis* [20; 21]. It was shown FSM in the diet induce to higher growth and feed efficiency compared to control diet for yellowtail [29]. Also Kader reported the beneficial effect of dietary FSM on the growth of flounder [13]. Wee suggested that nutrient value of plant ingredient improved during fermentation period by microbial activities [32].

It was observed that hepatosomatic index and plasma triglyceride content of juvenile olive flounder fed diet containing 240-320 g kg^{-1} FSM were higher than fish fed diet containing 0-80 g kg^{-1} FSM and 0-160 g kg^{-1} FSM, respectively. Recent evidences showed that the increasing hepatosomatic index of fish was related to liver lipid content [34]. Panserat [24] observed an over-expression of genes involved in liver lipid biosynthesis in rainbow trout fed the plant protein based diet. In addition, it was reported liver lipid content was positively correlated with serum triglyceride content and negatively correlated with cholesterol content [16]. Also it was shown the higher amount of soybean protein induced to decrease plasma and liver cholesterol concentrations in rat [35]. Therefore, the increased hepatosomatic index and triglyceride content of fish fed high level of FSM may be due to some disorder in lipid metabolism leading to hyperlipidemia.

Also, lower growth rate of flounder fed the diet containing 320 g kg^{-1} FSM could be related to non-starch polysaccharides content in FSM. It was reported non-starch polysaccharides fraction of soybean meal had negatively effect on growth performance of Atlantic salmon and European sea bass [11; 28]. It has been suggested that non-starch polysaccharides in soybean lead to reduce bioavailability of nutrients and energy through mechanisms involving a binding action with bile salts [9].

It has been reported that using vegetable products from fermentation processing could enhance non-specific immune response, activity of antioxidant enzymes and disease resistance for fish [2; 25]. Lee announced that using Meju which is a traditional Korean fermented soybean paste by *Aspergillus oryzae* and *B. sabtilis* increased antioxidant activity like activity of superoxide dismutase in liver of olive flounder [15]. Also Kader reported that biological antioxidant activity significantly increased with FSM in diet of flounder. It was not observed significant effect of FSM on the activity of antioxidant enzymes in liver and plasma of olive flounder in this experiment [13].

CONCLUSION

We concluded that using FSM produced by *Bacillus sabtilis* had beneficial effect on the nutritional quality of soybean meal. Therefore, fish meal protein could be replaced up to 240 g kg^{-1} by FSM without negative effect on the growth performance of juvenile olive flounder.

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