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RESEARCH ARTICLE

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Effects of soybean oligosaccharides on lipid metabolism of Japanese flounder (*Paralichthys olivaceus* Temminck et Schlegel) fed animal or plant protein source-based diets

Abstract A feeding trial was conducted to investigate the effects of dietary soybean oligosaccharides (SBOS) on the levels of cholesterol in plasma and liver, and the fatty acid composition in muscle and liver of juvenile Japanese flounder (*Paralichthys olivaceus* Temminck et Schlegel). Four isonitrogenous and isocaloric practical diets (crude protein 49%, gross energy 19 kJ/g) including only fish meal (FM) or soy protein isolate (SPI) as sole dietary protein source with (Diets FMO and SPIO) or without (Diets FM and SPI) supplemented SBOS (stachyose, 2.61%; raffinose, 0.61%) were formulated. Each diet was randomly assigned to triplicate aquaria stocked with 30 fish (initial body weight 1.93 ± 0.02 g) each. Fish were maintained in the flow-through aquaria at water temperature ranging from 22.0°C to 25.0°C and fed twice (08:00, 16:00) daily to apparent satiation for 8 weeks. Dietary SBOS supplementation significantly increased the levels of total cholesterol (TC) and high-density lipoprotein cholesterol (HDL-C) in plasma of the fish fed FM-based diets ($P < 0.05$), but no significant differences were observed at the levels of plasma triacylglycerol (TG) and low-density lipoprotein cholesterol (LDL-C) and LDL-C/HDL-C ratio ($P > 0.05$). The levels of TC, TG, HDL-C and LDL-C, and LDL-C/HDL-C ratio in plasma of the fish fed SPI-based diets were not significantly influenced by the supplemented SBOS ($P > 0.05$). Supplementation of SBOS did not influence the hepatic TC, TG and lipid contents and lipid droplet accumulation in fish fed FM-based diets, but significantly decreased the hepatic TC, TG and lipid contents and lipid droplet accumulation in fish fed SPI-based diets ($P < 0.05$). In contrast, supplemented SBOS significantly increased fecal cholesterol extraction in the fish fed FM-based diets ($P < 0.05$), but no significant difference was observed in the fish fed SPI-based diets ($P > 0.05$). The fatty acid composition in muscle and liver was not significantly affected by supplemental SBOS regardless of dietary protein source ($P > 0.05$). These results indicate that dietary SBOS supplementation (stachyose, 2.61%; raffinose, 0.61%) does not negatively affect the lipid metabolism of the fish fed FM-based diets, but decrease the incidences of fatty liver of the fish fed SPI-based diets.

Keywords Japanese flounder (*Paralichthys olivaceus* Temminck et Schlegel), soybean oligosaccharides, lipid metabolism, cholesterol, fatty liver

1 Introduction

The hypocholesterolemic effect of substituting soy protein for animal protein has been well demonstrated in terrestrial (Carroll and Kurowska, 1995; Madani et al., 2004) and aquatic animals (Kaushik et al., 1995; Dongmeza et al., 2006; Romarheim et al., 2006). Previous studies suggested that the hypocholesterolemic effect was largely attributable to the differences in the amino acid profiles (such as lysine/arginine ratio) of soy protein and animal protein (Forsythe et al., 1986; Park et al., 1987; Kaushik et al., 1995). However, studies with diets based on highly purified plant and animal proteins have suggested that highly purified soy protein (98% of purity) and casein play only minor roles in the regulation of serum cholesterol concentration (Madani et al., 1998; 2000). In addition, previous studies in our laboratory showed that soy protein concentrate (SPC) versus soy protein isolate (SPI) had a plasma cholesterol-lowering effect on Japanese flounder (*Paralichthys olivaceus* Temminck et Schlegel) (Deng, 2006; Mai et al., 2006). Therefore, some authors suggested that some non-protein components such as non-starch polysaccharides (NSP) and soybean oligosaccharides (SBOS) associated with these proteins may be responsible for the hypocholesterolemic effect (Tasker and Potter, 1993; Hossain

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et al., 2001; Madani et al., 2004). It has also recently been demonstrated that NSP have the hypocholesterolemic effect on Atlantic salmon (*Salmo salar* L.) (Refstie et al., 1999) and common carp (*Cyprinus carpio* L.) (Hossain et al., 2001). However, little information is available concerning the effects of specific SBOS on the plasma cholesterol levels in fish (Refstie et al., 1999).

Soybean oligosaccharides refer to oligosaccharides found in soybeans, which contain primarily sucrose, raffinose, and stachyose (Hymowitz et al., 1972). Raffinose contains a fructose, a glucose, and a galactose, while stachyose contains one more galactose (Fig. 1). Both have a β-1, 2-fructosidic linkage and an α-1, 6-galactosidic linkage, which cannot be cleaved in the digestive tract of monogastric animals and fish due to the lack of the enzyme α-galactosidase that is necessary to break down raffinose and stachyose into sucrose and galactose (Neus et al., 2005). Thus, SBOS, mainly raffinose and stachyose, are deemed as one of the antinutritional factors present in soybean meal (Francis et al., 2001). Apparently, SBOS are low molecular weight carbohydrates. At the same time, based on the physical properties, the carbohydrates can be classified as water-soluble and water-insoluble ones (Bach-Knudsen, 1997). Soybean oligosaccharides belong to the water-soluble carbohydrate, which account for approximately 10% of dry weight of soybean meal (sucrose 5%, raffinose 1% and stachyose 4%) (Kennedy et al., 1985).

Due to the decreasing ocean fisheries resources, and increasing price of fish meal in recent years, many studies have been conducted on fish meal substitution in aquafeed with the lower price protein source (e.g. soybean meal). The antinutritional factors in soybean meal must be considered when evaluating the effects of fish meal (FM) substitution with soybean meal. Japanese flounder is a commercially important carnivorous fish widely cultured in China, Japan, and South Korea because of its delicious meat and rapid growth. The aim of this study was to evaluate the effects of SBOS on the lipid metabolism in Japanese flounder fed animal or plant protein source-based diets.

2 Materials and methods

2.1 Soybean oligosaccharides

Soybean oligosaccharides, a by-product made from soy protein concentrate whey, was supplied by Dong Ying Wonderful Vegetable Protein Science and Technology Co., Ltd. (Shandong, China). The proximate compositions of SBOS are shown in Table 1.

2.2 Experimental diets

Using either fish meal (71.2% crude protein) or SPI (91.0% crude protein) as a sole protein source, fish oil and soybean lecithin as lipid sources, and wheat flour (15.2% crude protein) as a carbohydrate source, respectively, four isonitrogenous (crude protein 49%) and isocaloric (gross energy 19 kJ/g) practical diets were formulated to meet the protein and energy requirements of Japanese flounder (Yigit et al., 2004). Diets FM or SPI included only FM or SPI as a sole dietary protein source, respectively. Diets FMO or SPIO were supplemented with a 10% SBOS mixture (stachyose, 2.61%; raffinose, 0.61%) based on either Diets FM or SPI, respectively. The ingredients and proximate composition of diets are presented in Table 2.

Experimental ingredients were ground into fine powder through 320-μm mesh. All the ingredients were thoroughly mixed with fish oil, and water was added to produce stiff

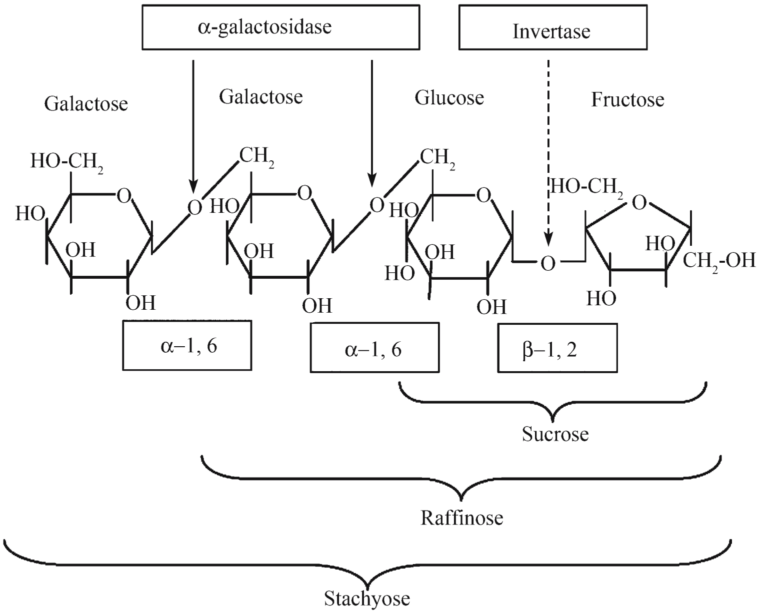


Fig. 1 The structure of α-galactosides raffinose and stachyose and the enzymes that catalyze their hydrolysis

Table 1 Analytical composition of soybean oligosaccharides (%, dry weight)

Composition	Moisture1)	Crude protein1)	Crude lipid1)	Ash1)	Stachyose1)	Raffinose1)	Sucrose2)	Other sugars2)	Others
Content	3.07	1.05	1.7	7.8	26.1	6.1	45.0	7.2	1.98

Notes: 1) stands for values analyzed by the company laboratory; and 2) for values adapted from Dong Ying Wonderful Vegetable Protein Science and Technology Co., Ltd. Other sugars mainly include glucose, fructose, vabascose, etc.

Table 2 Formulation and proximate composition of experimental diets (%, dry weight)

Ingredients	Diets			
	FM	FMO	SPI	SPIO
Fish meal1)	65.0	65.0		
Soy protein concentrate2)			51.0	51.0
Soybean oligosaccharides2)		10.0		10.0
Wheat flour	18.0	18.0	18.0	18.0
Fish oil1)	2.0	2.0	8.0	8.0
Soybean lecithin	2.0	2.0	2.0	2.0
α-Cellulose	10.0	0.0	16.0	6.0
Dextrin			1.0	1.0
Cholesterol			1.0	1.0
Mineral premix3)	1.0	1.0	1.0	1.0
Vitamin premix3)	1.0	1.0	1.0	1.0
Choline chloride /50%	0.5	0.5	0.5	0.5
Vitamin C4)	0.2	0.2	0.2	0.2
$Ca(H_2PO_4)_2$	0.3	0.3	0.3	0.3
Total	100.0	100.0	100.0	100.0
Proximate composition				
Dry matter /%	93.6	94.1	92.9	89.9
Crude protein /%	48.7	49.1	47.8	48.2
Crude lipid /%	9.0	8.6	8.1	8.3
Gross energy /$MJ\cdot kg^{-1}$	19.5	19.4	20.1	19.9

Notes: 1) stands for ingredients supplied by Liuhe Feed Co., Ltd. (Shandong, China), with crude protein (71.2% dry matter) and crude lipid (12.5% dry matter); 2) for ingredients supplied by Dong Ying Wonderful Vegetable Protein Science and Technology Co., Ltd. (Shandong, China), with soy protein concentrate of crude protein (91.0% dry matter) and crude lipid (2.0% dry matter), soybean oligosaccharides of crude protein (1.05% dry matter), crude lipid (1.7% dry matter), stachyose (26.1% dry matter) and raffinose (6.1% dry matter), and others (52.2% dry matter); 3) for ingredients supplied by Qingdao Marine Science & Technology Bio-tech Co., Ltd., Shandong, China; and 4) for L-Ascor-bate-2-polyhosphate (35%).

dough. The dough was then extruded using a twin-screw extruder [F-26 (II)] (South China University of Technology, China), and extruded through a 3-mm die. The moist pellets were dried in a forced air oven at room temperature for about 12 h, and then stored at −20°C until they were used.

2.3 Experimental procedures

Juvenile Japanese flounder were obtained from a commercial farm. Prior to the start of the experiment, fish were transported to the experiment station (Yellow Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, Qingdao, China), stocked into experimental tanks to adjust to the new environment for two weeks and fed twice daily with a commercial diet (Nisshin Flour Milling Co., Ltd., Japan) to satiation during this period. Fiberglass circular tanks (100 L capacity filled to 80 L) were used. Seawater, continuously pumped from the coast adjacent to the experiment station, passed through sand filters into each tank at approximately 1.5 L/min. All rearing tanks were provided with continuous aeration and maintained under natural photoperiod (14 h light/10 h dark at the end of August and 12 h light/12 h dark at the end of October).

At the start of the experiment, the fish were fasted for 24 h before weighing. Fish of similar sizes (initial mean body weight 1.93 ± 0.02 g) were distributed into 12 tanks with 30 juveniles per tank. Each diet was randomly assigned to triplicate tanks. Fish were hand-fed to apparent satiation twice daily (08:00 and 16:00). The feeding trial lasted for 8 weeks. During the experimental period, the temperature ranged from 22.0°C to 25.0°C, salinity 30‰–33‰, and pH 7.7–7.9.

2.4 Sample collection and chemical analysis

Continuous feces collection was begun within 2 h after feeding. Feces was removed by siphon and separated from the remaining feed by a mesh screen. Fecal samples were rinsed with distilled water, pooled and homogenized from each tank, frozen (−20°C) and lyophilized. At the end of the feeding trial, the fish were fasted for 24 h before harvest. Seven fish per tank were sampled for analyses of cholesterol, total lipid and fatty acid composition. Blood was collected from the caudal vein with a heparinized syringe and transferred into a heparinized tube. Plasma was recovered after centrifugation (6 000 g, 10 min) and immediately stored at −70°C until analysis. Liver and muscle samples were removed and stored frozen (−20°C) for subsequent determination of the lipid composition and fatty acids profiles. Analyses of dry matter (105°C, 24 h), crude protein (Kjeldahl nitrogen × 6.25), crude lipid (ether extraction by Soxhlet method) and ash (550°C, 18 h) in feed ingredients and experimental diets were performed following standard laboratory procedures (AOAC, 1995). Gross energy in diets was determined by an adiabatic bomb calorimeter (Parr 1281, USA). Raffinose and stachyose contents in SBOS were measured using high-performance liquid chromatography (HPLC) following ethanol extraction of the samples (Liu and Markakis, 1987). Total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and triglyceride (TG) levels in plasma were determined without extraction by using a commercial enzymatic kit (supplied by Shanghai Jiemen Bio-Tech Co., China). Plasma low-density lipoprotein cholesterol (LDL-C) level and LDL-C/HDL-C ratio were calculated according to the method of Friedewald et al. (1972). Total cholesterol contents in liver, diets and feces, and TG content in liver were determined using the same enzymatic kits as in the plasma, but the diet and feces samples were extracted with solvents (Folch et al., 1957). Total lipid content in fish liver was determined gravimetrically (Folch et al., 1957). Assay of fecal bile acid was performed according to the method described by Madani et al. (1998).

Fatty acids were extracted from the diets, muscle and liver samples with solvents (Folch et al., 1957). The procedures for analysis of the fatty acid profiles in diets, muscle and liver of fish were modified from those described by Metcalfe et al. (1966). Briefly, fatty acid methyl esters were separated and quantified by a gas chromatogram equipment (HP 5890, USA) with a fused silica capillary column (007-CW, HP) and a flame ionization detector. The column temperature was programmed to rise from 150 up to 200°C at a rate of 15°C/min, from 200°C to 250°C at a rate of 2°C/min. Fatty acids were identified by comparison with retention times of the standard fatty acids methyl esters (Sigma Chemical Co.).

2.5 Liver histology

At the end of the experimental period, liver from three fish per tank were removed for histological examinations. Liver samples were fixed in Bouin's solution, dehydrated in a graded ethanol series and embedded in paraffin (Robaina et al., 1995). A series of 7-μm sections were stained with hematoxylin and eosin (H&E) for histology analysis with light microscopy (Teh et al., 1997).

2.6 Statistical analysis

All data are presented as means ± SEM. Data were analyzed by students' *t*-test to examine the effect of dietary SBOS within the same dietary protein source. Differences were considered significant when $P<0.05$. Statistical analysis was performed using SPSS 11.0 for Windows.

3 Results

3.1 Levels of TC, TG, HDL-C and LDL-C in plasma

It can be seen from Table 3 that dietary SBOS supplementation significantly increased the TC and HDL-C levels in plasma of fish fed FM-based diets ($P<0.05$), but did not significantly affect the TG and LDL-C levels and LDL-C/HDL-C ratio ($P>0.05$). However, the plasma TC, TG, HDL-C and LDL-C levels and LDL-C/HDL-C ratio in fish fed SPI-based diets were not significantly affected by supplemented SBOS ($P>0.05$).

3.2 TC, TG and total lipid contents in liver

SBOS supplementation significantly decreased the contents of hepatic TC, TG and total lipid in fish fed SPI-based diets ($P<0.05$), but no significant differences were observed in fish fed FM-based diets ($P>0.05$) (Table 3).

Table 3 Concentration of cholesterol (CHOL) in feces, and triglycerides (TG) and CHOL in plasma and liver lipid from Japanese flounder fed various protein sources-based diets with or without soybean oligosaccharides

Diets	FM	FMO	SPI	SPIO
Feces/ mg · g^{-1} dry matter				
Total cholesterol	0.14 ± 0.03	$1.19\pm0.05^{\dagger}$	7.52 ± 0.18	7.69 ± 0.05
Bile acid	5.66 ± 0.10	7.01 ± 0.77	20.04 ± 1.48	26.47 ± 1.16
Plasma /mmol · L^{-1}				
Triglycerides	2.91 ± 0.37	4.14 ± 0.25	3.72 ± 0.57	3.35 ± 0.65
Total cholesterol	6.12 ± 0.27	$7.63\pm0.34^{\dagger}$	7.04 ± 0.26	6.37 ± 1.48
HDL cholesterol (HDL-C)	4.69 ± 0.16	$5.58\pm0.21^{\dagger}$	1.73 ± 0.09	1.08 ± 0.24
LDL cholesterol (LDL-C)	0.16 ± 0.03	0.17 ± 0.06	3.68 ± 0.23	4.76 ± 0.54
LDL-C/HDL-C	0.04 ± 0.01	0.03 ± 0.01	2.15 ± 0.29	2.59 ± 0.28
Liver /mg · g^{-1} wet liver				
Lipid	45.37 ± 2.61	41.36 ± 4.48	218.81 ± 11.49	$59.91\pm6.93^{\ddagger}$
Total cholesterol	4.29 ± 0.36	4.63 ± 0.47	24.14 ± 3.12	$6.45\pm0.08^{\ddagger}$
Triglycerides	29.29 ± 2.70	25.24 ± 2.70	198.98 ± 6.88	$36.11\pm3.83^{\ddagger}$

Notes: values are presented as means ± SEM ($n = 3$). $^{\dagger}P<0.05$, FMO diet versus FM diet. $^{\ddagger}P<0.05$, SPIO diet versus SPI diet.

3.3 Fecal cholesterol and bile acid excretion

SBOS supplementation significantly increased the fecal cholesterol content in fish fed FM-based diets ($P<0.05$), but no significant difference was observed in fish fed SPI-based diets ($P>0.05$) (Table 3). The fecal bile acid content in fish fed FM- or SPI-based diets was slightly increased by supplemental SBOS, but the difference was not significant ($P>0.05$).

3.4 Fatty acid composition

The fatty acid composition in the muscle of fish fed FM- or SPI-based diets was not significantly affected by dietary SBOS supplementation ($P>0.05$) (Table 4). Similarly, supplementation with SBOS did not significantly affect the fatty acid composition of liver in fish fed FM-based diets (Table 5). However, supplemental SBOS significantly increased the hepatic C18 : 1*n*–9/C18 : 0 ratio in fish fed SPI-based diets ($P<0.05$), but no significant differences were observed in the contents of individual fatty acids, saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) and the C20 : 4*n*–6/C18 : 2*n*–6 ratio in the fish liver ($P>0.05$).

3.5 Liver histology

In the fish fed FM-based diets regardless of SBOS levels, hepatocytes contained few lipid droplets, which did not show any alterations compared with the normal histology (Fig. 2A and B). However, hepatic lipodosis (fatty liver) was observed in the fish fed Diet SPI, in which lipid deposition was quite uniform throughout the hepatic parenchyma (Fig. 2C). In the fish fed Diet SPIO, the hepatocytes with large amounts of lipid droplets were several times larger than nonfatty

Table 4 Fatty acid composition (% of total fatty acids) of the muscle of Japanese flounder fed various protein sources-based diets with or without soybean oligosaccharides

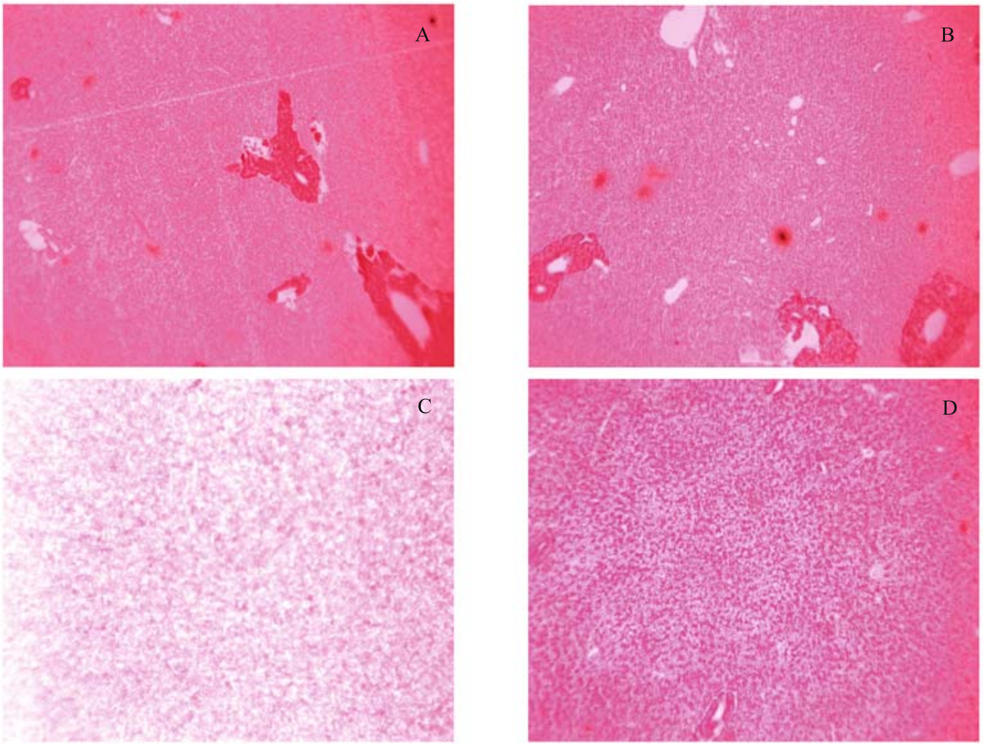
Fatty acids	FM	FMO	SPI	SPIO
14 : 1	3.63±0.34	3.27±0.30	2.74±0.08	2.94±0.15
16 : 0	22.56±3.14	18.12±1.47	18.67±0.33	17.79±0.45
16 : 1	4.92±0.24	4.53±0.49	3.53±0.32	3.72±0.25
18 : 0	6.08±0.36	5.89±0.59	6.69±0.21	6.47±0.34
18 : 1n–9	11.06±0.16	11.01±0.56	13.12±0.90	11.87±0.42
18 : 1n–7	3.06±0.07	2.86±0.15	3.42±0.17	3.62±0.06
18 : 2n–6	6.52±0.06	6.76±0.57	11.46±1.41	9.60±0.38
18 : 3n–3	0.92±0.06	1.06±0.12	0.96±0.23	0.84±0.11
20 : 0	0.28±0.03	0.33±0.01	0.40±0.01	0.40±0.01
20 : 4n–6	1.40±0.04	1.34±0.09	1.71±0.17	1.80±0.12
20 : 5n–3	7.53±0.81	8.26±0.23	6.44±0.54	7.79±0.04
22 : 6n–3	20.05±2.50	20.78±1.78	18.57±1.63	19.83±0.62
ΣTotal	88.00±0.85	84.20±2.94	87.70±0.49	86.66±0.25
ΣSFA	28.92±3.47	24.33±1.39	25.76±0.51	24.66±0.80
ΣMUFA	22.67±0.72	21.67±1.44	22.80±1.00	22.15±0.76
ΣPUFA	36.41±3.34	38.19±1.47	39.13±0.94	39.85±0.20
Σn–3	28.49±3.35	30.09±1.62	25.97±1.97	28.46±0.47
Σn–6	7.92±0.05	8.10±0.52	13.16±1.24	11.40±0.27
Ratio n–3/n–6	3.60±0.43	3.75±0.37	2.03±0.31	2.50±0.10
18 : 1n–9/18 : 0	1.92±0.06	1.93±0.31	1.77±0.02	1.84±0.16
20:4n–6 /18:2n–6	0.21±0.01	0.20±0.03	0.19±0.01	0.19±0.02

Notes: values are presented as means±SEM ($n = 3$). SFA stands for saturated fatty acids; MUFA for monounsaturated fatty acids and PUFA for polyunsaturated fatty acids.

Table 5 Fatty acid composition (% of total fatty acids) of the liver of Japanese flounder fed various protein sources-based diets with or without soybean oligosaccharides

Fatty acids	FM	FMO	SPI	SPIO
14 : 1	4.47±0.14	4.16±0.42	2.76±0.23	3.23±0.15
16 : 0	16.81±1.10	16.56±0.36	18.16±1.19	17.73±0.38
16 : 1	7.18±0.30	7.44±0.81	5.17±0.02	5.81±0.19
18 : 0	4.02±0.18	5.04±1.13	5.36±0.18	5.45±0.05
18 : 1n–9	15.20±0.71	15.08±2.36	12.68±0.81	15.61±0.20
18 : 1n–7	2.83±0.15	2.85±0.89	3.62±0.45	4.18±0.09
18 : 2n–6	8.99±0.41	9.08±0.62	9.18±2.45	8.86±1.11
18 : 3n–3	1.15±0.06	1.53±0.21	1.01±0.02	1.15±0.26
20 : 0	0.20±0.06	0.28±0.12	0.15±0.00	0.18±0.18
20 : 4n–6	1.42±0.15	1.08±0.15	1.53±0.17	1.47±0.08
20 : 5n–3 (EPA)	6.45±0.46	6.45±0.18	6.05±0.88	8.19±1.24
22 : 6n–3 (DHA)	10.78±0.12	10.41±1.18	12.71±2.14	10.85±1.83
ΣTotal	79.51±0.66	79.95±3.86	78.36±8.52	82.68±0.51
ΣSFA	21.03±1.12	21.87±0.76	23.67±1.37	23.35±0.51
ΣMUFA	29.69±0.94	29.53±4.39	24.22±1.50	28.82±0.33
ΣPUFA	28.79±0.77	28.55±1.01	30.47±5.65	30.52±0.70
Σn–3	18.38±0.43	18.39±0.85	19.77±3.04	20.19±0.34
Σn–6	10.41±0.36	10.16±0.74	10.70±2.61	10.33±1.03
Ratio n–3/n–6	1.77±0.03	1.83±0.18	1.89±0.18	1.98±0.23
18:1n–9/18:0	3.79±0.08	4.42±0.53	2.37±0.08	2.87±0.02[‡]
20:4n–6 /18:2n–6	0.16±0.02	0.12±0.01	0.18±0.03	0.17±0.03

Notes: values are presented as means±SEM ($n = 3$). SFA stands for saturated fatty acids, MUFA for monounsaturated fatty acids and PUFA for polyunsaturated fatty acids. [‡] $P<0.05$, SPIO diet versus SPI diet.



Notes: A and B stand for normal liver; C for fatty liver (numerous hepatocytes filled with clear vacuoles presenting fat); and D for fatty liver (few hepatocytes filled with vacuoles presenting fat).

Fig. 2 Liver histology (H&E, 100×) of Japanese flounder fed the experimental diets
(A) Diet FM; (B) Diet FMO; (C) Diet SPI; and (D) SPIO

hepatocytes (Fig. 2A and B), whereas a lesser number of lipid droplets around the pancreatic tissue were observed in the liver of fish fed Diet SPIO (Fig. 2D) compared with Diet SPI (Diet 2C).

4 Discussion

4.1 Effects of dietary SBOS supplementation on the cholesterol level

SBOS are water-soluble carbohydrates (Li, 2003). However, SBOS (mainly stachyose and raffinose) cannot be cleaved in the digestive tract by endogenous enzymes due to the absence of α-galactosidase in the intestinal mucosa of monogastric animals or fish, and directly enter into the hindgut (Smiricky et al., 2002). Some anaerobic bacteria such as *Bifidobacteria* and *Lactobacteria* present in the gut contain α-galactosidase and may thus utilize galacto-oligosaccharides (namely raffinose and stachyose) for the production of volatile fatty acid (VFA) and gas (e.g. carbon dioxide, hydrogen, methane, etc.) (Tortuero et al., 1997; Kihara and Sakata, 2002; Lan et al., 2007). In addition, dietary indigestible and fermentable SBOS can lead to an increase in the proportion of the *Bifidobacteria* and *Lactobacteria* and to a decrease in potentially toxic microbial products in the gut (Cole et al., 1989; Tortuero et al., 1997; Tarasewicz et al., 2004; Lan et al., 2007). It was reported that the cholesterol-lowering effects of SBOS were at least partly due to the balance of intestinal bacteria (Gilliland et al., 1985; Lan et al., 2007).

In this study, adding 10% SBOS (stachyose, 2.61%; raffinose, 0.61%) to FM-based diets significantly increased the plasma TC level of Japanese flounder, but slightly decreased the TC level of fish fed SPI-based diets. Similarly, in our other study, the plasma TC level of Japanese flounder fed FM-based diets supplemented with stachyose slightly increased with increasing dietary stachyose content (0%–1.5%), but no significant difference was found among dietary treatments (Deng, 2006). Maisuo (1996) also reported that the plasma TC level in rats fed OC (*Okara*, an insoluble residue of homogenized soybean, containing stachyose 2.88% and raffinose 13.43%) diet was significantly higher than that of rats fed OK (*Okara Koji*, fermented soybean product, containing stachyose 0.75% and raffinose 0.84%) diet. However, Refstie et al. (1999) reported that the plasma TC level was slightly lower in Atlantic salmon fed the soybean meal (SBM) diet than that of fish fed the ROM diet (reduced oligosaccharide content). Similarly, the TC level in the quail fed plant protein source-based diets was slightly decreased by supplemental 3% SBOS (raffinose 0.15%, stachyose 1.58%, verbascose 0.62%) (Tarasewicz et al., 2004). In terrestrial mammals, the hypocholesterolemic effect of SBOS in rats with hyperlipidemia was well documented (Tortuero et al., 1997; Wang et al., 1996, 1997; Anderson and Hanna, 1999; Xie and Shi, 2006). From these above-mentioned results, the hypocholesterolemic effect of SBOS seems to be related to animal species (Refstie et al., 1999) and dietary protein sources (Deng, 2006). There are three characteristics of SBOS that are believed to be responsible for their hypocholesterolemic properties: (1) water solubility; (2) fermentability and (3) viscosity (Davidson and Maki, 1999), but the mechanism by which this occurred is not known. To this day, there are four hypotheses regarding the hypocholesterolemic effect of SBOS: (1) to accelerate the production of VFA, which enhances the excretion of cholesterol and bile acids (Tarasewicz et al., 2004; Terada et al., 1994) and decreases the production of endogenous cholesterol (Tortuero et al., 1997; Tarasewicz et al., 2004); (2) to increase the population of a group of available bacteria such as *Bifidobacteria* and *Lactobacteria*, which perhaps directly affects the activity of β-hydroxide-β-methylglutary-CoA reductase, accordingly restrains the synthesis of cholesterol and decreases the blood cholesterol content (Gilliland et al., 1985; Tasker and Potter, 1993); (3) to bind bile salts in the gut due to high viscosity, which increases the fecal steroid excretion, in particular as bile acids (Gatchalian-Yee et al., 1997; Moundras et al., 1997; Refstie et al., 1999); and (4) to activate the hepatic antioxidative defense system (Kim et al., 2005).

It is well known that LDL causes cholesterol to build up inside blood vessels, together with other substances which can form plaque, a thick, hard deposit that can clog arteries. This condition is known as atherosclerosis. Whereas, HDL removes cholesterol from the walls of blood vessels and brings cholesterol back to the liver to be safely excreted. Therefore, LDL-C/HDL-C ratio is measured as an index of risk for arteriosclerosis lesion (Goldstein and Brown, 1984). In addition, there are many evidences to support the proposition that the elevated blood TG level leads to the generation of a blood lipid profile that is strongly linked with the increased risk of arteriosclerosis lesion (Williams, 1997). In this study, dietary SBOS supplementation did not affect the plasma TG and LDL-C levels and LDL-C/HDL-C ratio of Japanese flounder regardless of dietary protein source. Similarly, no statistically significant differences were found in the plasma HDL-C and TG levels of the quail fed diets with graded levels of pea-isolated oligosaccharides (Tarasewicz et al., 2004). Thus, these observations suggest that dietary SBOS supplementation has no negative influence on the arteriosclerosis lesion in Japanese flounder or quail. However, most studies on terrestrial mammals showed that dietary SBOS supplementation significantly increased the plasma HDL-C level, but decreased the TG and LDL-C levels and LDL-C/HDL-C ratio (Tortueroe et al., 1997; Wang et al., 1996, 1997; Anderson and Hanna, 1999; Xie and Shi, 2006), which restrain the arteriosclerosis lesion.

4.2 Effects of dietary SBOS supplementation on the fatty acid composition of fish muscle and liver

To our knowledge, so far, very few studies have dealt with the effects of dietary SBOS on the fatty acid composition of fish or monogastric animals. In the present study, except that dietary SBOS supplementation significantly increased the hepatic desaturation index for stearic acid (C18 : 1n–9/C18 : 0 ratio) in the fish fed SPI-based diets, suggesting that the higher Δ^9 desaturase activity, the fatty acid compositions in the muscle and liver of fish fed FM- or SPI-based diets were not affected by the supplemented SBOS. These observations suggest that the dietary SBOS supplementation has a minor impact on the fatty acid composition of Japanese flounder.

4.3 Effect of dietary SBOS supplementation on liver lipid content and histology

Fatty liver disease is one of the common nutritional diseases of cultured fish, especially for marine fish such as Atlantic salmon (*Salmo salar* L.) (Menoyo et al., 2006), sea bass (*Dicentrarchus labrax* L.) (Spisni et al., 1998), gilthead seabream (*Sparus auratus* L.) (Robaina et al., 1995; Spisni et al., 1998), rainbow trout (*Salmo gairdneri* R.) (Yang and Dick, 1994), haddock (*Melanogrammus aeglefinus* L.) (Nanton et al., 2003) and white sturgeon (*Acipenser transmontanus* R.) (Watson et al., 1998). The most common cause of fatty liver disease was a diet with high saturated fats (Spisni et al., 1998; Menoyo et al., 2006) and/or carbohydrate (Robaina et al., 1995), or deficiencies in biotin (Halver, 2003), choline (Weisman and Miller, 2006) and/or essential fatty acids (Yang and Dick, 1994), which gave rise to several alterations such as poor feeding and swimming activities, growth retardation, immunologic dysfunction and high mortality (Gaylord and Gatlin III, 2000; Halver, 2003). Histologically, the main feature is larger liver, often white or pale in color, hepatocellular alterations including increased lipid storage, uneven deposition, and large vacuoles (Moseoni-Bac, 1987). Although bacterial diseases and parasitic infections claim many more fish than nutritional deficiencies (Francis-Floyd and Klinger, 2003), fatty liver disease is probably one of the most common fatal nutritional problems. The major sites for de novo fatty acid synthesis depend on the species. In rats, both adipose tissue and liver have been reported to be important sites of fatty acid synthesis. In rabbits, pigs, sheep and cows, the major site of fatty acid synthesis appears to be adipose tissue; whereas in chicken, pigeons and fish, the major site of fatty acid synthesis is the liver (Lin et al., 1977a). In addition, a rapid reduction occurs in the rates of fatty acid synthesis when rats are fasted or fed a high-fat or high-carbohydrate diet. This change in metabolic enzyme activities in the rat occurs within hours after treatment. However, lipogenic enzymes in fish might not be rapidly responsive to switching from one diet to another. In general, fish require several weeks for hepatic lipogenic enzyme activities to change when diets are switched (Lin et al., 1977b). Therefore, the incidence of fatty liver disease in fish is relatively higher than in most homoiothermous animals (Lin et al., 1977b).

Liver is an important organ for lipid metabolism. The hepatic lipid comes from exogenous supply of feed and endogenous translation from excess protein and carbohydrate (Lie et al., 1988). Fatty liver in fish results from the excessive accumulation of fats in hepatocytes when lipoprotein transport is disrupted. The cause of fatty liver disease suffered by the breeding fish is over-ingestion of carbohydrates (Halver, 2003) and/or fat (Nanton et al., 2001), and the lack of the phospholipids (Poston, 1991; Salhi et al., 1999) or protein (Burtle and Liu, 1994) involved in the lipometabolism. The fatty degeneration and necrosis in hepatocytes and liver decline and failure are the results of overaccumulation of fats in the hepatocytes. In this study, dietary SBOS supplementation did not affect the total lipid, TC and TG contents in liver of fish fed FM-based diets, whereas it significantly decreased the total lipid, TC and TG contents in fish fed SPI-based diets. A similar trend was observed in the liver histology. The hepatocytes of fish fed FM-based diets were not destroyed regardless of whether adding SBOS or not. However, the symptom of fatty liver of fish fed SPI-based diets was alleviated by supplemental SBOS. These results indicate that SBOS can protect liver function and lessen hepatic lipodosis in Japanese flounder with hepatocellular vacuoles. It was well demonstrated that indigestible and fermentable SBOS could protect the liver function by the following pathways. First, SBOS can restrain harmful metabolite production by proliferating *Bifidobacteria*. Some saprophytic bacteria (e.g. *Escherichia coli*, *Clostridium* sp. and *Streptococcus faecalis*) in the intestinal tract accelerate the production of harmful substances such as ammonia, indole and hydrogen sulfide, which are hazardous to animals. *Bifidobacteria* can suppress the production of saprophytic bacteria, and reduce the amount of the toxins going into the liver (Cole et al., 1989; Martin, 1998). Second, dietary SBOS can accelerate the proliferation of *Bifidobacteria* and *Lactobacteria*, which can utilize toxins such as ammonia by using it as an important source of nitrogen for their own protein synthesis during their growth phase. *Bifidobacteria* and *Acidophilus* bacteria decompose nitrosamines and can also suppress the production of nitrosamines in the intestine and liver (Cole et al., 1989; Martin, 1998). Thirdly, supplementation with oligosaccharides reduces oxidative damage to liver by activating the hepatic antioxidative defense system (Kim et al., 2005). Finally, SBOS can accelerate the excretion of fecal toxins (Li, 2003).

In conclusion, dietary SBOS supplementation had a minor impact on the fatty acid composition in the muscle and liver of Japanese flounder regardless of dietary protein source. Similarly, except for TC and HDL-C levels in fish fed FM-based diets, the plasma lipoprotein levels were not affected by the supplemental SBOS, indicating that SBOS have no negative influence on the arteriosclerosis lesion. In addition, the dietary SBOS supplementation may decrease the hepatic TC, TG and total lipid contents and hepatocellular vacuoles of fish fed SPI-based diets, which indicates that SBOS can alleviate the incidence of fatty liver in fish fed SPI-based diets and thus protect liver function.

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