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Effect of Perilla Oil in Diet on the Biochemical Property of Cultured Sweet Smelt *Plecoglossus altivelis*

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The effect of perilla oil added in diet on the biochemical properties of cultured sweet smelt, *Plecoglossus altivelis*, was investigated. The cultured fish were fed two different diets for 8 weeks; a control diet was a commercial diet, which was low in the content of docosahexaenoic acid (DHA, 22:6n-3) and eicosapentaenoic acid (EPA, 20:5n-3) less than approximately 2% (CO group) and an experimental diet (PO group) was added perilla oil as a lipid source in the diet of the CO group. The PO group was superior in growth rate and feed efficiency compared with CO group. This trend showed markedly in female of both groups. The fatty acid composition in the muscle of PO group was closely related with those of the diet, while those of CO group were not. For plasma components, total cholesterol (CHOL) of PO group was higher than that of CO group. Thiobarbituric acid-reactive substances (TBARS), hydroxyl (OH) radical levels and superoxide dismutase (SOD) activity of plasma were higher in PO group than CO group. The intensity of watermelon-like or cucumber-like aroma was much stronger in PO group with higher level of TBARS and OH radical in plasma compared CO group. Survival rate was also high in PO group with high levels of phagocytic rate, CHOL and SOD activity. These results suggest that perilla oil might be usefulness as a lipid source of the cultured sweet smelt diet, in which result in high quality of the cultured fish.

Key words: Aroma, Fatty acid, Perilla oil, Phagocytic rate, Plasma, Sweet smelt

Introduction

Sweet smelt *Plecoglossus altivelis* is a freshwater fish, which possesses watermelon-like or cucumber -like aroma (Chyung, 1991). Recently, the production of cultured sweet smelt has been increased rapidly in Korea, but the cultured fish have been produced at the inferior in terms of quality, including the intensity of aroma. It has been reported that the aroma compounds are low molecular volatile nine-carbon aldehydes and alcohols (Fross et al., 1962; Hirano et al., 1992; Zhang et al., 1992). These low molecular materials are the breakdown products of hydroperoxide, which is generated from certain polyunsaturated fatty acids (PUFA) by fish lipoxygenase (Josephson et al., 1984). In the previous study, Jeong et al. (2000, 2001) reported that the wild fish muscle contained high levels of alpha -linolenic acid (ALA) and eicosapentaenoic acid (EPA), and high or similar level of docosahexaenoic acid (DHA) compared with the cultured fish. This therefore suggests that ALA or EPA might play potential precursors role for the generation of the aromatic compounds in the wild fish. The fatty acid compositions including the n-3 PUFA are usually related with the fish diets, e.g., sessile algae for the wild fish (Shimma et al., 1984; Jeong et al., 2001) and artificial diet for the cultured fish (Jeong et al., 2000, 2001). Perilla oil contains a large amount of ALA (approximately 62% of total fatty acids) (Jeong et al., 1997). Therefore, it is of interest how

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a large amount of ALA in diet affects on the quality and growth of the cultured fish.

The present study is conducted to demonstrate effects of perilla oil in diet on the biochemical properties such as the growth, intensity of aroma, plasma component level, lipid peroxidation and non-specific immune potential such as superoxide dismutase activity and phagocytosis, fatty acid and proximate compositions in the cultured fish.

Materials and Methods

Experimental condition

Juvenile sweet smelts were purchased from a hatchery and reared up to 36.2 g on a commercial diet for approximately 4 months at Kyeongsangnam-do Hatchery, Korea. The fish were randomly divided into two groups with different diets. Each group of 500 fish was reared for 8 weeks in a polypropylene tank (ID, 5.3 m) in duplicate. Fish were fed a control diet (CO group) poor in EPA (1.33%) and DHA (1.70%), and a diet (PO group) that contained high level of ALA (41.2%) but similar levels of EPA and DHA compared to those of CO group. Diet of CO group was a commercial diet containing EPA and DHA less than approximately 2%. Diet of PO group was prepared by impregnation of perilla oil (10%) as a lipid source to the diet of CO group.

Proximate compositions of the diets are shown in Table 1. The fish were fed three times a day at 2.0~2.4% of body weight in total. The tanks were continuously supplied with freshwater at 19±2℃ and photoperiod was controlled at 16 L and 8 D throughout rearing period. Body length and body weight of fish were measured and then transported to a laboratory over dry ice. The fish muscle and liver were removed from 20 specimens of male and female, respectively, in each tank and stored at 80℃ until analysis. The fish muscle was thoroughly mixed with a speed cutter (National, MK-K51, Japan) prior to assay.

Analysis of proximate composition

Moisture and ash contents were determined by atmospheric heat drying method at 105℃ and 550℃, respectively. Protein content was determined by Kjeldahl method. Total lipid (TL) was extracted and purified according to the method of Bligh and Dyer (1959), and the content was determined gravimetrically.

Table 1. Proximate compositions of experimental diets for cultured sweet smelt

(wt %)

	CO group[1]	PO group[2]
Moisture	4.27±0.08	4.10±0.07
Protein	43.8±0.27	41.0±0.74
Lipid	10.3±0.19	13.5±0.91
Ash	8.20±0.02	8.13±0.05

[1]Diet of CO group was a commercial diet containing EPA and DHA less than approximately 2%.

[2]Diet of PO group was prepared by impregnation of perilla oil (10%) as a lipid source to the diet of CO group.

Analysis of fatty acid compositions

Fatty acid composition of TL was analyzed on its methyl esters by a gas-liquid chromatograph (Shimadzu GC 14A, Shimadzu Seisakusho, Co. Ltd., Kyoto, Japan) equipped with an Omegawax 320 fused silica capillary column (30 m×0.32 mm, ID, Supelco, Bellefonte, PA, USA). Injector and flame-ionization detector were held at 250℃, and the column oven temperature was programmed from 180℃ (initial time 8 min) to 230℃ at 3℃/min and final temperature was held for 15 min. Helium was used as a carrier gas at the constant column inlet pressure of 1.0 kg/cm^2 with split ratio of 1:50. Fatty acids were identified by comparison with authentic standards (Sigma Chemical Co., St. Louis, MO, USA) and oyster fatty acids, which was analyzed by Koizumi et al. (1990).

Blood sampling and measurement of plasma component levels

Fish were starved for 24 h before blood sampling. Ten fish in each tank were caught randomly and blood was taken from the caudal vein with heparinized syringes fitted with 23 G needles. An aliquot of the blood was used to determine the hematocrit (Ht) and the remainder was centrifuged at 1,000 g for 10 min to obtain plasma which was then stored at 80℃ until analysis. Plasma component levels were determined with an automatic biochemical analyzer

(CL-7100, Shimadzu Co. Ltd., Kyoto, Japan) using the Biuret method (total protein) and the enzymatic method (urea nitrogen, glucose, total cholesterol and triglyceride).

Phagocytosis Assay

Fifty L of blood was mixed with an equal volume of yeast cell suspension consisting of 5 mg of yeast cells (Zymosan A, Sigma) suspended in 1 mL of phosphate buffered saline. The mixture was then incubated for 30 min at 20℃ and then smeared onto a microscope slide. The smear was stained with May-Grunwald Giemsa and observed under a light microscope. Phagocytic activity was determined by counting the number of phagocytes containing yeast cells as a proportion of a total of 200 cells.

Thiobarbituric acid-reactive substances, hydroxyl radical and superoxide dismutase activity assay

Thiobarbituric acid-reactive substances (TBARS), hydroxyl (OH) radical and superoxide dismutase (SOD) activity were assayed in the fish plasma. TBARS were assayed according to the method of Ohkawa et al. (1979). The concentration of TBARS, a decomposition product of lipid hydroperoxide, was calculated from a standard curve generated using malondialdehyde (MDA), and expressed as MDA μg/mL. OH radical activity was assayed according to the method of Halliwell et al. (1987), which is based on the degradation of deoxyribose and expressed as MDA nmol/mg protein. SOD activity was assayed using a method based on the formation of nitrite, as described by Oyanagui (1984) and expressed as NU/mg protein. Protein content of plasma was measured using the method of Lowry et al. (1951).

Sensory evaluation

The flavor was evaluated by a sensory panel consisted of five staff members and graduate students who showed consistency in flavor description. The flavor intensity such as watermelon-like aroma or cucumber-like aroma of cultured sweet smelt was ranked from "+" to "+++++" with increasing intensity. The flavor intensity of wild fish was designated by "+++++".

Results

Typical biological factors of the cultured fish fed different diets for 8 weeks rearing; growth rate (GR), feed efficiency (FE) and survival rate are shown in Table 2. The GR of PO group accounted for 92~102% and three to four times as much as that of CO group. This was well coincided with the result in respect with FE. In both groups, GR was higher in female fish (33.4~102%) than male fish (21.4~92.3%).

Proximate compositions of the cultured fish muscle and gonad are shown in Table 3. Protein and ash contents in muscle were 14.9~15.3% and 1.01~1.27%, respectively, and had no difference between both groups. However, lipid content in muscle showed a great difference between PO and CO groups, accounting for 8.65~10.10% and 4.30~5.57%, respectively, and decreased with increasing moisture content in both groups. The female fish muscle contained slightly low level of lipid compared with male fish muscle. In gonad, protein and ash contents were 14.4~23.4% and 1.49~3.37%, respectively, and much more than in muscle, except the protein of ovary in CO group. Lipid content of gonad was low level compared with that of muscle, except that of ovary in CO group and that of PO group contained slightly high level of lipid compared with CO group. In contrast to the muscle

Table 2. Biological factors of cultured sweet smelt fed different diets for 8 weeks

Biological factor	Sex	Initial	Final	
			CO group[1]	PO group[2]
Body length (cm)	Male	14.3 ± 1.09	15.2 ± 1.38	17.4 ± 1.20
	Female	14.3 ± 0.82	15.1 ± 1.29	17.3 ± 0.86
Body weight (g)	Male	35.0 ± 5.86	42.5 ± 10.3	67.3 ± 10.5
	Female	37.4 ± 7.14	49.9 ± 12.1	75.5 ± 10.9
Growth rate (%)	Male		21.4	92.3
	Female		33.4	102.0
Feed efficiency (%)	Male		16.6	70.2
	Female		27.3	82.7
Survival rate (%)			86.0	94.8

[1]Diet of CO group was a commercial diet containing EPA and DHA less than approximately 2%.

[2]Diet of PO group was prepared by impregnation of perilla oil (10%) as a lipid source to the diet of CO group.

Table 3. Proximate composition of muscle and gonad of sweet smelt fed different diets for 8 weeks (wt %)

		Moisture	Protein	Lipid	Ash
Muscle					
CO group[1]	Male	75.9±0.16	15.3±0.33	5.57±0.07	1.26±0.03
	Female	78.0±0.84	14.9±0.61	4.30±0.15	1.27±0.08
PO group[2]	Male	70.6±0.11	15.0±0.40	10.10±0.50	1.01±0.05
	Female	69.5±0.14	15.0±0.19	8.65±2.34	1.01±0.09
Gonad					
CO group	Testis	71.3±0.05	21.3±0.22	3.32±0.21	3.30±0.06
	Ovary	70.6±0.90	14.4±0.15	6.23±0.28	1.49±0.05
PO group	Testis	69.3±0.12	23.4±0.19	3.38±0.37	3.37±0.06
	Ovary	66.3±0.40	18.6±0.33	8.28±0.02	1.81±0.01

[1]Diet of CO group was a commercial diet containing EPA and DHA less than approximately 2%.
[2]Diet of PO group was prepared by impregnation of perilla oil (10%) as a lipid source to the diet of CO group.

lipid, ovary contained approximately 2 times of lipid as much as testis. Moisture content of gonad showed inversely lipid content likewise muscle.

Table 4 shows fatty acid compositions of muscle in both groups. The prominent fatty acids in the muscle lipids of CO group were 16:0, 18:1n-9, 18:2n-6, DHA, 16:1n-7, 14:0 and 18:0. On the other hand, the prominent fatty acids in the muscle lipids of PO group were similar to those of CO group but ALA was added to them; these fatty acid proportions of the fish muscle of both groups were almost no difference by sex. PO group was rich in ALA and 18:2n-6, while CO group was rich in 16:0, DHA, 14:0, 16:1n-7 and EPA. PO group was much more in PUFA (38.9~40.5% vs. 25.9~28.3% in CO group) due to a large amount of ALA (17.2~17.9% vs. 0.91~0.97% in CO group) than CO group. The fatty acid composition of PO group was closely related with that of the diet. However, CO group showed considerable difference in fatty acid composition between muscle and diet. Particularly DHA, which contained less than 2% in the diet of CO group, was found 8.33~9.75% in the muscle.

As shown in Table 5, the prominent fatty acids of gonad were similar to those of muscle, but considerably different between testis and ovary, and between both groups. Testis of both groups was rich in PUFA such as DHA, EPA, 20:4n-6 and 22:5n-3, of which content was approximately 2 times as much as ovary. On the other hand, ovaries of both groups were rich in 18:1n-9, 18:2n-6, 16:1n-7 and ALA (only in PO group). Testis showed similar levels of PUFA (54.9~55.9%), monounsaturated fatty acids (MUFA, 16.4~17.2%) and saturated fatty acids (SFA, 27.7~28.0%) in both groups. Ovary however showed a considerable difference between both groups. In particular, ovary of PO group contained a large amount of PUFA (44.7% vs. 33.6% in CO group) due to a high level of ALA (11.8%).

Plasma components, especially total protein and glucose, showed similar levels in the fish of both groups, but total cholesterol (CHOL) level was high in PO group (626 vs. 475 mg/100 mL plasma in CO group) (Table 6). Ht value in fish blood was also no deference between both groups. The SOD activity in plasma and phagocytic rate were also high in PO group (8.31 NU/mg protein and 34.6%, respectively) compared to CO group (3.94 NU/mg protein and 32.8%, respectively) (Table 7). As shown in Table 8, TBARS value and OH radical level in plasma, as lipid peroxidation index, were considerably high in PO group (28.9 MDA μg/mL plasma and 21.8 MDA nmol/mg protein, respectively) compared with CO group (19.0 MDA μg/mL plasma and 5.34 nmol/mg protein, respectively). The intensity of fish aroma was also much strong in PO group, which contained large amount of lipid peroxides in plasma, TBARS value and OH radical compared with CO group.

Discussion

The watermelon-like or cucumber-like aroma compounds have been identified as nine-carbon aldehydes and alcohols such as (*E*)-2-nonenal, (*E,Z*)-2, 6-nonadienal and 3,6-nonadien-1-ol. These key compounds were found in aromatic fish including sweet smelt (Fross et al., 1962; Hirano et al., 1992; Zhang et al., 1992). These aroma compounds were generated from the breakdown products of certain polyunsaturated fatty acid hydroperoxides oxidized by fish lipoxygenase. In general, the characteristic aroma of sweet smelt is much strong in wild sweet smelt than cultured one. This might be due to difference between lipid compositions of their original diet, especially PUFA compositions. In the

Table 4. Fatty acid composition of total lipid of the muscle in cultured sweet smelt fed different diets for 8 weeks (wt %)

Fatty acids	CO group[1]			PO group[2]		
	Male	Female	Diet	Male	Female	Diet
14:0	4.57±0.08	4.40±0.09	8.19±0.07	2.89±0.04	3.16±0.11	1.11±0.00
14:1n-7	0.10±0.00	0.10±0.01	0.09±0.01	0.08±0.00	0.09±0.00	0.03±0.00
15:0 iso	0.13±0.00	0.12±0.01	0.39±0.03	0.07±0.00	0.06±0.01	0.04±0.00
15:0 anteiso	0.03±0.00	0.03±0.01	0.13±0.02	0.02±0.00	0.01±0.00	0.02±0.00
15:0	0.36±0.01	0.36±0.01	0.96±0.01	0.20±0.00	0.21±0.01	0.10±0.00
16:0 iso	0.06±0.00	0.06±0.01	0.15±0.00	0.03±0.00	0.03±0.00	0.02±0.00
Pristanic	0.09±0.01	0.12±0.00	ND[3]	0.04±0.01	0.03±0.01	ND
16:0	25.4±0.22	23.3±0.21	28.4±0.19	20.9±0.12	22.3±0.88	11.1±0.07
16:1n-13	0.22±0.04	0.26±0.00	ND	0.17±0.02	0.20±0.01	ND
16:1n-7(+9)	8.40±0.07	7.85±0.10	8.29±0.08	6.26±0.01	6.72±0.03	1.60±0.02
16:1n-5	0.11±0.03	0.12±0.01	0.23±0.00	0.08±0.02	0.10±0.01	0.05±0.00
17:0 iso	0.14±0.03	0.16±0.01	0.28±0.01	0.08±0.01	0.11±0.02	0.06±0.00
17:0 anteiso	0.04±0.00	0.04±0.01	0.08±0.02	0.06±0.01	0.10±0.01	0.09±0.01
16:2n-4	0.21±0.01	0.23±0.01	0.23±0.00	0.16±0.00	0.16±0.01	0.12±0.00
Phytanic	0.20±0.00	0.24±0.01	0.36±0.00	0.13±0.00	0.13±0.00	0.09±0.01
17:0	0.31±0.01	0.34±0.00	1.03±0.00	0.18±0.00	0.19±0.02	0.19±0.00
17:1n-10	0.10±0.01	0.12±0.01	0.09±0.01	0.08±0.01	0.08±0.01	0.09±0.01
17:1n-8	0.24±0.00	0.25±0.00	0.39±0.01	0.19±0.00	0.21±0.00	0.11±0.00
17:2n-8	0.11±0.00	0.10±0.01	0.35±0.02	0.02±0.00	0.04±0.01	0.02±0.00
16:4n-3	0.14±0.00	0.18±0.01	0.13±0.02	0.11±0.01	0.11±0.00	0.15±0.00
18:0	4.09±0.03	3.89±0.03	6.13±0.04	3.15±0.04	3.13±0.17	3.05±0.04
18:1n-9	18.9±0.04	19.6±0.07	18.2±0.04	18.1±0.03	20.1±1.45	15.2±0.05
18:1n-7	2.62±0.00	2.83±0.03	3.25±0.04	1.82±0.07	0.95±1.32	1.45±0.02
18:1n-5	0.18±0.01	0.21±0.01	0.44±0.02	0.12±0.01	0.12±0.00	0.06±0.01
18:2n-9	0.15±0.03	0.13±0.02	ND	0.09±0.00	0.10±0.01	ND
18:2n-6	9.02±0.11	9.02±0.02	7.01±0.09	12.0±0.03	11.7±0.08	16.4±0.10
18:2n-4	0.12±0.04	0.14±0.00	ND	0.10±0.00	0.10±0.01	ND
19:0	0.19±0.03	0.19±0.00	0.17±0.00	0.16±0.00	0.22±0.00	0.02±0.00
18:3n-4	0.14±0.01	0.15±0.00	ND	0.08±0.00	0.08±0.01	ND
18:3n-3	0.97±0.06	0.91±0.00	0.52±0.01	17.9±0.07	17.2±0.17	41.2±0.18
18:4n-3	0.38±0.01	0.38±0.01	0.29±0.01	0.78±0.02	0.88±0.02	0.45±0.01
18:4n-1	0.08±0.01	0.10±0.01	ND	0.07±0.00	0.06±0.00	ND
20:0	0.22±0.00	0.21±0.01	0.83±0.00	0.16±0.00	0.14±0.01	0.20±0.00
20:1n-11	2.52±0.44	1.97±0.05	0.68±0.00	1.91±0.02	0.09±0.10	0.02±0.00
20:1n-9	2.46±0.54	1.93±0.03	2.99±0.00	1.40±0.02	1.33±0.01	0.82±0.02
20:1n-7	0.10±0.01	0.17±0.05	0.40±0.01	0.06±0.00	0.06±0.00	0.05±0.00
20:2n-9	0.12±0.01	0.14±0.04	ND	0.08±0.00	0.05±0.06	ND
20:2n-6	0.27±0.01	0.26±01	0.14±0.01	0.22±0.01	0.16±0.08	0.08±0.00
20:3n-6	0.25±0.01	0.25±00	ND	0.19±0.00	0.22±0.01	ND
20:4n-6	0.59±0.05	0.72±00	0.14±0.01	0.28±0.00	0.24±0.03	0.18±0.00
20:3n-3	0.06±0.02	0.07±0.02	ND	0.34±0.00	0.28±0.01	ND
20:4n-3	0.43±0.00	0.34±0.13	0.05±0.03	0.68±0.01	0.67±0.18	0.10±0.00
20:5n-3	2.82±0.02	3.47±0.00	1.33±0.06	1.84±0.01	1.26±0.01	1.94±0.02
22:0	0.05±0.04	0.07±0.00	0.34±0.03	0.02±0.00	0.06±0.01	0.07±0.01
22:1n-11	0.96±1.25	2.31±0.01	4.69±0.05	0.06±0.01	0.17±0.00	0.95±0.01
22:1n-9	0.89±1.24	0.02±0.00	ND	0.89±0.01	0.82±0.01	ND
22:1n-7	0.02±0.00	0.02±0.00	0.17±0.06	0.02±0.00	0.01±0.00	0.02±0.00
21:5n-3	0.14±0.01	0.18±0.00	ND	0.09±0.00	0.08±0.00	ND
22:4n-6	0.06±0.06	0.08±0.08	ND	0.02±0.00	0.01±0.00	ND
22:5n-6	0.18±0.00	0.21±0.00	ND	0.08±0.02	0.08±0.00	ND
22:5n-3	1.37±0.00	1.55±0.01	0.11±0.01	0.93±0.00	0.95±0.01	0.19±0.02
24:0	0.02±0.00	0.02±0.00	ND	0.02±0.00	0.01±0.00	ND
22:6n-3	8.33±0.02	9.75±0.08	1.70±0.06	4.46±0.02	4.45±0.03	2.42±0.01
24:1n-9	0.31±0.00	0.35±0.01	0.71±0.23	0.19±0.00	0.18±0.00	0.16±0.01
24:1n-7	0.02±0.00	0.02±0.00	ND	0.02±0.00	0.01±0.00	ND
Saturates	35.9	33.6	47.4	28.1	29.9	16.2
Monoenes	38.1	38.1	40.6	31.4	31.2	20.6
Polyenes	25.9	28.3	12.0	40.5	38.9	63.2

[1] Diet of CO group was a commercial diet containing EPA and DHA less than approximately 2%.
[2] Diet of PO group was prepared by impregnation of perilla oil (10%) as a lipid source to the diet of CO group.
[3] ND, not detected.

Table 5. Fatty acid composition of testes and ovaries in cultured sweet smelt fed different diet for 8 weeks (wt %)

Fatty acids	CO group[1]		PO group[2]	
	Testis	Ovary	Testis	Ovary
14:0	1.16±0.02	3.46±0.04	1.03±0.09	2.37±0.16
15:0 iso	0.03±0.01	0.15±0.03	0.02±0.00	0.07±0.04
15:0	0.20±0.00	0.44±0.00	0.14±0.00	0.22±0.00
16:0 iso	0.03±0.00	0.06±0.02	0.03±0.00	0.03±0.00
Pristanic	0.17±0.00	0.06±0.00	0.15±0.01	0.05±0.00
16:0	22.2±0.29	24.7±0.04	22.4±0.62	21.9±0.48
16:1n-13	0.41±0.00	0.53±0.02	0.29±0.02	0.28±0.00
16:1n-9	0.48±0.00	0.02±0.00	0.33±0.01	0.48±0.03
16:1n-7	1.97±0.08	7.19±0.06	1.80±0.13	5.03±0.15
16:1n-5	0.10±0.00	0.15±0.04	0.08±0.02	0.10±0.00
17:0 iso	0.14±0.00	0.36±0.07	0.09±0.02	0.14±0.01
17:0 anteiso	0.05±0.04	0.09±0.05	0.06±0.05	0.09±0.01
16:2n-4	0.39±0.00	0.40±0.04	0.16±0.19	0.24±0.00
17:0	0.27±0.01	0.36±0.06	0.21±0.00	0.21±0.00
17:1n-8	0.18±0.00	0.41±0.00	0.11±0.01	0.26±0.00
16:4n-3	0.11±0.01	0.02±0.00	0.11±0.01	0.02±0.00
18:0	3.47±0.02	2.77±0.01	3.46±0.02	2.47±0.04
18:1n-9	9.94±0.26	20.2±0.16	10.4±0.21	17.6±0.03
18:1n-7	2.66±0.02	3.13±0.00	2.32±0.04	2.03±0.00
18:1n-5	0.16±0.02	0.29±0.10	0.10±0.01	0.16±0.01
18:2n-9	0.11±0.00	0.37±0.01	0.06±0.01	0.13±0.01
18:2n-6	3.91±0.07	6.43±0.02	5.22±0.06	9.40±0.00
18:2n-4	0.09±0.00	0.19±0.06	0.10±0.02	0.13±0.03
19:0	0.15±0.00	0.41±0.00	0.13±0.00	0.28±0.04
18:3n-4	0.11±0.01	0.19±0.00	0.07±0.01	0.15±0.06
18:3n-3	0.25±0.05	0.58±0.02	3.46±0.08	11.8±0.04
18:4n-3	0.05±0.04	0.27±0.01	0.21±0.01	0.74±0.02
18:4n-1	0.02±0.00	0.09±0.00	0.02±0.00	0.08±0.00
20:0	0.04±0.02	0.08±0.00	0.02±0.00	0.07±0.00
20:1n-11	ND[3]	ND	ND	0.40±0.03
20:1n-9(+11)	0.86±0.03	0.99±0.02	0.60±0.19	0.65±0.00
20:1n-7	0.05±0.00	0.13±0.00	0.02±0.00	0.07±0.00
20:2n-9	0.13±0.01	0.26±0.01	0.06±0.01	0.09±0.00
20:2n-6	0.50±0.02	0.60±0.02	0.29±0.01	0.28±0.00
20:3n-6	0.88±0.00	0.55±0.01	0.67±0.01	0.42±0.02
20:4n-6	3.58±0.04	1.61±0.02	2.38±0.02	0.98±0.07
20:3n-3	0.04±0.03	0.14±0.10	0.36±0.04	0.32±0.02
20:4n-3	0.22±0.01	0.28±0.00	0.68±0.03	0.74±0.04
20:5n-3	7.15±0.04	3.18±0.05	8.75±0.18	3.34±0.10
22:1n-11	0.16±0.00	0.33±0.02	0.20±0.00	0.27±0.01
21:5n-3	0.09±0.01	0.19±0.00	0.07±0.01	0.13±0.00
22:4n-6	0.40±0.01	0.17±0.00	0.25±0.01	0.16±0.02
22:5n-6	0.50±0.02	0.28±0.00	0.34±0.00	0.14±0.00
22:5n-3	3.47±0.02	1.68±0.05	3.63±0.10	1.72±0.03
22:6n-3	32.9±0.07	16.1±0.26	29.0±0.62	13.7±0.57
24:1n-9	0.21±0.07	0.17±0.00	0.20±0.07	0.17±0.03
Saturates	28.0	32.9	27.7	27.9
Monoenes	17.2	33.5	16.4	27.5
Polyenes	54.9	33.6	55.9	44.7

[1] Diet of CO group was a commercial diet containing EPA and DHA less than approximately 2%.
[2] Diet of PO group was prepared by impregnation of perilla oil (10%) as a lipid source to the diet of CO group.
[3]ND, not detected.

Table 6. Plasma component levels of cultured sweet smelt fed different diets for 8 weeks

Parameters	CO group[1]	PO group[2]
Hematocrit value (%)	50.8±11.4	49.3±8.90
Total protein (g/100 mL)	3.8±0.6	3.8±0.6
Glucose (mg/100 mL)	56±10	55±8.0
Urea nitrogen (mg/100 mL)	1.8±0.5	2.2±0.5
Total cholesterol (mg/100 mL)	475±108	626±173
Triglyceride (mg/100 mL)	501±212	483±164

[1]Diet of CO group was a commercial diet containing EPA and DHA less than approximately 2%.
[2]Diet of PO group was prepared by impregnation of perilla oil (10%) as a lipid source to the diet of CO group.

Table 7. Phagocytic rate and SOD activity of cultured sweet smelt fed different diets for 8 weeks

Parameters	CO group[1]	PO group[2]
Phagocytic rate (%)	32.8±6.22	34.6±6.23
SOD activity (NU/mg protein)	3.94±0.80	8.31±3.86

[1]Diet of CO group was a commercial diet containing EPA and DHA less than approximately 2%.
[2]Diet of PO group was prepared by impregnation of perilla oil (10%) as a lipid source to the diet of CO group.

Table 8. TBARS and OH radical level of plasma and intensity of aroma in cultured sweet smelt fed different diets for 8 weeks

Parameters	CO group[1]	PO group[2]
TBARS (MDA μg/mL plasma)	19.0±4.8	28.9±10.9
OH radical (MDA nmol/mg protein)	5.34±2.35	21.8±8.52
Intensity of aroma	+	++++

[1]Diet of CO group was a commercial diet containing EPA and DHA less than approximately 2%.
[2]Diet of PO group was prepared by impregnation of perilla oil (10%) as a lipid source to the diet of CO group.

previous paper (Jeong et al., 2000), the wild fish muscle contained high levels of ALA and EPA compared with the cultured fish. This suggests that ALA and EPA might be prominent precursors of the characteristic aroma in the wild fish. In the present study, the intensity of aroma was much stronger in PO group than in CO group, suggesting

that ALA played an important role for generation of the aroma in PO group. On the other hand, the muscle of CO group contained approximately 2 times high levels of DHA (8.33~9.75% vs. 4.45~4.46% in PO group) and EPA (2.82~3.47% vs. 1.26~1.84% in PO group) as much as those of PO group, despite the same level of DHA and EPA in diets of both groups, accounting for less than 2%, respectively. Such results were also shown in a study of Kayama et al. (1963) in which in a model experiment of aquatic food chain, despite trace amount of ALA in *Chaetoceros*, which is a feed for *Artemia*, the large amount of EPA was found in *Artemia* oil. Therefore, they suspected that some fatty acids in *Chaetoceros* oil served as the parent fatty acid of EPA, and that a small amount ALA in *Chaetoceros* was spared and converted to EPA efficiently. Although testis of CO group contained slightly high level of DHA (32.9% vs. 29.0% in PO group) as well, the EPA content had almost no difference between the testes of both groups. The high level of DHA found in the cultured fish testes was also found in human (Nissen and Kreysel, 1983; Conquer et al., 1999). Likewise human the concentration of the DHA in fish may be positively correlated with sperm density, the number of motile sperm and sperm motility. Moreover, proportions of DHA, EPA, 20:4n-6 and 22:5n-3 in testis were high approximately 2 times compared with ovary of both groups, while ovary contained much more in 18:1n-9, 16:1n-7 and 18:2n-6, and ALA (only in PO group) than testis. These results suggest that these fatty acids may be preferentially incorporated into testis or ovary in order to a different reproductive function each other.

On the other hand, CHOL level in the fish plasma were considerably high in PO group compared with CO group. Furthermore, survival rates of both groups were positively related with CHOL level. Therefore, the levels of plasma CHOL may be used as an index of the cultured fish healthy. Yellowtail and rainbow trout containing the low level of plasma CHOL are reduced on disease resistance (Maita et al., 1998a, b). McDonald and Milligan (1992) reported that the levels of plasma lipid components of fish, such as TG, CHOL and phospholipid, were decreased by the effect of inanition. In the present study, the fish of CO group was seemed to be received almost no effect of malnutrition, in particular, as shown in the TG level, which showed slightly high level in CO group compared with PO group, though FE of CO group was low level compared with PO group. Coldwater disease is often accompanied with anemia (Iita and Mizokami, 1996), which reduces oxygen transport and causes various alterations in fish. The anemic fish is susceptible to the pathogen (Piacentini et al., 1989), in which plasma CHOL and urea nitrogen are significantly low compared to those of normal fish (Maita et al., 1996).

In biological defense system, SOD plays a scavenging role of free radicals developed during aerobic metabolism with catalase and glutathion peroxidase. Phagocytosis is an index of non-specific immune, concerned with engulfing and destruction of foreign materials. In the present study, high level of SOD activity and phagocytic rate in PO group were resulted in healthy fish, so high level of survival rate might be kept during rearing period. The TBARS and OH radical levels in plasma were higher in PO group than CO group. This might imply that PO group has a strong lipoxigenase or due to large amount of ALA compared with CO group. Therefore, the levels of these lipid peroxides were greatly related with the intensity of the fish aroma. These results were supported by the study of Kaewsrithong et al. (2000), in which they reported that some aromatic fish including sweet smelt contained a large amount of hydroperoxide in liver compared to non-aromatic fish, and suggested that biogeneration of lipid hydroperoxide is an initial step in the development of certain volatile compounds in the aromatic fish. These results suggest that perilla oil might be usefulness as a lipid source of the cultured fish diet, in which result in high quality of the cultured fish.

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