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Effects of Dietary Perilla Oil and *Enteromorpha compressa* Meal on Growth, Fatty Acid Composition and Hematology of the Cultured Sweet Smelt (*Plecoglossus altivelis*)

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Sweet smelt (*Plecoglossus altivelis*) were fed four different diets supplemented with either perilla oil (2.0%) rich in 18:3n-3 (CP), and perilla oil and *Enteromorpha compressa* meal (2.0%) (CPA), soybean oil rich in 18:2n-6 (CO), or soybean oil and algal meal (CA) for 4 weeks. The growth performance, fatty acid composition of muscle, plasma lipid peroxidation and blood components of the sweet smelt were then determined. The specific growth rate and feed efficiency in the fish fed the CPA diet were the highest, while the other groups showed similar results. The fatty acid composition of muscle in sweet smelt reflected the dietary lipids; 18:3n-3 was higher in the fish fed the CP and CPA diets, and 18:2n-6 was higher in the fish fed the CO and CA diets. The other fatty acid profiles presented almost no differences with respect to the diet composition. The fish fed the CA, CP and CPA diets contained significantly lower levels of triglyceride, thiobarbituric acid-reactive substances and hydroxyl radical in their plasma than that fed the CO diet. Phagocytic activity was the highest in the fish fed the CPA diet and higher in those of the fish fed the CP and CA diets compared to the CO diet group. The results from this study suggest that a dietary supplement of 2.0% perilla oil together with 2.0% *E. compressa* meal may improve the growth and health of cultured sweet smelt.

Key words: *Enteromorpha compressa*, Fatty acid, Perilla oil, Phagocytic activity, *Plecoglossus altivelis*

Introduction

Consumers are interested in the safety and quality of cultured fish, and producers need to pay close attention to ensure health and quality of the cultured fish. Sweet smelt (*Precoglossus altivelis*) has been an important freshwater fish cultured species in Korea as well as Japan. Wild sweet smelt possesses a characteristic smell that has been referred to as a watermelon-like or cucumber-like aroma. The intensity of this aroma is generally weak in cultured sweet smelt compared to wild one. The compounds responsible for the aroma are known to be nine-carbon aldehydes and alcohols (Hirano et al., 1992). These are generated through the action of fish lipoxygenase, which peroxidizes polyunsaturated fatty acids (PUFA), such as 20:5n-3 and 22:6n-3, to generate low molecular weight volatile carbonyl compounds through progressive decomposition of hydroperoxides (Fross et al., 1962; Josephson et al., 1984; Hirano et al., 1992; Zhang et al., 1992; Kaewsrithong et al., 2000). From this information and the previous study (Jeong et al., 2000), we speculated that 18:3n-3

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and/or 20:5n-3 would play an important role in the production of the characteristic aroma of sweet smelt. It is considered that the fatty acid composition of muscle from the cultured fish should be brought close to that of wild fish in order to improve the quality of the cultured fish.

On the other hand, it is reasonable to assume that the levels and ratios of available dietary nutrients would influence fish health, and reviews of Blazer (1991) and Waagbo (1994) highlight the importance of this line of aquaculture research. Among macronutrients lipids, dietary PUFA has been demonstrated to be a modulator of the immune response (Schroit and Gallily, 1979; Calder et al., 1990; Kiron et al., 1995). We therefore consider that the influence of dietary fatty acids on the health condition of sweet smelt is worthy of further investigation.

Many workers have shown that fish fed on diets supplemented with marine algae improved fish quality (muscle taste) and increased resistance to stress and disease (Yone et al., 1986a, b; Nakagawa et al., 1984, 1985, 1987; Mustafa et al., 1995). Because the green alga *Enteromorpha compressa* is harvested in large quantities in Korea, we considered the practical application of this alga as a feed additive to improve the efficacy of fish culture.

The aim of this study was to ensure that the fatty acid composition (18:3n-3) of the muscle from cultured fish approximates to that of wild fish. In addition, the aim was also to keep the fish healthy through modification of dietary ingredients during the test period. Therefore, in the present study, we investigated the effects of perilla oil which is rich in 18:3n-3 and an algal *E. compressa* meal which contains various physiologically beneficial components on the muscle fatty acid compositions and blood components of the cultured sweet smelt during the 4 weeks feeding trial.

Materials and Methods

Experimental conditions

Juvenile sweet smelts were purchased from a private fish farm and reared on a commercial diet prior to experiment at Kyeongsangnam-Do Hatchery, Korea. The fish weighing about 39.8 g were randomly divided into 8 rectangular concrete tanks (5,000 L), each holding 200 fish. They were cultured on the experimental diets for 4 weeks and each diet had 2 populations assigned to it. The fish were fed 3 times a day with a total daily feed representing 2.0-2.4% of their body weights. The tanks were continuously supplied with underground water at 19±2℃ and the photoperiod was controlled at 16 L and 8 D throughout the feeding trial. Oxygen was supplied by aeration. Each tank was inspected daily and dead fish were counted and removed from the tank.

Experimental diets

Four experimental white fish meal-based diets were prepared; a control diet (CO) supplemented with soybean oil (2.0%) to approximate the 18:2n-6 rich commercial diet for sweet smelt commonly used in Korea, a diet (CA) supplemented with soybean oil (2.0%) and alga *E. compressa* meal (2.0%), a diet (CP) to which perilla oil (2.0%) was added instead of soybean oil, and a diet (CPA) supplemented with the algal meal (2.0%) and perilla oil (2.0%). The ingredient and proximate composition of experimental diets is shown in Table 1.

Table 1. Ingredient and proximate composition of experimental diets for cultured sweet smelt (wt %)

	CO	CA	CP	CPA
Ingredient				
White fish meal	65.00	65.00	65.00	65.00
Wheat flour	22.00	20.00	22.00	20.00
Potato starch	5.00	5.00	5.00	5.00
NaCl	0.50	0.50	0.50	0.50
Soybean oil	2.00	2.00	-	-
Perilla oil	-	-	2.00	2.00
Alga meal[1]	-	2.00	-	2.00
Choline chloride	0.50	0.50	0.50	0.50
Vitamin premixture[2]	2.49	2.49	2.49	2.49
Mineral premixture[3]	2.50	2.50	2.50	2.50
Alpha-tocopheryl acetate	0.01	0.01	0.01	0.01
Proximate composition				
Moisture	20.4	17.3	19.5	17.8
Protein	37.3	37.3	36.9	35.5
Lipid	7.37	7.51	7.07	6.60
Ash	12.1	13.6	12.5	12.3

CO, a control diet supplemented with soybean oil; CA, a diet with supplemented with soybean oil and alga *Enteromorpha compressa* meal; CP, a diet supplemented with perilla oil instead of soybean oil; CPA, a diet with supplemented with perilla oil and the alga meal.

[1]Alga, *Enteromorpha compressa*, was purchased from a market, washed in a freshwater, dried in the shade and grinded in 40 mesh.

[2,3]Vitamin and mineral premixtures were purchased from ABANK Co. in Korea.

Sampling

After 4 weeks feeding trial, body length and body weight were measured, and then the fish were transported to a laboratory in dry ice. Muscle was removed from 20 male and 20 female specimens from each tank and then stored at -80℃ prior to analysis. The fish muscle was blended with a speed cutter (National, MK-K51, Japan) prior to assay.

Analysis of fatty acid composition

Total lipid (TL) was extracted and purified (Bligh and Dyer, 1959) and was determined gravimetrically. Fatty acid was determined after methylation (Jeong et al., 2000). Fatty acid composition of TL was analyzed using gas-liquid chromatography (Shimadzu GC 14A, Shimadzu Seisakusho, Co. Ltd., Japan) fitted with an Omegawax 320 fused silica capillary column (30 m×0.32 mm, ID, Supelco, USA). The injector and flame-ionization detectors were held at 250℃, and the column oven temperature was programmed to increase from 180℃ (initial time 8 min) to 230℃ at 3℃/min with the final temperature held for 15 min. Helium was used as a carrier gas at a constant column inlet pressure of 1.0 Kg/cm^2 with a split ratio of 1:50. Fatty acids were identified by comparison with authentic standards (Sigma Chemical Co., USA) and oyster fatty acids, which have previously been analyzed by Koizumi et al. (1990).

Methyl tricosanoate (99%, Aldrich Chem. Co., USA) was used as an internal standard.

Blood sampling and measurement of plasma constituent 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 and the remainder was centrifuged at 1000×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., Japan) using the Biuret method (total protein) and the enzymatic method (urea nitrogen, glucose, total cholesterol and triglyceride).

Phagocytic activity

Fifty μL of blood was mixed with an equal volume of yeast cell suspension consisting of 5 mg of yeast cells (Zymosan A, Sigma Chemical Co., USA) suspended in 1 mL of phosphate buffered saline. The mixture was incubated for 30 min at 20℃ and then smeared onto a microscopic 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, OH 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 (Ohkawa et al., 1979). Concentration of TBARS, a decomposition product of lipid hydroperoxide, was calculated from a standard curve generated using malonaldehyde (MDA), and expressed as MDA μg/mL. The OH radical activity was assayed (Halliwell et al., 1987) which is based on the degradation of deoxyribose and expressed as MDA nmol/mg protein. The 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).

Analysis of tocopherol analogues

An aliquot of TL from fish muscle was dissolved in n-hexane, and an aliquot of this solution was injected into an HPLC instrument (LC-10Avp series, Shimadzu Seisakusho, Co. Ltd., Japan) equipped with a Shim-pack CLC-NH_2 column (5 μm particle size, 6.0×150 mm, Shimadzu Seisakusho, Co. Ltd., Japan). Materials were eluted with a mobile phase of hexane-isopropanol (25:1, v/v). All HPLC procedures were carried out at ambient temperature and monitored for absorbance at 295 nm using a photodiode array UV detector (SPD-M10Avp, Shimadzu Seisakusho, Co. Ltd., Japan).

The flow rate was 0.8 mL/min. α-, β- and γ-tocopherols in the TL sample were identified by comparing the retention time and diode array UV spectra of the peaks to those of the corresponding authentic materials (Eisai Co. Ltd., Japan) run under the same conditions. Tocopherols were quantified by peak area measurements. The areas were compared with areas of known amounts of standards using 2,2,5,7,8-pentamethyl-6-hydroxychroman as an internal standard. The data were expressed as μg/g muscle (dry matter).

Statistical analysis

Analyses were performed using a SAS (version 6.12) statistical package. Significance differences between dietary groups ($P<0.05$) were determined by analysis of variance (ANOVA), followed, when pertinent, by Turkey's multiple comparison test.

Results

Growth and feed performance

The results of the 4-weeks feeding trial are shown in Table 2. Daily feed consumption, specific growth rate and feed efficiency were not significantly different among three diet groups, CO, CA and CP. However, fish fed on the CPA diet, which was supplemented with perilla oil and *Enteromorpha compressa* meal showed a significantly higher growth rate and feed performance.

Fatty acid compositions of diets and sweet smelt muscles

Tables 3 and 4 show the fatty acid compositions of the diets and the muscles from the cultured sweet smelt fed on each experimental diet. The prominent fatty acids in all diets and muscles were 16:0, 18:1n-9, 18:2n-6, 22:6n-3, 16:1n-7, 20:5n-3, 14:0, 18:0 and 18:1n-7 or 18:3n-3. In all diets, PUFA was found at the highest level, while saturated fatty acids (SFA) were found at the lowest level. In particular, the CP and CPA diets had higher levels of PUFA because perilla oil contains high levels of 18:3n-3, resulting in higher levels of n-3 PUFA. In contrast, the CO and CA diets contained higher levels of n-6 PUFA as a result of the soybean oil which contains higher levels of 18:2n-6.

On the other hand, there were no differences in the muscle levels of SFA, monounsaturated fatty acids (MUFA) and PUFA between the diet groups. PUFA showed the lowest level in the fish muscles of all diet groups and MUFA and SFA showed similar levels. Although PUFA was present at a similar level in the muscles of all diet groups, CO and CA diet groups supplemented with soybean oil had significantly higher levels of 18:2n-6 (8.84-8.51% vs. 6.57-6.69% in CP and CPA diet groups), while those in the CP and CPA diet groups supplemented with perilla oil had significantly higher levels of 18:3n-3 (3.02-3.19% vs. 1.06-1.8% in CO and CA diet groups). The unsaturation index was much higher in the CP and CPA diets with perilla oil than in the CO and CA diet with soybean oil. On the other hand, in the fish muscle, the unsaturation index was low in the CA and CPA diet groups dieting on algal meal.

Hematocrit values, plasma components and phagocytosis

The hematocrit (Ht) values, plasma components and phagocytic rate of cultured sweet smelt are shown in Table 5. Ht values did not significantly differ between the different diet groups ranging from 34.9 (CPA diet group) to 37.4% (CO diet group). There were almost no differences in the total protein, glucose and triglyceride (TG) levels of plasma. However, the urea nitrogen level of plasma was higher in the CO and CA diet groups compared to the CP and CPA diet groups. On the other hand, the plasma of CA and CPA diet groups supplemented with *E. compressa* meal, and the fish plasma of the CP diet group had a slightly low level of total cholesterol (CHOL) compared to the CO diet group fed without

Table 2. Results of the 4 weeks feeding trial on the growth and feed efficiency in the cultured sweet smelt[1]

	CO	CA	CP	CPA
Body weight (g)				
Initial	39.8±7.31	39.8±7.31	39.8±7.31	39.8±7.31
Final	53.0±2.12^{b}	52.6±0.92^{b}	52.5±3.25^{b}	60.1±0.35^{a}
Total feed intake (g)	10,273	10,192	10,426	10,406
Specific growth rate (%)	1.06±0.15^{b}	1.03±0.06^{b}	1.02±0.23^{b}	1.52±-0.02^{a}
Feed efficiency (%)	64.1±11.2^{b}	60.7±4.38^{b}	60.9±15.6^{b}	95.5±0.96^{a}
Survival rate (%)	94.6	96.5	96.0	96.8

For abbreviations of diet, refer to Table 1.
[1]Data are expressed as mean±SD from 80 fish in each group (two tanks×40 fish) and different superscript letters indicate statistically significant difference ($P<0.05$).

Table 3. Total fatty acid compositions (wt %) of diets for cultured sweet smelt[1]

Fatty acid	CO	CA	CP	CPA
14:0	2.65±0.05^{a}	2.47±0.04^{b}	2.59±0.05^{ab}	2.46±0.12^{b}
16:0	15.1±0.16^{a}	15.2±0.15^{a}	14.0±0.17^{b}	14.1±0.46^{b}
16:1n-7	5.05±0.06^{a}	4.81±0.05^{ab}	4.97±0.05^{a}	4.65±0.27^{b}
17:0	1.02±0.01^{a}	1.00±0.02^{ab}	1.02±0.02^{a}	0.98±0.02^{b}
18:0	3.39±0.02^{a}	3.46±0.04^{a}	2.86±0.04^{b}	2.84±0.03^{b}
18:1n-9	16.2±0.12^{a}	16.2±0.18^{a}	14.0±0.22^{b}	13.7±0.20^{b}
18:1n-7	4.21±0.04^{a}	4.22±0.09^{a}	4.13±0.03^{a}	4.16±0.02^{a}
18:2n-6	17.9±0.02^{b}	18.1±0.08^{a}	7.72±0.10^{d}	8.31±0.06^{c}
18:3n-3	2.08±0.04^{c}	2.26±0.12^{c}	16.8±0.25^{b}	17.4±0.08^{a}
18:4n-3	0.65±0.00^{ab}	0.67±0.01^{a}	0.59±0.02^{c}	0.63±0.03^{b}
20:1n-11	3.09±0.02^{b}	2.90±0.04^{b}	3.44±0.76^{b}	4.46±0.05^{a}
20:1n-9	1.71±0.01^{a}	1.65±0.02^{a}	1.90±0.44^{a}	0.00±0.00^{b}
20:4n-6	0.54±0.01^{b}	0.59±0.02^{a}	0.54±0.01^{b}	0.58±0.01^{a}
20:5n-3	8.66±0.04^{a}	8.69±0.06^{a}	8.44±0.17^{a}	8.67±0.23^{a}
22:1n-11	2.16±0.03^{a}	2.02±0.05^{b}	2.01±0.04^{b}	1.83±0.11^{c}
22:1n-9	0.63±0.02^{a}	0.56±0.00^{bc}	0.59±0.02^{ab}	0.53±0.03^{c}
22:5n-3	0.61±0.01^{a}	0.63±0.01^{a}	0.60±0.02^{a}	0.62±0.03^{a}
22:6n-3	9.87±0.18^{a}	10.2±0.17^{a}	9.61±0.22^{a}	10.0±0.60^{a}
24:1n-9	0.65±0.04^{a}	0.64±0.03^{a}	0.68±0.05^{a}	0.63±0.05^{a}
Saturates[2]	23.2±0.32^{a}	23.2±0.37^{a}	21.4±0.40^{b}	21.3±0.71^{b}
Monoenes[3]	34.9±0.38^{a}	34.0±0.55^{a}	32.9±1.72^{b}	31.1±0.82^{c}
Polyenes[4]	41.9±0.37^{c}	42.8±0.55^{c}	45.7±0.86^{b}	47.6±1.10^{a}
n-3 PUFA	22.6±0.30^{c}	23.2±0.39^{c}	36.7±0.70^{b}	38.0±0.98^{a}
n-6 PUFA	18.6±0.04^{b}	18.9±0.10^{a}	8.48±0.12^{d}	9.11±0.08^{c}
Unsat. index[5]	192	195	210	216

For abbreviations of diet, refer to Table 1.
[1]Data are expressed as mean±SD of four determinations (two groups x two determination), only the values over than 0.50% are presented and different superscript letters indicate statistically siginificant difference ($P<0.05$).
[2]includes 15:0 iso, 15:0, 16:0 iso, 17:0 iso and anteiso, phytanic, 19:0, 20:0 and 22:0.
[3]includes14:1n-7, 16:1n-5, 17:1n-10, 17:1n-8, 18:1n-5, 20:1n-7 and 22:1n-7.
[4]includes 16:2n-4, 17:2n-8, 16:4n-3, 18:2n-4, 18:3n-1, 18:4n-1, 20:2n-6, 20:4n-3, 21:5n-3 and 22:5n-6.
[5]Sum of percentages of individual fatty acids×number of double bonds.

the addition of *E. compressa* meal. The CPA diet group supplemented with alga meal and perilla oil had the highest phagocytic rate of all the diet groups (49.2%), being four times higher than the CO diet group. Moreover, the phagocytic activity of the CA and CP groups was also 2.3-2.7 times higher than that of the CO diet group, and that of the CA diet group fed with *E. compressa* meal was slightly higher than that of the CP diet group.

Proximate compositions, tocopherol, lipid peroxidation and superoxide dismutase activity

Table 6 shows the proximate compositions and molar ratios of PUFA/alpha-tocopherol (α-Toc) in fish muscle, and the plasma lipid peroxidation and superoxide dismutase activities of sweet smelt. Protein content was highest in fish muscle of the CA diet group but there was almost no difference between the other groups. Lipid contents in the fish muscles of the CP and CPA diet groups fed with perilla oil were 4.3-4.6% (dry matter) higher than that of the CO diet group, and the CA diet group had a 1.9% higher lipid content than the CO diet group. The fish muscle of the CO diet group had the lowest levels of PUFA and therefore the highest PUFA/α-Toc ratio of all of the diet groups. Conversely, the fish muscles of the CPA and CA diet groups, despite having higher levels of PUFA, had the lowest PUFA/α-Toc ratio, 707 and 713 respectively, due to the higher level of α-Toc compared to the CO diet group. In contrast to the PUFA/α-Toc ratio in muscle, TBARS (MDA μg/mL plasma) and OH radical (MDA nmol/mg protein in plasma) levels in the fish plasma of the CO diet group were the highest

Table 4. Total fatty acid compositions (wt %) of muscles from cultured sweet smelt fed different diets[1]

Fatty acid	CO	CA	CP	CPA
14:0	4.45±0.16^{ab}	4.43±0.21^{b}	4.56±0.11^{ab}	4.59±0.15^{a}
16:0	25.9±1.11^{b}	26.9±1.31^{a}	26.9±0.63^{a}	27.2±0.58^{a}
16:1n-7	8.40±0.41^{c}	8.45±0.27^{c}	8.78±0.29^{b}	9.30±0.21^{a}
18:0	3.37±0.09^{ab}	3.41±0.14^{a}	3.31±0.09^{b}	3.16±0.08^{c}
18:1n-9	19.5±1.45^{a}	19.3±1.22^{a}	18.5±1.00^{a}	18.9±1.09^{a}
18:1n-7	3.03±0.20^{a}	3.07±0.20^{a}	2.95±0.14^{a}	2.92±0.18^{a}
18:2n-6	8.48±0.25^{a}	8.51±0.46^{a}	6.69±0.17^{b}	6.57±0.21^{b}
18:3n-3	1.08±0.05^{b}	1.06±0.07^{b}	3.02±0.31^{a}	3.19±0.24^{a}
18:4n-3	0.54±0.03^{a}	0.53±0.03^{a}	0.55±0.01^{a}	0.54±0.02^{a}
20:1n-11	1.69±0.07^{a}	1.59±0.10^{bc}	1.63±0.07^{ab}	1.56±0.07^{c}
20:1n-9	1.56±0.07^{a}	1.50±0.09^{ab}	1.55±0.06^{a}	1.46±0.04^{b}
20:4n-3	0.48±0.02^{ab}	0.45±0.02^{c}	0.50±0.02^{a}	0.48±0.02^{b}
20:5n-3	3.86±0.18^{a}	3.78±0.27^{a}	3.87±0.17^{a}	3.70±0.18^{a}
22:1n-11	1.20±0.06^{a}	1.16±0.07^{a}	1.16±0.05^{a}	1.10±0.05^{b}
22:5n-3	1.54±0.04^{ab}	1.49±0.07^{ab}	1.56±0.06^{a}	1.45±0.18^{b}
22:6n-3	8.87±0.32^{a}	8.40±0.48^{bc}	8.63±0.33^{ab}	8.16±0.25^{c}
Saturates[2]	35.3±1.55^{b}	36.3±1.78^{a}	36.2±0.99^{ab}	36.4±0.99^{a}
Monoenes[3]	37.4±2.39^{a}	37.1±2.16^{a}	36.5±1.73^{a}	37.1±1.76^{a}
Polyenes[4]	27.4±1.15^{a}	26.6±1.58^{a}	27.2±1.21^{a}	26.5±1.24^{a}
n-3 PUFA	16.9±0.68	16.3±0.99	18.8±0.94	18.1±0.92
n-6 PUFA	9.63±0.35	9.60±0.52	7.70±0.21	7.56±0.25
Unsat. index[5]	150	146	150	146

For abbreviations of diet, refer to Table 1.
[1]Data are expressed as mean±SD of eight determinations (two tank in each dietary group x two specimens, 20 fish each×two determinations), only the values over than 0.50% are presented and different superscript letters indicate statistically significant difference ($P<0.05$).
[2]includes 15:0 iso and anteiso, 15:0, 16:0 iso, pristanic, 17:0 iso and anteiso, phytanic, 17:0, 19:0, 20:0 and 22:0.
[3]includes 14:1n-7, 16:1n-13, 16:1n-5, 17:1n-10, 17:1n-8, 18:1n-5, 20:1n-7, 22:1n-9, 22:1n-7 and 24:1n-9.
[4]includes 16:2n-4, 17:2n-8, 16:4n-3, 18:2n-9, 18:2n-4, 18:4n-1, 20:2n-6, 20:3n-6, 20:4n-6, 20:3n-3, 21:5n-3, 22:4n-6 and 22:5n-6.
[5]Sum of percentages of individual fatty acids×number of double bonds.

Table 5. Hematocrit value, plasma components and phagocytic activity in cultured sweet smelt fed different diets[1]

Parameter	CO	CA	CP	CPA
Hematocrit value (%)	37.4±1.91^{a}	36.5±4.74^{a}	36.1±5.61^{a}	34.9±5.91^{a}
Total protein (g/100 mL)	5.3±0.3^{a}	4.8±0.5^{b}	4.9±0.3^{b}	4.7±0.3^{b}
Glucose (mg/100 mL)	62±10^{a}	41±9^{b}	47±7^{b}	48±13^{b}
Urea nitrogen (mg/100 mL)	2.4±0.3^{b}	2.9±0.3^{a}	1.9±0.3^{c}	2.3±0.3^{b}
Total cholesterol (mg/100 mL)	703±41^{a}	653±136^{ab}	606±156^{b}	689±58^{ab}
Triglyceride (mg/100 mL)	386±55^{a}	307±91^{b}	320±104^{b}	319±55^{b}
Phagocytic rate (%)	11.3±8.48^{c}	30.6±8.11^{b}	26.4±6.58^{b}	49.2±4.82^{a}

For abbreviations of diet, refer to Table 1.
[1]Data are expressed as mean±SD from 10 fish in each group and different superscript letters indicate statistically significant difference ($P<0.05$).

in all diet groups at 37.0 and 19.2, respectively. However, the fish of the other groups had similar levels of TBARS and OH radicals, which were 19.2-22.5 and 9.28-12.6, respectively. SOD activity was not significantly different in the fish plasma of all diet groups, although the activity was slightly high

Table 6. Proximate composition, TFA, PUFA and molar ratio PUFA/alpha-tocopherol of muscle and the level of plasma TBARS, OH radical and SOD activity from cultured sweet smelt fed different diets[1]

Parameter	CO	CA	CP	CPA
Proximate composition (wt %)				
Moisture	71.8±0.61	72.0±0.49	70.8±0.31	71.5±0.50
Protein	15.6±0.14	16.5±0.13	15.8±0.45	15.4±0.40
Ash	1.33±0.26	1.48±0.03	1.26±0.08	1.40±0.24
Lipid	7.56±0.36[b]	8.04±0.25[b]	9.09±0.39[a]	8.96±0.72[a]
TFA[2] (g/100 g, dry basis)	25.3	27.4	29.6	29.3
PUFA (mg/g, dry basis)	69.3	72.9	80.6	77.4
PUFA (mmol)	0.24	0.25	0.27	0.26
Alpha-Toc[3] (μg/g, dry basis)	106	150	156	160
Alpha-Toc (μmol)	0.25	0.35	0.36	0.37
Molar ratio PUFA/alpha-Toc (μmol/μmol)	961	713	755	707
TBARS[4] (MDA[5] μg/mL)	37.0±16.6[a]	22.5±12.5[b]	19.2±9.84[b]	20.1±9.79[b]
OH radical (MDA nmol/mg protein)	19.2±6.98[a]	10.8±2.77[b]	12.6±2.72[b]	9.28±5.09[b]
SOD[6] activity (NU/mg protein)	6.78±1.72	6.59±1.35	6.20±0.85	7.48±1.31

For abbreviations of diet, refer to Table 1.
[1]Proximate compositions are expressed as mean±SD from four determinations (two tanks×two specimens, 20 fish each) and different superscript letters indicate statistically significant difference ($P<0.05$).
[2]TFA, total fatty acids. [3]Toc, tocopherol. [4]TBARS, thiobarbituric acid reactive substanses.
[5]MDA, malondialdehyde. [6]SOD, superoxide dismutase.

in the CPA diet group.

Discussion

It is considered that supplementing fish diets of perilla oil together with the algal meal improves the growth and health of the cultured sweet smelt. Growth and feed performance are not affected by supplementation with these ingredients. Fatty acid composition of fish is influenced by dietary fatty acids (Watanabe, 1982; Henderson and Tocher, 1987), and this was confirmed by the fact that the muscle fatty acid composition of the fish in each experimental group in this study reflected that of the experimental diet. The level of 18:3n-3 in the muscle from CP and CPA diet groups was higher than that from the CO and CA groups. Perilla oil would be a good lipid source to increase the level of 18:3n-3 in the muscle of the cultured fish. Although we did not objectively measure the quantity of aroma compounds in the present study, the aroma of fish in the former groups was stronger than those in the latter by using smell as an indicator. This suggests that 18:3n-3 may be an important precursor of fish aroma. However, the levels of 18:3n-3 in the cultured fish muscle was lower compared to that of the wild one (Jeong et al., 2000). It is suggested that the amount of perilla oil in the diet should be increased over 2% in order to improve the quality of the cultured sweet smelt.

Supplementation of diets with algal meal or perilla oil had a positive effect on the health of the cultured sweet smelt as measured by phagocytic activity. In fact, these additives had a synergistic effect, because phagocytic activity was significantly stimulated by the diet supplemented with algal meal (CA diet group) or perilla oil (CP diet group). The fish fed the CPA diet containing both perilla oil and algal meal showed the highest level of phagocytic activity among the experimental groups. It has been shown that rainbow trout fed linseed oil, which is rich in 18:3n-3, elevated phagocytic activity compared to fish fed Alaska pollack liver oil or sunflower oil which are rich in 18:2n-6 (V. Kiron, per. comm., 2001). Kiron et al. (1995) reported that the in vitro bactericidal activity of macrophages from the rainbow trout fed dietary n-3 PUFA was higher than in those obtained from fish fed dietary n-6 PUFA. Furthermore, chemotactic activity of neutrophils was enhanced by dietary n-3 PUFA rather than by n-6 PUFA in the rainbow trout (Ashton et al., 1994). The dietary amount of n-3 PUFA in the CP and CPA groups was higher than that of n-6 PUFA compared to amounts found in

the CO and CA diet groups. The higher levels of n-3 PUFA resulted from the elevation of 18:3n-3 as a result of the perilla oil supplement. Therefore, it is considered that the effect of perilla oil on the elevation of phagocytic activity may be due to dietary 18:3n-3.

Maita et al. (1998a,b) suggested that high levels of fish plasma lipid components such as CHOL or TG in the rainbow trout and the yellowtail were closely related to resistance to bacterial infection. In addition, the level of plasma CHOL could also be used as an indicator of latent poor health in the sweet smelt (Maita and Lee, 2000). The levels of plasma CHOL and TG in the fish of the CO diet group were the highest among all the groups, while a significant difference was not observed in the other groups. The crude lipid level of a diet influences plasma lipid component levels. Furthermore, supplementation of *Ulva* meal to the diet significantly lowered plasma lipid levels and increased muscle lipid levels in cultured red sea bream (Nakagawa and Kasahara, 1986), black sea bream (Nakagawa et al., 1984) and yellowtail (Nakagawa et al., 1985). In the present study, muscle lipid levels of the CA and CPA diet groups were higher than that of the CO group. It is suggested that *E. compressa* meal has similar effects on lipid accumulation and mobilization in the cultured fish to that of *Ulva* meal in other fish species. Therefore, it seems that the levels of plasma CHOL and TG in the fish fed the CA and CPA diets may be reduced due to algal meal supplementation. The results of hematological parameters reflect the proximate composition of the diets, but do not reflect the better health condition of the CO diet group in the present study.

A greater amount of PUFA in fish, unlike in terrestrial animals, could result in the animal being highly susceptible to lipid peroxidation, with a sub- sequent loss of quality. The sweet smelt has a high content of hydroperoxides in their tissues (Kaewsrithong et al., 2000). In the present study, plasma TBARS and OH radical levels were significantly lower in the fish fed the diets containing algal meal (CA and CPA diet group) and perilla oil (CP diet group) compared to the fish fed the CO diet. This result suggests that lipid peroxidation was inhibited by algal meal and/or perilla oil supplementation. Stephan et al. (1995) and Mourente et al. (2000) stressed the importance of an α-Toc/PUFA ratio with respect to lipid peroxidation in turbot (*Scophthalmus maximus*) and gilthead sea bream (*Sparus aurata*). The high α-Toc/PUFA ratio suppressed lipid peroxidation of fish muscle and liver. In the present study, the α-Toc/PUFA ratio in the muscles of fish fed the CPA, CA and CP diets was higher than that of the fish fed the CO diet. Therefore, a high α-Toc/PUFA ratio may be related to the suppression of lipid peroxidation. Nakagawa et al. (1981) showed that the sweet smelt fed *Chlorella* extract had decreased levels of serum TBARS, but the effective components of this extract have not been identified. There may be other antioxidants contained in algal meal or perilla oil that also prevent lipid peroxidation. In the present study, perilla oil contained a small amount of γ-tocopherol (326 ng/mL).

The results presented in this study suggest that diets supplemented with algal meal or perilla oil, particularly with both perilla oil and algal meal, improved the quality of the cultured sweet smelt. It is reeded to investigate the optimum quantity of perilla oil and algal meal in the cultured fish diets.

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