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Effect of α-Tocopherol Level in Diet on the Biochemical Property of Cultured Sweet Smelt, *Plecoglossus altivelis*

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The effect of α-tocopherol (α-Toc) level in diet on the biochemical property of sweet smelt, *Plecoglossus altivelis*, was investigated. The cultured sweet smelt fish were fed two different diets for 8 weeks; a control diet was added 0.01% of α-Toc (CO group) and an experimental diet was added 1.00% of α-Toc (HT group). Both diets were rich in docosahexaenoic acid (DHA, 22:6n-3) and eicosapentaenoic acid (EPA, 20:5n-3), accounting for 12.3~13.2% and 12.1~12.4%, respectively. Growth rate (GR) and feed efficiency (FE) were almost no difference between both groups, but female fish of both groups were superior to male fish in GR and FE. Lipid contents of muscle and ovary were high in CO group compared with HT group, but that of testis showed a similar level in both groups. The proportion of polyunsaturated fatty acids (PUFA) in muscle showed almost no difference in sex and between both groups. In testes, the proportion of PUFA was 1.35 times for HT group to 1.54 times for CO group as much as in ovaries, in which was high approximately 1.5 times compared with muscle. Thiobarbituric acid-reactive substances (TBARS) and hydroxyl (OH) radical levels of plasma were higher in CO group than HT group and superoxide dismutase activity was also slightly high in the former. The intensity of watermelon-like or cucumber -like aroma was related positively with TBARS and OH radical levels in plasma. The level of triglyceride (TG) and total cholesterol (CHOL) in plasma of CO group was higher than those of HT group. Survival rate was high in CO group with higher level of TG and CHOL in plasma.

Key words: α-Tocopherol, Aroma, Fatty acid, Growth rate, Plasma, Sweet smelt

Introduction

Defense system on peroxidation of organisms acts synergistically through joint action of antioxidants such as α-tocopherol (α-Toc), ascorbic acid, ubiquinol and glutathione, and enzymes such as superoxide dismutase, catalase and glutathione peroxidase etc. However, when the balance of biological defense system is destroyed, biological lipids are oxidized by active oxygen species or free radicals and consequently lipid peroxides and its breakdown products are implicated in the cause of pathology such as cancer, aging and chromic inflammation (Niki et al., 1996). Moreover α-Toc is a classical lipophilic antioxidant well known as a scavenger of free radicals in a hydrophobic milieu (Niki et al., 1984). The primary function of α-Toc is to stabilize cellular and subcellular membrane by preventing peroxidative damage of structural polyunsaturated fatty acids (PUFA). However, α-Toc can develop both antioxidant and prooxidant activities *in vitro* in isolated low density lipoprotein (LDL) (Kontush et al., 1996) depending on oxidative conditions and presence of co-antioxidants and *in vivo* (Robert and Knight, 1987). In the one hand, it has been known that a large amount of α-tocopheroxyl radical generated from the result of antioxidation of α-Toc participates as a prooxidant in human LDL

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(Browry et al., 1992; Browry and Stocke, 1993). Ito et al. (1999) reported that high level of mortality was recognized in yellowtail injected with causative bacteria of fish jaundice after rearing with a diet contained high level of α-Toc because of prooxidant activity and highly accumulation of α-Toc in fish tissues. However, a suitable amount of α-Toc in fish diet increases the resistance of fish diseases (Sekiya et al., 1991; 1994) and dietary α-Toc is closely related with fish muscle (Murata and Yamauchi, 1989).

Sweet smelt *Plecoglossus altivelis* is a freshwater fish, which inhabits in the Far East, mainly Korea and Japan where the fish is a favorite dish with the peoples due to possessing a watermelon-like or cucumber-like aroma (Chyung, 1991). The characteristic aroma of the fish is known as oxidative breakdown products of PUFA ironically. The key compounds of the fish aroma have been reported to be volatile nine-carbon aldehydes and alcohols such as (*E*)-2-nonenal, (*E,Z*)-2,6-nonadienal and 3,6-nonadien-1-ol (Fross et al., 1962; Hirano et al., 1992; Zhang et al., 1992). Moreover, Hu and Pan (2000) reported that melon aroma such as 2-butoxyethanol, (*E,Z*)-3,5-octadiene-2-one and (*E,Z*)-2,4-octadienal were generated from cod liver oil by algal lipoxygenase treatment. These low molecular materials are the breakdown products of hydroperoxide, which is generated from certain PUFA by fish lipoxygenase (Josephson et al., 1984). The fact was supported indirectly from the results of Kaewsrithong et al. (2000), in which they found that unusual amounts of lipid hydroperoxides were accumulated in the tissues of aromatic fish including sweet smelt. Unlikely non-aromatic fish, when α-Toc is overdosed to aromatic fish, lipid peroxidation may be suppressed excessively in the fish tissues and subsequently the generation of aroma may be also suppressed.

In the present study, effects of overdose of α-Toc on the intensity of aroma, growth, lipid peroxidation, plasma component, non-specific immune system, proximate composition and fatty acid composition in cultured sweet smelt were investigated.

Materials and Methods

Experimental condition

Juvenile sweet smelts were purchased from a hatchery and reared up to 36.2 g by a commercial diet for approximately 4 months prior to experiment at Kyeongsangnam-do Hatchery, Korea. The fish were randomly divided into two dietary groups. Each group was reared for 8 weeks in duplicate polypropylene tanks (ID, 5.3 m) of 500 fish each. Fish were fed a control diet (CO group, α-tocopherol 0.01%) and experimental diet (HT group, α-tocopherol 1.00%). Diet compositions of each group are shown in Table 1. The fish were fed three times a day at 2.0~2.4% of body weight. The tanks were continuously supplied with freshwater at 19±2℃ and photoperiod was controlled at 16 L and 8 D throughout rearing period. Each tank was inspected daily and dead fish was counted and removed from the tank. Blood samples were collected from a representative sample of the experimental fish. The fish samples were measured for body length and body weight, 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℃. The

Table 1. Compositions of experimental diets for sweet smelt (%)

Ingredient	CO group[1]	HT group[2]
White fish meal	65.00	65.00
Wheat flour	19.95	19.90
Potao starch	5.00	5.00
Defatted rice bran	7.00	7.00
NaCl	0.50	0.50
Ascorbic mono-phosphate Mg	0.05	0.10
Choline chloride	0.50	0.50
Vitamin C-free vitamin mixture	1.00	1.00
P-free mineral mixture[3]	1.00	1.00
Alpha-Tocopheryl acetate	0.01	1.00
Proximate compositions (%)		
Moisture	4.91±0.24	6.01±0.05
Protein	44.1±0.00	41.2±0.10
Lipid	8.12±0.32	7.19±0.03
Ash	13.0±0.05	11.1±0.03

[1]Diet of CO group is a control group and contains 0.01% of alpha-tocopherol.
[2]Diet of HT group contains 1.00% of alpha-tocopherol.
[3]P-free meneral mixture contains following ingredients (g/100 g): NaCl, 5; $MgSO_4$ $7H_2O$, 74.5; $FeC_6H_5O_7$ nH_2O, 12.5; trace element mixture, 5 ($ZnSO4$ $7H_2O$, 1,765 mg; $MnSO_4$ $5H_2O$, 810 mg; $CuSO_4$ $5H_2O$, 155 mg; $AlCl_3$ $6H_2O$, 50 mg; $CoCl_2$ $6H_2O$, 15 mg; KIO_3, 5 mg; Cellulose, 2,200 mg); Cellulose, 3.0.

fish muscle was thawed and 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 the Kjeldahl method. Lipid content was extracted with chloroform-methanol by the method of Bligh and Dyer (1959) and determined gravimetrically.

Analysis of fatty acid composition

Fatty acid was determined after methylation (Jeong et al., 2000). Fatty acid composition of total lipid (TL) was analyzed by a gas-liquid chromatography (Shimadzu GC 14A, Shimadzu Seisakusho, Co. Ltd., Kyoto, Japan) fitted 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 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 (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 malonaldehyde (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).

Analysis of α-tocopherol content

An aliquot of TL was dissolved in n-hexane and an aliquot of the solution was injected into HPLC (LC-10Avp series, Shimadzu Seisakusho, Co. Ltd., Kyoto, Japan) equipped with a Shim-pack CLC -NH_2 column (5 μm particle size, 6.0×150 mm, Shimadzu Seisakusho, Co. Ltd., Kyoto, Japan), and materials were eluted with a mobile phase of hexane -isopropanol (25:1, v/v). All HPLC procedures were carried out at the ambient temperature and monitored for absorbance at 295 nm using a photodiode array UV detector (SPD-M10Avp, Shimadzu Seisakusho, Co. Ltd., Kyoto, Japan). The flow rate was 0.8 mL/min. α-, β-, γ-tocopherols were identified by comparing to the retention time and diode array UV spectra of the peaks with those of the corresponding authentic materials (Eisai Co. Ltd., Tokyo, Japan) run under the same conditions. The toco-

pherols were quantified by peak area measurements. The areas were compared with the areas of known amounts of standards using 2,2,5,7,8-pentamethyl-6-hydroxychroman as internal standard. The results were expressed in μg per g tissue or diet.

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 and that of wild sweet smelt was designated by "+++++".

Results

Growth rate (GR), feed efficiency (FE) and survival rate of cultured sweet smelt are shown in Table 2. After rearing for 8 weeks, both groups showed similar levels of GR, but female fish were superior to male fish in GR and FE. CO group showed 96.3% of survival rate by 8 weeks, while HT group showed 79.9% by that time.

Table 3 shows proximate composition of the muscle and gonad in the cultured fish after rearing for 8 weeks. In the muscle, the content of protein (15.5~16.8%) and ash (1.05~1.21%) was almost no difference between both groups and in sex. However, lipid content was slightly high level in CO group (8.64~10.60%) compared with HT group (7.95~8.79%) and was high level in female fish compared with male fish in both groups. Moisture content of muscle was contrast to lipid content in both groups. On the other hand, moisture content of gonad was similar level, accounting for 69.2~70.2 %, and showed low level compared with that of muscle in both groups. Testes contained high level of protein (21.7~23.6% vs. 14.9~17.3% in ovaries) and ash (3.50~3.67% vs. 1.31~1.74% in ovaries) but low level of lipid (3.68~3.84% vs. 6.39~8.21% in ovaries) compared with ovaries. The testis of CO group contained high level of lipid (8.21%) compared with HT group (6.39%).

The fatty acid composition of the muscle and diet of the cultured fish were shown in Table 4. The prominent fatty acids of both the muscle and diet were 16:0, 18:1n-9, 16:1n-7, 18:2n-6, 22:6n-3, 14:0, 20:5n-3, 18:0 and 18:1n-7. The proportion of monounsaturated fatty acids (MUFA) in muscle showed a similar level (37.9~38.2%) in both groups, while that of polyunsaturated fatty acids (PUFA) was slightly higher level in HT group (27.7~27.9% vs. 24.2~24.9% in CO group) and in male fish compared with female fish. These PUFA proportion was contrast to that of saturated fatty acids (SFA) in both groups. On the other hand, the prominent fatty acids of gonads were similar to those of the muscle, but remarkably different in proportions (Table 5). The SFA proportion was 28.8~32.1%, slightly high level in ovaries of both groups. PUFA was the highest fatty acid group, accounting for 50.9~54.3% in testes and 35.1~37.8% in ovaries due to high level of 22:6n-3, 20:5n-3, 22:5n-3 and 20:4n-6. The PUFA proportion was contrast to MUFA proportion in both groups. Testis of CO group contained higher level of PUFA and lower level of MUFA than HT group, respectively. In both groups, testes were rich in 22:6n-3 (30.6~33.6% vs. 17.1~19.5% in ovaries), 20:5n-3 (7.54~8.25% vs. 4.32~4.47% in ovaries), 22:5n-3 (3.50~3.78% vs. 2.16~2.18% in ovaries) and 20:4n-6 (2.53~2.87% vs. 1.16~1.41% in ovaries), while ovaries were rich in 18:2n-6 (5.99~6.39% vs. 2.94~3.64% in testes) and 18:1n-9 (18.3~19.6% vs. 9.82~11.3% in testes). These fatty acid compositions of muscle were closely related with those of diet. However, incorporation of dietary fatty acids to muscle was more effective in 22:6n-3 than

Table 2. Growth, feed efficiency and survival rate of sweet smelt fed different diets for 8 weeks

	Initial body weight (g)		Final body weight (g)		Feed efficiency (%)		Surval rate (%)
	Male	Female	Male	Female	Male	Female	
CO group[1]	35.0±5.86	37.4±7.14	50.7±10.3	66.3±11.1	29.1	58.2	96.3
HT group[2]	35.0±5.86	37.4±7.14	51.1±12.2	63.8±13.3	33.2	59.2	79.9

[1] Diet of CO group is a control group and contains 0.01% of alpha-tocopherol.
[2] Diet of HT group contains 1.00% of alpha-tocopherol.

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	72.2±0.08	16.5±0.18	8.64±0.12	1.13±0.05
	Female	69.4±0.18	16.7±0.01	10.60±0.24	1.21±0.07
HT group[2]	Male	73.2±0.03	16.8±0.05	7.95±0.06	1.05±0.09
	Female	72.0±0.08	15.5±0.45	8.79±0.31	1.07±0.07
Gonad					
CO group	Testis	70.2±0.07	21.7±0.04	3.68±0.15	3.50±0.02
	Ovary	69.3±0.21	17.3±0.21	8.21±0.11	1.74±0.10
HT group	Testis	69.3±0.30	23.6±0.12	3.84±0.15	3.67±0.17
	Ovary	69.2±0.07	14.9±0.44	6.39±0.69	1.31±0.03

[1]Diet of CO group is a control group and contains 0.01% of alpha-tocopherol.
[2]Diet of HT group contains 1.00% of alpha-tocopherol.

20:5n-3, because despite the similar amount of the both fatty acids (12.1~13.2%) the latter presented in muscle a half as much as the former. Difference of PUFA proportion in both groups was dependant upon 22:6n-3, 20:5n-3 and 22:5n-3.

Hematocrit (Ht) value and plasma components are shown in Table 6. Ht value and plasma triglyceride (TG) and total cholesterol (CHOL) levels were higher in CO group than HT group. However, other plasma components showed similar level in both groups. SOD activity of plasma and phagocytic rate showed similar levels in both groups (Table 7). As shown in Table 8, TBARS and OH radical in plasma were higher levels in CO group (26.5 MDA μg/mL plasma and 7.89 MDA nmol/mg protein, respectively) than HT group (22.3 MDA μg/mL plasma and 4.65 MDA nmol/mg protein, respectively). The α-Toc content of the fish muscle and gonad was closely related to their dietary α-Toc content. Male muscles contained higher level of α-Toc than female muscles in both groups, but in gonad α-Toc content was higher level in ovaries than testes. On the other hand, CO group possessed much strong characteristic aroma, watermelon-like or cucumber-like aroma, and the intensity of the aroma marked "+++" for CO group and "++" for HT group, when compared with that of wild sweet smelt (+++++).

Discussion

In the present study, a main finding was that an excess supplementation of α-Toc to diet of sweet smelt was undesirable in the growth and quality of the cultured fish, in particular, the characteristic aroma such as watermelon-like and cucumber-like aroma. The watermelon-like or cucumber-like aroma compounds have been well known as nine-carbon aldehydes and alcohols such as (*E*)-2-nonenal, (*E*, *Z*)-2,6-nonadienal and 3,6-nonadien-1-ol, of which were found in aromatic fish including sweet smelt (Fross et al., 1962; Hirano et al., 1992; Zhang et al., 1992). These aroma compounds are the breakdown products of certain PUFA hydroperoxides oxidized by fish lipoxygenase. In general, the characteristic aroma of sweet smelt is much stronger 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 previous paper, Jeong et al. (2000) reported that the wild fish muscle contained high levels of alpha-linolenic acid (ALA) and eicosapentaenoic acid (EPA) compared with the cultured fish. Therefore, this suggests that ALA and EPA might be potential precursors of the characteristic aroma in the wild fish. However, in the present study, the proportion of n-3 PUFA was slightly lower in CO group than HT group. On the other hand, gonad of CO group contained slightly high level of n-3 PUFA including 20:5n-3 compared with HT group. Such a close difference might be resulted from similar dietary fatty acid compositions.

Despite a close difference in n-3 PUFA, the fish of CO group was occurred remarkably a strong aroma compared with HT group. This might be due to the fact in which precursors of some aroma compounds such as n-3 PUFA were oxidized more in CO group by the fish lipoxygenase and resulted in high level of TBARS in plasma as described in later. In the present study, CO group contained high level of plasma TBARS and OH radical, the strongest lipid peroxidation initiator, compared with HT group. This indicates that α-Toc in the diet plays an antioxidant role in the fish tissues, as described by Yamauchi et al. (1980). The levels of the lipid peroxides were decreased with increasing of α-Toc content in their diet, but the intensity of

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

Fatty acids	CO group[1]			HT group[2]		
	Male	Female	Diet	Male	Female	Diet
14:0	4.08 ± 0.10	4.15 ± 0.04	2.88 ± 0.07	3.83 ± 0.05	4.02 ± 0.05	2.82 ± 0.00
14:1n-7	0.12 ± 0.01	0.12 ± 0.00	ND3	0.10 ± 0.01	0.09 ± 0.00	ND
15:0 iso	0.08 ± 0.00	0.08 ± 0.00	0.12 ± 0.00	0.08 ± 0.00	0.09 ± 0.00	0.10 ± 0.00
15:0 anteiso	0.02 ± 0.00	0.02 ± 0.00	ND	0.02 ± 0.00	0.02 ± 0.00	ND
15:0	0.23 ± 0.00	0.25 ± 0.00	0.28 ± 0.00	0.25 ± 0.00	0.27 ± 0.00	0.29 ± 0.01
16:0 iso	0.04 ± 0.00	0.04 ± 0.00	0.05 ± 0.00	0.04 ± 0.00	0.04 ± 0.00	0.05 ± 0.00
Pristanic	0.07 ± 0.03	0.04 ± 0.01	0.09 ± 0.01	0.05 ± 0.00	0.03 ± 0.02	0.10 ± 0.01
16:0	28.4 ± 0.25	28.9 ± 0.08	18.7 ± 0.31	25.2 ± 0.15	25.3 ± 0.41	18.6 ± 0.18
16:1n-13	0.35 ± 0.02	0.38 ± 0.01	ND	0.27 ± 0.01	0.34 ± 0.01	ND
16:1n-7(+9)	9.72 ± 0.10	9.41 ± 0.04	3.84 ± 0.12	7.54 ± 0.09	7.71 ± 0.13	3.57 ± 0.04
16:1n-5	0.13 ± 0.03	0.13 ± 0.01	0.17 ± 0.00	0.13 ± 0.00	0.12 ± 0.01	0.16 ± 0.00
17:0 iso	0.13 ± 0.02	0.15 ± 0.00	0.13 ± 0.01	0.15 ± 0.00	0.18 ± 0.01	0.14 ± 0.01
17:0 anteiso	0.04 ± 0.00	0.06 ± 0.00	0.05 ± 0.00	0.06 ± 0.00	0.06 ± 0.01	0.05 ± 0.01
16:2n-4	0.17 ± 0.01	0.18 ± 0.01	0.24 ± 0.02	0.21 ± 0.01	0.21 ± 0.00	0.23 ± 0.01
phytanic	0.16 ± 0.02	0.16 ± 0.01	0.23 ± 0.03	0.19 ± 0.00	0.17 ± 0.02	0.24 ± 0.00
17:0	0.19 ± 0.03	0.23 ± 0.01	1.06 ± 0.06	0.24 ± 0.07	0.30 ± 0.00	0.99 ± 0.01
17:1n-10	0.08 ± 0.00	0.08 ± 0.01	0.11 ± 0.01	0.09 ± 0.00	0.09 ± 0.00	0.14 ± 0.01
17:1n-8	0.19 ± 0.01	0.24 ± 0.03	0.22 ± 0.02	0.21 ± 0.00	0.25 ± 0.01	0.20 ± 0.01
17:2n-8	0.02 ± 0.00	0.09 ± 0.09	0.06 ± 0.05	0.07 ± 0.07	0.09 ± 0.09	0.10 ± 0.10
16:4n-3	0.11 ± 0.01	0.12 ± 0.01	0.27 ± 0.01	0.15 ± 0.01	0.12 ± 0.04	0.28 ± 0.00
18:0	3.31 ± 0.09	3.29 ± 0.04	3.21 ± 0.03	3.56 ± 0.02	3.44 ± 0.07	3.26 ± 0.00
18:1n-9	19.8 ± 0.18	20.5 ± 0.00	14.9 ± 0.44	18.9 ± 0.04	20.0 ± 0.44	14.3 ± 0.03
18:1n-7	3.06 ± 0.12	3.09 ± 0.03	4.78 ± 0.06	3.48 ± 0.02	2.04 ± 2.34	4.38 ± 0.06
18:1n-5	0.25 ± 0.04	0.19 ± 0.01	0.29 ± 0.03	0.24 ± 0.02	0.26 ± 0.02	0.26 ± 0.00
18:2n-9	0.23 ± 0.05	0.18 ± 0.02	ND	0.14 ± 0.02	0.20 ± 0.01	ND
18:2n-6	7.47 ± 0.04	7.32 ± 0.01	8.87 ± 0.17	7.41 ± 0.00	7.28 ± 0.23	9.89 ± 0.03
18:2n-4	0.15 ± 0.02	0.10 ± 0.04	0.13 ± 0.04	0.14 ± 0.01	0.14 ± 0.02	0.11 ± 0.01
19:0	0.15 ± 0.03	0.12 ± 0.00	0.08 ± 0.01	0.11 ± 0.00	0.11 ± 0.01	0.09 ± 0.00
18:3n-4	0.13 ± 0.01	0.12 ± 0.01	0.12 ± 0.01	0.13 ± 0.00	0.15 ± 0.03	0.11 ± 0.01
18:3n-3	0.84 ± 0.01	0.82 ± 0.01	1.06 ± 0.02	0.84 ± 0.00	0.89 ± 0.04	1.19 ± 0.00
18:4n-3	0.48 ± 0.00	0.46 ± 0.00	1.45 ± 0.01	0.48 ± 0.00	0.48 ± 0.02	1.44 ± 0.00
18:4n-1	0.09 ± 0.01	0.08 ± 0.00	0.11 ± 0.00	0.11 ± 0.00	0.11 ± 0.00	0.10 ± 0.00
20:0	0.13 ± 0.00	0.12 ± 0.00	0.09 ± 0.01	0.15 ± 0.00	0.13 ± 0.00	0.11 ± 0.00
20:1n-11	1.17 ± 0.01	1.13 ± 0.02	2.43 ± 0.03	2.57 ± 0.03	2.39 ± 0.11	2.07 ± 0.02
20:1n-9	1.35 ± 0.00	1.32 ± 0.02	2.21 ± 0.23	1.68 ± 0.05	1.68 ± 0.04	2.11 ± 0.01
20:1n-7	0.10 ± 0.01	0.10 ± 0.01	0.20 ± 0.06	0.15 ± 0.01	0.13 ± 0.00	0.18 ± 0.02
20:2n-9	0.14 ± 0.00	0.16 ± 0.00	ND	0.07 ± 0.05	0.17 ± 0.01	ND
20:2n-6	0.24 ± 0.01	0.26 ± 0.00	0.17 ± 0.01	0.25 ± 0.01	0.29 ± 0.00	0.18 ± 0.01
20:3n-6	0.17 ± 0.00	0.19 ± 0.00	0.04 ± 0.02	0.16 ± 0.00	0.19 ± 0.00	0.07 ± 0.00
20:4n-6	0.47 ± 0.00	0.45 ± 0.01	0.92 ± 0.04	0.59 ± 0.02	0.58 ± 0.03	0.94 ± 0.01
20:3n-3	0.06 ± 0.00	0.06 ± 0.00	0.10 ± 0.00	0.11 ± 0.01	0.10 ± 0.01	0.10 ± 0.02
20:4n-3	0.50 ± 0.01	0.51 ± 0.00	0.41 ± 0.02	0.59 ± 0.01	0.57 ± 0.02	0.42 ± 0.00
20:5n-3	3.88 ± 0.03	3.60 ± 0.02	12.1 ± 0.54	4.83 ± 0.00	4.55 ± 0.30	12.4 ± 0.06
22:0	0.04 ± 0.03	0.03 ± 0.01	ND	0.05 ± 0.00	0.04 ± 0.02	ND
22:1n-11	1.16 ± 0.01	1.06 ± 0.01	3.36 ± 0.17	2.17 ± 0.25	2.21 ± 0.05	3.04 ± 0.04
22:1n-9	0.18 ± 0.04	0.16 ± 0.01	0.06 ± 0.05	0.16 ± 0.21	0.02 ± 0.00	0.02 ± 0.00
22:1n-7	0.04 ± 0.02	0.02 ± 0.00	0.21 ± 0.18	0.07 ± 0.01	0.07 ± 0.01	0.04 ± 0.01
21:5n-3	0.19 ± 0.02	0.18 ± 0.00	0.21 ± 0.15	0.23 ± 0.00	0.24 ± 0.01	0.32 ± 0.02
22:4n-6	0.09 ± 0.01	0.10 ± 0.01	ND	0.10 ± 0.00	0.11 ± 0.00	ND
22:5n-6	0.11 ± 0.01	0.10 ± 0.00	0.22 ± 0.15	0.14 ± 0.00	0.14 ± 0.00	0.16 ± 0.00
22:5n-3	1.61 ± 0.04	1.55 ± 0.02	0.88 ± 0.04	1.99 ± 0.01	1.99 ± 0.05	0.95 ± 0.00
24:0	0.02 ± 0.00	0.02 ± 0.00		0.02 ± 0.00	0.02 ± 0.00	
22:6n-3	7.72 ± 0.16	7.57 ± 0.07	12.3 ± 0.78	9.02 ± 0.07	9.27 ± 0.19	13.2 ± 0.12
24:1n-9	0.34 ± 0.01	0.32 ± 0.00	0.55 ± 0.01	0.43 ± 0.01	0.44 ± 0.01	0.53 ± 0.02
24:1n-7	0.02 ± 0.00	0.02 ± 0.00	ND	0.02 ± 0.00	0.02 ± 0.00	ND
Saturates	37.1	37.6	26.9	34.0	34.2	26.8
Monoenes	38.0	38.2	33.4	38.2	37.9	31.0
Polyenes	24.9	24.2	39.7	27.7	27.9	42.2

[1]Diet of CO group is a control group and contains 0.01% of alpha-tocopherol.
[2]Diet of HT group contains 1.00% of alpha-tocopherol.
[3]ND, not detected.

Table 5. Fatty acid composition of total lipid in the gonad of sweet smelt fed different diets for 8 weeks (wt %)

Fatty acids	CO group[1]		HT group[2]	
	Testis	Ovary	Testis	Ovary
14:0	1.04±0.01	3.14±0.01	1.34±0.01	2.85±0.02
15:0 iso	0.03±0.00	0.08±0.00	0.04±0.00	0.07±0.00
15:0	0.14±0.00	0.24±0.00	0.15±0.01	0.27±0.02
16:0 iso	0.03±0.00	0.04±0.01	0.03±0.01	0.05±0.01
pristanic	0.17±0.00	0.06±0.00	0.16±0.00	0.08±0.00
16:0	23.8±0.33	25.3±0.03	24.2±0.62	24.7±0.31
16:1n-13	0.39±0.02	0.46±0.01	0.37±0.01	0.51±0.02
16:1n-9	0.42±0.02	0.01±0.00	0.40±0.01	0.79±0.05
16:1n-7	1.88±0.04	7.50±0.03	2.78±0.12	5.72±0.14
16:1n-5	0.13±0.09	0.12±0.00	0.08±0.01	0.14±0.01
17:0 iso	0.12±0.03	0.17±0.01	0.10±0.02	0.22±0.01
17:0 anteiso	0.02±0.00	0.04±0.00	0.09±0.01	0.05±0.00
16:2n-4	0.28±0.01	0.27±0.02	0.29±0.00	0.32±0.00
17:0	0.23±0.01	0.25±0.01	0.22±0.01	0.28±0.01
17:1n-8	0.10±0.00	0.25±0.01	0.12±0.01	0.27±0.01
16:4n-3	0.09±0.00	0.01±0.00	0.09±0.00	0.02±0.00
18:0	3.14±0.05	2.57±0.01	3.12±0.01	2.56±0.00
18:1n-9	9.82±0.34	19.6±0.07	11.3±0.21	18.3±0.09
18:1n-7	2.98±0.10	2.94±0.06	2.86±0.10	3.35±0.01
18:1n-5	0.15±0.02	0.23±0.00	0.15±0.02	0.26±0.02
18:2n-9	0.07±0.03	0.20±0.01	0.10±0.02	0.20±0.02
18:2n-6	2.94±0.18	6.39±0.01	3.64±0.09	5.99±0.02
18:2n-4	0.08±0.02	0.14±0.00	0.10±0.02	0.15±0.03
19:0	0.04±0.03	0.14±0.01	0.09±0.01	0.14±0.01
18:3n-4	0.08±0.02	0.13±0.02	0.09±0.00	0.13±0.01
18:3n-3	0.24±0.09	0.69±0.03	0.27±0.01	0.69±0.05
18:4n-3	0.07±0.01	0.34±0.01	0.11±0.00	0.33±0.00
18:4n-1	0.02±0.00	0.09±0.01	0.03±0.01	0.12±0.00
20:0	0.02±0.00	0.07±0.00	0.03±0.02	0.04±0.02
20:1n-9(+11)	0.49±0.00	0.90±0.00	0.63±0.07	0.95±0.05
20:1n-7	0.05±0.00	0.08±0.00	0.07±0.03	0.10±0.00
20:2n-9	0.10±0.00	0.16±0.00	0.11±0.00	0.19±0.01
20:2n-6	0.23±0.11	0.36±0.01	0.33±0.00	0.43±0.00
20:3n-6	0.53±0.01	0.34±0.00	0.55±0.02	0.43±0.01
20:4n-6	2.87±0.04	1.16±0.00	2.53±0.03	1.41±0.03
20:3n-3	0.05±0.04	0.08±0.01	0.04±0.02	0.08±0.00
20:4n-3	0.23±0.01	0.51±0.02	0.26±0.00	0.51±0.02
20:5n-3	8.25±0.09	4.32±0.00	7.54±0.07	4.47±0.06
22:1n-11	0.23±0.01	0.51±0.00	0.42±0.01	0.29±0.00
21:5n-3	0.08±0.01	0.22±0.00	0.10±0.01	0.24±0.01
22:4n-6	0.33±0.03	0.21±0.01	0.30±0.02	0.23±0.02
22:5n-6	0.39±0.01	0.20±0.01	0.35±0.01	0.24±0.00
22:5n-3	3.78±0.11	2.18±0.01	3.50±0.08	2.16±0.03
22:6n-3	33.6±1.07	17.1±0.06	30.6±0.97	19.5±0.40
24:1n-9	0.26±0.06	0.22±0.00	0.28±0.00	0.21±0.05
Saturates	28.8	32.1	29.6	31.3
Monoenes	16.9	32.8	19.5	30.9
Polyenes	54.3	35.1	50.9	37.8

[1] Diet of CO group is a control group and contains 0.01% of alpha-tocopherol.
[2] Diet of HT group contains 1.00% of alpha-tocopherol.

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

Parameters	CO group[1]	HT group[2]
Hematocrit value (%)	54.5±10.0	46.8±7.70
Total proten (g/100 mL)	4.3±0.4	4.1±0.7
Glucose (mg/100 mL)	53±32	55±13
Urea nitrogen (mg/100 mL)	2.5±0.7	2.7±0.9
Total cholesterol (mg/100 mL)	838±197	599±139
Triglyceride (mg/100 mL)	436±179	355±162

[1] Diet of CO group is a control group and contains 0.01% of alpha-tocopherol.
[2] Diet of HT group contains 1.00% of alpha-tocopherol.

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

Parameters	CO group[1]	HT group[2]
Phagocytic rate (%)	31.6±8.11	31.6±5.77
SOD activity (NU/mg protein)	3.78±1.11	3.96±1.33

[1] Diet of CO group is a control group and contains 0.01% of alpha-tocopherol.
[2] Diet of HT group contains 1.00% of alpha-tocopherol.

Table 8. TBARS and OH radical level of plasma, alpha-tocopherol content of tissue and intensity of aroma in sweet smelt fed different diets for 8 weeks

Parameters	CO group[1]	HT group[2]
TBARS (MDA μg/mL plasma)	26.5±7.20	22.3±8.40
OH radical (MDA nmol/mg protein)	7.89±2.84	4.65±2.25
Alpha-tocopherol (μg/g tissue)		
Male muscle	68.9±2.69	577±0.35
Female muscle	50.9±1.48	500±3.89
Testis	82.9±0.28	467±7.07
Ovary	178±2.83	561±3.54
Intensity of aroma	+++	++

[1] Diet of CO group is a control group and contains 0.01% of alpha-tocopherol.
[2] Diet of HT group contains 1.00% of alpha-tocopherol.

aroma was strong with increasing of lipid peroxide level in plasma. Therefore, these results indicate that the supplementation of an excess of α-Toc to diet of the cultured fish is undesirable on the development of aroma. On the other hand, the levels of

TG and CHOL in the fish plasma were significantly high in CO group compared with HT group. Furthermore, survival rate of the cultured fish was increased with increasing of the plasma lipid contents. Therefore, the levels of plasma TG and CHOL may be used as indices of the cultured fish health. Yellowtail and rainbow trout with the low level of plasma CHOL are reduced in 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 HT group was seemed to be received almost no effect of malnutrition because of similar level of TG or CHOL and FE in both groups. 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).

It has known that α-Toc can develop both antioxidant and prooxidant activities *in vitro* in isolated low density lipoprotein (LDL) (Kontush et al., 1996), depending on oxidative conditions and presence of co-antioxidants and *in vivo* (Robert et al., 1987). On the other hand, it has been known that a large amount of α-tocopheroxyl radical generated from the result of antioxidation of α-Toc participates as a prooxidant in human LDL (Browry et al., 1992; Browry and Stocke, 1993) and in yellowtail injected with the causative bacteria of fish jaundice (Ito et al., 1999), of which mortality resulted in 40 times as much as yellowtail without injection of the fish bacteria. However, in the present study, an excess of α-Toc in HT group was obscured whether to be participated as prooxidant or not, though mortality was higher in HT group than CO group. Furthermore, plasma SOD activity, a scavenger of free radicals, was slightly high level in CO group and phagocytic rate, a non-specific immune parameter, had almost no difference between both groups. Therefore these biological defense systems might be acted similarly in both groups. Consequently, the supplementation of an excess of α-Toc in diet was undesirable in growth and quality, in particular, the aroma, in the cultured fish.

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