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Effect of Dietary Oxidized Squid Liver oil and DL-α-Tocopherol Level on Growth and Body Composition of Juvenile Flounder (*Paralichthys olivaceus*)

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This study was conducted to investigate the effect of dietary oxidized oil and α-tocopherol level on growth and body composition of juvenile flounder. To prepare oxidized diets, squid liver oil was oxidized by aeration at 25°C for 30 days. The six diets were prepared to contain 6% fresh or oxidized squid liver oil as the lipid sources in combination with three levels of α-tocopheryl acetate at 0, 80 and 800 mg/kg diet. Triplicate groups of fish (3.9±0.1 g) were fed to apparent satiation twice a day for 8 weeks. Survival was not significantly different among treatments. Weight gain, feed efficiency, daily feed intake, protein efficiency ratio and condition factor of fish fed the fresh oil diets were significantly higher than those of fish fed the oxidized oil diets ($P<0.05$). The increase of the vitamin E level in diets did not result in any significant improvement on growth performance of fish fed both oil diets. The vitamin E content of the liver and dorsal muscle increased with increasing dietary vitamin E level at both oil diet groups. A decreasing trend in vitamin E content of the tissues was observed in fish fed the oxidized oil diets at the same dietary vitamin E level. Significantly higher moisture content and lower crude lipid content were observed in the whole body of fish fed the oxidized oil diets than fish fed the fresh oil diets ($P<0.05$). Dietary lipid source affected the fatty acid content of the whole body; higher contents of saturated and monoenoic fatty acids, and lower n-3 HUFA contents such as 20:5n-3 and 22:6n-3 were observed in fish fed the oxidized oil diets than those of fish fed fresh oil diets. The results of this study suggest that the dietary oxidized oil may impair the growth performance, and an increase in α-tocopheryl acetate supplementation have no beneficial effect on growth and feed efficiency of juvenile flounder.

Keywords: Flounder, Oxidized oil, α-Tocopherol, Growth

Introduction

Most marine fish being farmed require n-3 highly unsaturated fatty acids (HUFA) such as eicosapentaenoic acid and docosahexaenoic acid in their diet to prevent deficiency in essential fatty acids. Fish oils containing high proportions of n-3 HUFA are commonly used as a component of fish diets for this reason. The HUFA, which are vital constituents for cell membrane structure and function, are easily oxidized by oxygen and other organic radicals. Resultant damage to HUFA in membrane phospholipids can have damaging consequences for cell membrane structure and fluidity, with potential pathological effects on cells and tissues (Sies, 1991). Most nutritional problems attributed to fish oils are related to their oxidations. Fish oils and their oil fatty acids are not toxic of themselves, but oxidized oils cause various symptoms in unprotected cultured fish (Hung et al., 1981; Moccia et al., 1984; Murai and Andrews, 1974).

Vitamin E is widely regarded as the primary lipid-soluble antioxidant. The antioxidant activities of tocopherols are imparted by their ability to donate their phenolic hydrogen atoms to lipid free radicals, resulting in the stabilization of lipid free radical and the termination of the lipid peroxidation chain reaction (Burton and Ingold, 1989). Vitamin E is commonly found in several natural formations such as α-tocopherol having the highest vitamin E activity. DL-α-tocopherol acetate, a stable form of α-tocopherol, is the most commonly used vitamin E supplement in fish feeds (NRC, 1993). Therefore, this study was conducted to investigate the effect of dietary oxidized oil with different α-tocopherol levels on the growth and body composition of juvenile flounder.

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Materials and Methods

Experimental diets

Vitamin free casein (USB corporation, Cleveland, OH, USA) and white fish meal (defatted by mixture of chloroform and methanol) as the protein sources were used for the basal diet. To prepare oxidized diets, squid liver oil was oxidized by aeration at 25°C for 30 days, and peroxide values for fresh and oxidized oils were 13 and 922 milliequivalent peroxide/kg, respectively. Six diets were prepared to contain 6% fresh (FR) or oxidized squid liver oil (OX) as the lipid sources in combination with three levels of vitamin E as DL-α-tocopheryl acetate at 0, 80 and 800 mg/kg diet (Table 1). The experimental diets were made using a laboratory pellet machine after 30~40 g of water was mixed with 100 g mixture of ingredients, and dried at room temperature overnight. Diets were stored at –30°C until use. The proximate composition, vitamin E level and fatty acids composition of the experimental diets are presented in Table 2. The oxidized oil diets had higher contents of saturated and monoenoic fatty acids, and lower polyunsaturated fatty acids content compared to the fresh oil diets.

Table 1. Composition of the basal diet

Ingredients	%
Vitamin free casein[1]	17.0
White fish meal[2]	40.0
Dextrin[1]	23.0
Lipid sources[3]	6.0
Vitamin E[4]	
α-Cellulose[5]	5.0
Mineral premix[6]	2.0
Vitamin premix (Vitamin E free)[7]	2.0
Carboxymethyl cellulose[5]	4.8
Choline chloride[5]	0.2

[1]United States Biochemical, Cleveland, Ohio, USA.
[2]Defatted with chloroform-methanol mixture (2:1, v/v).
[3]Fresh squid liver oil and oxidized squid liver oil represented as FR and OX, respectively, in other tables.
[4]Vitamin E is supplemented as DL-α-tocopheryl acetate, purity 25%: 0, 80 and 800 mg kg^{-1} diet, indicated as VE0, VE80 and VE800 in the other tables.
[5]Sigma, USA.
[6]Mineral premix contained the following ingredients (g/kg premix): $MgSO_4 \cdot 7H_2O$, 80.0; $NaH_2PO_4 \cdot 2H_2O$, 370.0; KCl, 130.0; Ferric citrate, 40.0; $ZnSO_4 \cdot 7H_2O$, 20.0; Ca-lactate, 356.5; CuCl, 0.2; $AlCl_3 \cdot 6H_2O$, 0.15; KI, 0.15; $Na_2Se_2O_3$, 0.01; $MnSO_4 \cdot H_2O$, 2.0; $CoCl_2 \cdot 6H_2O$, 1.0.
[7]Vitamin premix contained the following amount which were diluted in cellulose (g/kg premix): L-ascorbic acid, 121.2; thiamin hydrochloride, 2.7; riboflavin, 9.1; pyridoxine hydrochloride, 1.8; niacin, 36.4; Ca-D-pantothenate, 12.7; myo-inositol, 181.8; D-biotin, 0.27; folic acid, 0.68; p-aminobenzoic acid, 18.2; menadione, 1.8; retinyl acetate, 0.73; cholecalciferol, 0.003; cyanocobalamin, 0.003.

Fish and feeding trial

Juvenile flounder (*Paralichthys olivaceus*) were purchased from a local fish farm (Gyeongbuk, Korea). They were acclimated to laboratory conditions for 2 weeks before feeding trial. Juveniles (3.9±0.1 g) were randomly allocated in eighteen 300 L cylindrical plastic tanks (180 L water volume) with 30 fish each tank in a flow–through aquarium system. Triplicate groups of fish were hand–fed to apparent satiation twice a day (0900 and 1700 h, 6 days per week) for 8 weeks. Filtrated seawater was supplied at a flow rate of 5 L/min in each tank, and the mean water temperature was 20.1±1.5°C. Photoperiod was left at natural conditions during the feeding trail. All fish in each tank were collectively weighed at the beginning and the end of feeding trial after being fasted for 24 h. Records were kept for daily feed consumption, mortalities and feeding behavior.

Sample collection and chemical analysis

Thirty fish at the beginning and all fish in each tank at the end of the feeding trial were killed and stored at –75°C for chemical analysis. The ground and homogenized mixture of 5 fish/tank were analyzed for proximate composition and fatty acids composition. The livers and dorsal muscles of remaining fish in each tank were removed and pooled for vitamin E analysis. Crude protein was determined by the Kjeldahl method using an Auto Kjeldahl system (VAP50OT/TT125, Germany). Crude lipid was determined with ether extraction in a soxhlet extractor. Moisture was determined by drying in an oven (105°C for 6 h), and ash was determined by using a muffle furnace (550°C for 4 h). Peroxide value was determined by AOAC (1990) method. Lipid for fatty acids analyses was extracted by mixture of chloroform and methanol (2:1, v/v) according to the method of Folch et al. (1957). Fatty acid methyl esters were prepared by transesterification with 14% BF_3-MeOH (Sigma, St. Louis, USA), and analyzed by using a gas chromatograph (HP-5890 II; Hewlett-Packard, Palo Alto, CA, USA) with a flame ionization detector equipped with HP-INNOWax capillary column (30 m×0.32 mm i.d., film thickness 0.5 μm, Hewlett-Packard, USA). Injector and detector temperatures were 250 and 270°C, respectively. The column temperature was programmed from 170 to 225°C at a rate of 1°C/min. Helium was used as the carrier gas. Fatty acids were identified by comparison with known standards.

Vitamin E (α-tocopherol) content in diets, liver and dorsal

Table 2. Proximate composition, α-tocopherol level and major fatty acids composition of the experimental diets

	FR			OX		
	VE0	VE80	VE800	VE0	VE80	VE800
Proximate composition (%, DM)						
Crude protein	49.0	48.8	48.7	49.5	48.6	49.6
Crude lipid	5.7	5.6	5.8	5.6	5.6	5.6
Ash	8.6	8.5	8.6	8.6	8.7	8.5
Vitamin E level (mg/kg diet)						
α-tocopherol	14.7	89.0	830.1	1.8	82.4	775.6
Fatty acids composition (% of total fatty acids)						
14:0	4.7	5.0	5.2	8.0	7.9	7.8
16:0	16.9	17.2	16.8	24.1	24.1	23.9
16:1n-7	5.3	4.7	5.2	6.7	6.8	6.8
18:0	2.9	3.4	3.2	4.5	4.7	4.5
18:1n-9	17.5	18.1	17.9	23.1	23.5	24.1
18:2n-6	3.0	3.2	3.0	0.9	0.9	0.9
18:3n-3	2.6	1.0	1.0			
20:1n-9	9.3	8.7	8.7	13.0	12.6	12.6
20:4n-6	1.0	1.0	1.0			
20:5n-3	10.6	11.2	10.4	1.1	1.3	1.3
22:1n-9	8.2	7.9	8.4	13.6	12.8	13.0
22:5n-3	1.5	1.4	1.4			
22:6n-3	13.1	13.0	12.3	3.2	3.4	3.2
Saturates	25.1	28.3	28.2	37.1	37.4	36.9
Monoenes	40.6	39.6	40.5	56.9	56.0	56.9
n-3HUFA[1]	25.9	26.2	24.7	4.2	4.9	4.5

[1]Highly unsaturated fatty acid (C≥20).

muscle was analyzed by high-performance liquid chromatography. Samples of 0.5 g were dissolved in 5 ml ethanol with the addition of 0.1 g L-ascorbic acid. After well mixing, the samples were put into 80°C water bath for 10 minutes, and then 150 μl 80% KOH was added to saponify the samples for another 10 minutes. Saponified samples were cooled in ice bath, then 5 ml n-hexane was added and completely mixed with the samples. Homogenates were centrifuged at 900 rpm for 1 minute at 5°C, and supernatants were removed. The supernatants were washed three times by 5 ml water, and the final n-hexane solution was filtered through filter paper by adding Na_2SO_4. The n-hexane phase was dried under nitrogen, and reconstituted with 1 ml isooctane and filtered through a 0.20 μm membrane filter. The chromatographic separation was performed at room temperature on an Nova-Pak silica column (150×3.9 mm, 5 μm particle size, 60 Å nominal pore size). The mobile phase was a mixture of isooctane, acetic acid, ethyl acetate, and 2,2-Dimethoxypropane (985:7:7:1, v/v) at a flow rate of 1 ml/min. An aliquot of 20 μl of sample was injected. The fluorescence was measured at 290 nm excitation and 330 nm emission wavelengths. The quantification was made using external standard calibration.

Statistical analysis

The data was subjected to one and two-way analysis of variance (ANOVA) using the SPSS program version 11.5 for Windows. If significant differences ($P<0.05$) were found, Duncan's multiple range test (Duncan, 1955) was used to rank the groups.

Results

The growth performances of juvenile flounder fed the fresh or oxidized oil diets with three levels of vitamin E inclusion were presented in Table 3. Survival was significantly affected by dietary lipid ($P<0.05$), and survival of fish fed the fresh oil diets was slightly higher than that of fish fed the oxidized oil diets.

The weight gain, feed efficiency, protein efficiency ration, daily feed intake and condition factor of fish fed the oxidized

Table 3. Growth performance of juvenile flounder fed the experimental diets for 8 weeks[1]

	FR			OX		
	VE0	VE80	VE800	VE0	VE80	VE800
Initial weight (g/fish)	3.9±0.03	4.0±0.11	3.9±0.09	3.9±0.07	3.9±0.09	3.9±0.03
Survival (%)	93±1.9	96±2.2	94±1.1	73±13.8	80±13.3	80±6.4
Weight gain (g/fish)	20.2±0.59 ^{b}	20.4±1.04 ^{b}	19.0±0.65 ^{b}	9.0±0.43 ^{a}	8.6±1.09 ^{a}	9.1±0.11 ^{a}
Feed efficiency (%)[2]	106±3.3 ^{b}	100±3.2 ^{b}	99±3.8 ^{b}	77±5.5 ^{a}	81±2.2 ^{a}	75±4.3 ^{a}
Protein efficiency ratio[3]	2.20±0.07 ^{b}	2.06±0.07 ^{b}	2.03±0.08 ^{b}	1.54±0.11 ^{a}	1.66±0.05 ^{a}	1.50±0.09 ^{a}
Daily feed intake[4]	2.40±0.06 ^{b}	2.50±0.06 ^{b}	2.53±0.06 ^{b}	2.20±0.03 ^{a}	2.14±0.16 ^{a}	2.23±0.03 ^{a}
Hepatosomatic index[5]	1.45±0.03 ^{b}	1.37±0.10 ^{ab}	1.34±0.06 ^{ab}	1.29±0.05 ^{ab}	1.20±0.08 ^{ab}	1.19±0.07 ^{a}
Condition factor[6]	1.02±0.01 ^{b}	1.00±0.01 ^{b}	1.03±0.01 ^{b}	0.96±0.01 ^{a}	0.94±0.03 ^{a}	0.94±0.01 ^{a}

Two-way ANOVA

	Dietary lipid	Vitamin E	Interaction
Survival	$P<0.05$	$P<0.9$	$P<0.9$
Weight gain	$P<0.001$	$P<0.8$	$P<0.5$
Feed efficiency	$P<0.001$	$P<0.6$	$P<0.5$
Protein efficiency ratio	$P<0.001$	$P<0.5$	$P<0.4$
Daily feed intake	$P<0.001$	$P<0.6$	$P<0.7$
Hepatosomatic index	$P<0.05$	$P<0.4$	$P<0.9$
Condition factor	$P<0.001$	$P<0.7$	$P<0.7$

[1]Values (mean±SEM of three replications) in the same row not sharing a common superscript are significantly different (P<0.05).
[2]Fish wet weight gain×100/feed intake (dry matter).
[3]Fish wet weight gain×100/protein intake.
[4]Feed intake (dry matter)×100/[(initial fish wt.+final fish wt.+dead fish wt.)/2×days fed].
[5](Liver weight)×100/body weight.
[6](Body weight×100)/(Total body length)^{3}.

oil diets were significantly lower than those of fish fed the fresh oil diets (P<0.05). Hepatosomatic index of fish fed the fresh oil diet without vitamin E supplementation was significantly higher than that of fish fed the oxidized oil diet with 800 mg vitamin E supplementation (P<0.05), but not significantly different from that of fish fed the other diets. The increase of vitamin E level in diets did not result in any significant improvement on growth performance of fish fed both oil diets.

The vitamin E content of the liver and dorsal muscle, and proximate composition of the whole body of fish are shown in Table 4. The vitamin E content of the liver and dorsal muscle increased with increasing dietary vitamin E level at both oil diets groups. A decreasing trend in vitamin E content of the tissues was observed in fish fed the oxidized oil diets at the same dietary vitamin E level. Significantly higher moisture content and lower crude lipid content were observed in the whole body of fish fed the oxidized oil diets than those of fish fed the fresh oil diets (P<0.05). However, contents of crude protein and ash of the whole body were not significantly different among fish.

Fatty acids compositions of the whole body of fish are shown in Table 5. Significant effects of dietary lipid was observed for most fatty acids according to their relative values in the diets. The fish fed the oxidized oil diets showed higher contents of saturated fatty acids (16:0, 18:0) and monoenoic fatty acids (16:1n-7, 18:1n-9, 20:1n-9), but lower contents of 20:0, 20:4n-6, 20:5n-3, 22:5n-3 and 22:6n-3 than fish fed the fresh oil diets.

Discussion

No visible pathological signs were apparent in any group of fish fed oxidized oil diets in this study as reported in African catfish (Baker and Davies, 1996). However, channel catfish fed high level of oxidized menhaden oil showed gross syndromes such as muscular dystrophy and depigmentation (Murai and Andrews, 1974).

The inclusion of oxidized oil in diets affected on growth performance and body composition of juvenile flounder. In this study, the fish fed the oxidized oil diets showed lower weight gain, feed efficiency and daily feed intake compared to fish fed the fresh oil diets. Similarly, reduced growth and feed utilization, and loss of appetite had been reported for

Table 4. The vitamin E contents of liver and dorsal muscle and proximate composition of the whole body in juvenile flounder fed the experimental diets for 8 weeks[1]

	FR			OX		
	VE0	VE80	VE800	VE0	VE80	VE800
Vitamin E level (mg/kg)						
Liver	100±4.4^{a}	308±10.5^{a}	1908±41.2^{c}	85±1.6^{a}	122±2.0^{a}	1224±21.5^{b}
Dorsal muscle	4.6±0.15^{a}	16.5±0.06^{bc}	24.0±0.28^{c}	4.5±0.19^{a}	9.2±0.23^{ab}	20.2±0.48^{c}
Proximate composition (%)						
Moisture	76.4±0.37^{a}	76.4±0.42^{a}	76.9±0.05^{a}	78.1±0.47^{b}	78.9±0.15^{b}	78.1±0.52^{b}
Crude protein	17.2±0.44	17.2±0.17	17.1±0.24	16.1±0.56	16.2±0.41	16.2±0.06
Crude lipid	1.7±0.21^{b}	2.0±0.14^{b}	1.8±0.14^{b}	0.8±0.05^{a}	0.8±0.12^{a}	0.9±0.18^{a}
Ash	3.3±0.11	3.2±0.37	3.6±0.16	3.70±0.06	3.7±0.19	3.6±0.13

Two-way ANOVA	Dietary lipid	Vitamin E	Interaction
Vitamin E level			
Liver	$P<0.1$	$P<0.001$	$P<0.3$
Dorsal muscle	$P<0.2$	$P<0.001$	$P<0.5$
Proximate composition			
Moisture	$P<0.001$	$P<0.6$	$P<0.3$
Crude protein	$P<0.01$	$P<0.9$	$P<0.9$
Crude lipid	$P<0.001$	$P<0.6$	$P<0.8$
Ash	$P<0.1$	$P<0.8$	$P<0.5$

[1]Values (mean ±SEM of three replications) in the same row not sharing a common superscript are significantly different (P<0.05).

Table 5. Major fatty acids composition of the whole body in juvenile flounder fed the experimental diets for 8 weeks[1]

	FR			OX			Pooled SEM	Two-way ANOVA		
	VE0	VE80	VE800	VE0	VE80	VE800		Lipid	Vitamin E	Interaction
14:0	3.8	3.9	3.9	4.0	4.1	4.0	0.04	$P<0.2$	$P<0.6$	$P<0.8$
16:0	14.1^{c}	15.7^{bc}	16.0^{b}	17.8^{a}	18.5^{a}	17.2^{a}	0.39	$P<0.001$	$P<0.2$	$P<0.1$
16:1n-7	5.5^{c}	5.6^{c}	5.4^{c}	6.7^{b}	7.0^{b}	8.2^{a}	0.27	$P<0.001$	$P<0.2$	$P<0.1$
18:0	3.6^{c}	3.7^{c}	3.9^{c}	6.5^{a}	6.8^{a}	6.0^{b}	0.33	$P<0.001$	$P<0.2$	$P<0.05$
18:1n-9	20.1^{b}	20.2^{b}	20.5^{b}	31.0^{a}	31.1^{a}	31.3^{a}	1.32	$P<0.001$	$P<0.8$	$P<0.9$
18:2n-6	3.4^{b}	3.2^{b}	3.2^{b}	4.0^{a}	4.0^{a}	3.9^{a}	0.10	$P<0.001$	$P<0.7$	$P<0.8$
18:3n-3	1.1^{a}	0.9^{ab}	0.8^{abc}	0.1^{c}	0.1^{c}	0.3^{bc}	0.11	$P<0.01$	$P<0.9$	$P<0.6$
20:0	2.2^{a}	2.0^{ab}	1.8^{b}	0.1^{c}	0.2^{c}	0.2^{c}	0.22	$P<0.001$	$P<0.7$	$P<0.1$
20:1n-9	6.7^{bc}	6.2^{c}	6.5^{c}	7.6^{a}	7.4^{ab}	7.3^{ab}	0.16	$P<0.001$	$P<0.4$	$P<0.8$
20:2n-6	0.5^{a}	0.4^{ab}	0.5^{a}	0.3^{bc}	0.3^{bc}	0.2^{c}	0.03	$P<0.01$	$P<0.6$	$P<0.6$
20:3n-3	1.1	1.1	1.2	1.2	1.2	1.0	0.02	$P<0.6$	$P<0.9$	$P<0.3$
20:4n-6	1.2^{a}	1.1^{a}	1.1^{a}	0.3^{b}	0.3^{b}	0.3^{b}	0.10	$P<0.001$	$P<0.9$	$P<0.9$
20:5n-3	9.6^{a}	9.8^{a}	9.4^{a}	4.4^{b}	4.2^{b}	4.3^{b}	0.60	$P<0.001$	$P<0.5$	$P<0.4$
22:1n-9	4.3^{a}	4.1^{a}	3.9^{ab}	3.4^{ab}	3.2^{b}	3.5^{ab}	0.13	$P<0.01$	$P<0.7$	$P<0.7$
22:5n-3	3.3^{b}	3.3^{b}	3.6^{a}	1.3^{c}	1.3^{c}	1.3^{c}	0.25	$P<0.001$	$P<0.2$	$P<0.1$
22:6n-3	16.3^{a}	17.0^{a}	16.5^{a}	9.6^{b}	9.1^{b}	9.1^{b}	0.90	$P<0.001$	$P<0.9$	$P<0.4$
Saturates	25.5^{a}	26.5^{ab}	26.5^{ab}	29.1^{cd}	30.1^{d}	28.2^{bc}	0.43	$P<0.001$	$P<0.2$	$P<0.3$
Monoenes	37.1^{a}	36.2^{a}	36.4^{a}	49.4^{b}	49.2^{b}	50.9^{b}	1.64	$P<0.001$	$P<0.6$	$P<0.5$
n-3HUFA[2]	30.2^{b}	31.3^{b}	30.7^{b}	16.5^{a}	15.8^{a}	15.6^{a}	1.80	$P<0.001$	$P<0.9$	$P<0.3$

[1]Values (mean of three replications) in the same row not sharing a common superscript are significantly different (P<0.05).
[2]Highly unsaturated fatty acid (C≥20).

other species of fish fed the oxidized oil diets (Murai and Andrews, 1974; Baker and Davies, 1996; Ketola et al., 1989). It is possible that palatability was an influencing factor governing feed intake and thus growth, since the diets containing oxi-

dized oils possessed strong rancid aromas. Another possible reason for poor growth and feed utilization of fish fed the oxidized oil diets could be related to decreased dietary n-3 HUFA content such as EPA or DHA. In this study, oxidized oil diets contained about 0.3% n-3 HUFA. However, Kim and Lee (2004) reported that the requirement of dietary n-3 HUFA for juvenile flounder is approximately 0.8~1.0%, and flounder fed the diets with low level of n-3HUFA showed reduced weight gain and feed efficiency. Also, the presence of oxidized lipid in diet can have toxic consequences for cultured fish, whether they arise from direct dietary input or via deficiencies in essential antioxidant nutrients.

In this study, the increment of vitamin E level in diets did not affect the weight gain and feed efficiency of fish fed the fresh or oxidized oils diets. However, other studies showed that inclusion of proper level of vitamin E level into the oxidized oil diet improved growth and feed utilization (Murai and Andrews, 1974; Baker and Davies, 1996). Wang and Bai (unpublished data) reported that juvenile flounder fed the diet containing high level of vitamin E (600 mg/kg diet) showed reduced growth and feed utilization. The difference in responses to supplemental vitamin E by fish fed the diet containing oxidized oil appear to be due to differences in fish species, the degree of lipid oxidation, rearing conditions or the dietary composition used in the studies.

The vitamin E content of the liver and dorsal muscle of fish was associated with an increase in dietary vitamin E level, and lower vitamin E content of liver was observed in fish fed the oxidized oil diets compared to that of fish fed the fresh oil diets at the same vitamin E level in this study. Similar results have been reported in other fish species (Hung et al., 1980; Stephan et al., 1993; Sakai et al., 1992). The decrease in vitamin E of tissues in fish fed oxidized oil diet is probably due to its being utilized in halting the free-radical cascade initiated by lipid peroxyl radicals and other assorted products of lipid peroxidation (McDowell, 1989). Dietary oxidized oil decreased the lipid content of the whole body in this study. On the other hand, fat accumulation in livers of channel catfish fed oxidized oil diets has been reported (Murai and Andrews, 1974).

The fatty acid composition of the whole body well reflected the fatty acid composition of dietary lipid. Similar results had been founded in previous studies (Kim et al., 2002; Kim and Lee, 2004). In this study, oxidized oil in diets influenced the fatty acid content of the whole body of fish, and lowered contents of 20:4n-6, 20:5n-3, 22:5n-3 and 22:6n-3. Similarly, decreased contents of 20:5n-3 and 22:6n-3 in the whole body were reported in gilthead sea bream (Baker, 1997). The ratios of dietary levels of 22:6n-3 and 20:5n-3 in fresh oil to oxidized oil diets were about 4:1 and 8:1, respectively. However, both of the ratios of 22:6n-3 and 20:5n-3 contents in the body lipid of flounder fed the fresh to oxidized oil diets were about 2:1. Similar results of high incorporation of 22:6n-3 and 20:5n-3 into body lipid were found in the previous flounder study (Kim and Lee, 2004). It has been suggested that these fatty acids are important structural components of cell membranes, and thought that they play an important role in permeability, enzyme activity and other functions in polar lipids of biomembranes (Stubbs and Smith, 1984; Bell et al., 1986; Lee, 2001)

The results of this study suggest that the dietary oxidized oil may impair the growth performance, and an increase in α-tocopheryl acetate supplementation have no beneficial effect on growth and feed efficiency of juvenile flounder.

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