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Effects of Dietary Lipid Peroxides Contents on *In Vivo* Lipid Peroxidation, α-Tocopherol Contents, and Superoxide Dismutase and Glutathione Peroxidase Activities in the Liver of Yellowtail

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Yellowtail *Seriola quinqueradiata* were fed on brown fish meal diets supplemented with 40 mg/100 g of α-tocopherol (α-Toc) and 9% of sardine oil whose POV was 4.7 (group I) or 100 (group II) for 30 days, and changes in α-Toc contents, 2-thiobarbituric acid reactive substances (TBARS) values, and superoxide dismutase (SOD), and glutathione peroxidase (GSH-Px) activities in the liver were analyzed. No pathological changes were observed in either group after the experiment. TBARS value and both enzyme activities of group I were lower and the α-Toc content of the group was higher than those of group II. These results suggest that *in vivo* lipid peroxidation may progress in the liver of yellowtail fed on a diet containing lipid peroxides whose contents are lower than those bringing about pathological changes, and that to suppress *in vivo* lipid peroxidation α-Toc may be consumed while SOD and GSH-Px might be induced.

Reactive oxygen species such as the superoxide anion and hydroxyl radical may start radical chain reactions leading to the extensive formation of lipid hydroperoxides.[1,2] Lipid hydroperoxides and their breakdown products change the physicochemical properties of biomolecules and have been involved in the cause or pathology of cancer, aging, and chronic inflammation.[1,2] Because fish contain a large amount of longchain polyunsaturated fatty acids being labile and easily oxidizable in their lipids, they seem to be susceptible to *in vivo* lipid peroxidation, which is a principal cause of several severe diseases such as nutritional muscular dystrophy[3,4] and jaundice.[5] To prevent *in vivo* lipid peroxidation, like all other aerobic living organisms, fish also possess enzymatic and nonenzymatic antioxidant defenses. However information on relation to *in vivo* lipid peroxidation and antioxidant defenses of cultured fish is quite limited.[6,7] Therefore we undertook the present study. *In vivo* lipid peroxidation progressed in the liver of yellowtail *Seriola quinqueradiata* fed on a diet containing lipid peroxides. α-Tocopherol may be consumed and superoxide dismutase and glutathione peroxidase may be induced to suppress *in vivo* lipid peroxidation.

Materials and Methods

Experimental Condition

Before starting the experiment, yellowtail were fed a pre-feeding diet (single brown fish meal supplemented with 12 mg/100 g diet of α-tocopherol and 9% sardine oil whose POV was 4.7) for two weeks to control *in vivo* lipid peroxidation levels. Then the fish were randomly divided into two groups (groups I and II) of 60 fish each. Average initial weight of group I was 168 g while that of group II was 165 g. Each group of fish was held in a net cage (2×2×2 m). The composition of the experimental diets is shown in Table 1. Diet 1 contained 40 mg/100 g diet of α-tocopherol and 9% sardine oil whose POV was 4.7, while diet 2 contained 40 mg/100 g diet of α-tocopherol and 9% sardine oil whose POV was 100. The sardine oil (POV 100) was made from sardine oil (POV 4.7) autoxidized by the bubbling of dried air at 50°C for 96 h. The experimental diets was provided to fish in a single

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Table 1. Composition of the test diets

(%)

Ingredient	Diets	
	1	2
Brown fish meal	65	65
Active wheat gluten	10	10
White gluten	6.5	6.5
Vitamin Mixture (TH-4*[1])	3	3
α-Tocopherol (mg/100 g)	40	40
Mineral Mixture (T-4*[2])	2	2
Mineral Mixture (O-1*[3])	0.5	0.5
CMC	2	2
Cellulose	2	2
Sardine Oil (POV 4.7)	9	0
Sardine Oil (POV 100)	0	9
Water	50	50

*1 Contained 675,000 IU vitamin A, 60,000 IU vitamin D3, 24.77 g DL-α-tocopherol acetate, 2.567 g menadione sodium hydrogen sulfite, 0.8 g thiamine nitrate, 1.467 g riboflavin, 0.8 g pyridoxine hydrochloride, 2.38 g nicotinamide, 2.381 g pantothenic acid, 4.667 g calcium salts, 194.7 g choline chloride, 0.8 g folic acid, 0.011 g cyanocobalamine, 0.0467 g D-biotin, 56.34 g inositiol, 59.34 g L-ascorbic acid, 419 g cellulose per kg of vitamin mixture.

*2 Contained 206 g KH_2PO_4, $Ca(CH_3CHOHCOO)_2 \cdot 5H_2O$, 83 g iron proteinate, 309 g $Ca_2H_4PO_4$, 261 g cellulose per kg of mineral mixture.

*3 Contained 12.5 g $MgSO_4$, 0.1 g $CoCl_2$, 4 g $CuSO_4$, 0.3 g KIO_3, 900 g dextrin, 63.13 g cellulose per kg of mineral mixture.

moist pellet form. Fish were fed twice a day, each time to satiation. The daily feeding rate ranged from 4–7%. The fish were fed the experimental diets for 30 days. The water temperature was 20.4–20.6°C during the feeding experiment. After the experiment, the blood and liver were obtained from ten fish from each group. Because fish contain a large amount of polyunsaturated fatty acids being very labile and easily oxidizable in their tissues, accurate 2-thiobarbituric acid reactive substances (TBARS) values may be obtained from a few methods. We used to analyze TBARS values by the distillation method of Yamauchi *et al.*,[8] which is one of these methods,*4 although the method requires 10 g of tissues. For this reason, the livers of 10 specimens were combined and analyzed.

Hematological and Blood Biochemical Analysis as Indices of Physiological Condition of Fish

Hematocrit values and hemoglobin contents of the blood were analyzed by the method of Kawatsu.[9] The activities of plasma glutamic-oxaloacetic transaminase (GOT) and glutamic-pyrubic transaminase (GPT) were measured with a Transaminase Iatrozyme kit (Iatron Laboratories).

Analysis of 2-Thiobarbituric Acid Reactive Substances Values and α-Tocopherol Contents

TBARS values in the liver were measured by the distillation method of Yamauchi *et al.*,[8] and expressed as μg of malonaldehyde (MA)/g tissue. The α-tocopherol contents of the liver were measured by the HPLC method of Yamauchi *et al.*[10] and expressed as μg/g. Analytical conditions were: column, Zorbax BP-NH_2 (4×150 mm); detection wavelengths, excitation 295 nm and emission 325 nm; flow rate, 1.5 ml/min; mobile phase, 0.5% isopropyl alcohol in *n*-hexane.

Superoxide Dismutase and Glutathione Peroxidase Assay

Superoxide dismutase (SOD) activities of the liver and muscle were assayed by the nitrite method of Ôyanagi[11] and expressed as NU/mg protein. The KCN sensitive enzyme was defined as Cu·Zn SOD. Selenium dependent glutathione peroxidase (GSH-Px) activities of the liver were measured by use of *t*-butyl hydroperoxide as substrate.[12] Results were expressed as U/mg Protein. Protein was determined with a Bio-Rad protein assay kit (Bio-Rad Laboratories).

Results and Discussion

Results of the feeding experiment are summarized in Table 2. Growth rate, feeding efficiency, and mortality were not significantly different between both groups. Results of hematological and blood biochemical analyses are summarized in Table 3. Judging from hematological results, hemolysis which is thought to be caused by *in vivo* lipid peroxidation[8] did not occur in either group. Liver injuries may not occur in fish from group II, because GOT and GPT activities were not significantly different between both groups. Nutritional muscular dystrophy and other pathological changes were also not recognizable with the naked eye in the fish of group II. In addition, histologically no pathological changes were observed in the liver of group II (data not shown). Dietary levels of lipid peroxides used in the present experiment did not affect the growth and physiological conditions of the fish.

*4 Yamauchi *et al.*, unpublished results.

Table 2. Effects of dietary lipid peroxides on growth of yellowtail

Group	Av. Body weight (g)		Growth rate (%)	Feeding efficiency* (%)	Mortality (%)
	Initial	Final			
I	168	327	94	0.57	1.7
II	165	327	97	0.60	1.7

* g gain/g feed (dry basis).

Table 3. Effects of dietary lipid peroxides on hematological and blood biochemical values of yellowtail

Groups	Ht*1 (%)	Hb*2 (mg/m*l*)	GOT (Karmen U)	GPT (Karmen U)
I	43.8±6.2*3	118±17	34±25	3±2
II	46.8±2.6	126± 9	66±75	6±3

*1 Hematocrit value.
*2 Hemoglobin content.
*3 Mean±S.D. (n=10)

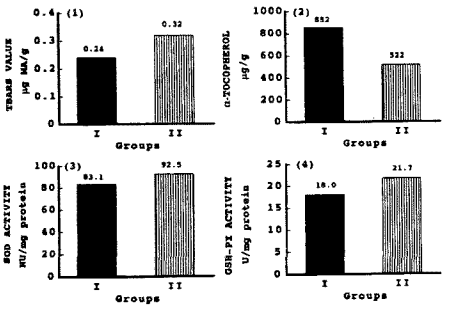

Fig. 1. Effects of dietary lipid peroxides on TBARS values (1), α-Toc contents (2), SOD (3), and GSH-Px (4) activities in the liver of yellowtail.
■: Group I. ▨: Group II.

Figure 1 shows the effects of dietary lipid peroxides on *in vivo* lipid peroxidation, α-tocopherol contents, and SOD and GSH-Px activities. Similar results were obtained from a preliminary feeding experiment in which only dietary α-tocopherol contents (12 mg/100 g diet) were different from the present experiment (data not shown). The TBARS value of group II was higher than that of group I. *In vivo* lipid peroxidation may progress even in the liver of fish fed on a diet containing lipid peroxides under the level which brings about pathological changes, such as jaundice or nutritional muscular dystrophy. Recently jaundice has occurred frequently in cultured yellowtail and causes enormous loss to farming. The main cause of this disease is thought to be *in vivo* lipid peroxidation.[5] However no preventive or therapeutical methods for the disease have been established until now. Early diagnosis may be the only countermeasure for the disease, and it may be necessary for early diagnosis to measure routinely *in vivo* lipid peroxidation levels in the tissues.

The α-tocopherol content of group I was higher than that of group II. In the liver of yellowtail fed on lipid peroxides, α-tocopherol seemed to be consumed to prevent *in vivo* lipid peroxidation. This result suggests that α-tocopherol may be supplied constantly to control *in vivo* lipid peroxidation. In addition, Murata *et al.* have reported that *in vivo* lipid peroxidation was suppressed in the liver of red sea bream *Pagrus major* fed on a diet containing high levels of α-tocopherol.[6] Enriching feed with α-tocopherol might be one useful countermeasure for the prevention of jaundice.

SOD and GSH-Px activities of group II were higher than those of group I. Because both enzymes in mammals are known to be induced in tissues suffering from oxidative stress,[13,14] they might be induced to suppress *in vivo* lipid peroxidation progressing in the liver, although their information in fish[7,15-18] is too little to elucidate any significance in preventing *in vivo* lipid peroxidation in fish tissues.

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