

Short Paper

Effect of Diet Supplemented with a Fermented Vegetable Product on Lipid Peroxidation in Liver of Japanese Flounder, *Paralichthys olivaceus*

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Abstract: We investigated the effects of Manda[®], a fermented vegetable product (FVP), on the growth and lipid peroxidation of Japanese flounder (*Paralichthys olivaceus*). Fish were fed on diets containing FVP (3, 6, and 15 mg/kg body weight/day) for 8 weeks. The final body weights of these fish were slightly influenced by the dietary treatments, whereas the level of lipid peroxidation in the livers was significantly ($p < 0.01$) lower than that in control fish (without FVP). These results suggested that the feeding of the FVP to fish reduces oxidative stress, and may be useful for the rearing of cultured fish.

Key Words: Fermented vegetable product; Japanese flounder; Lipid peroxidation.

Manda[®] is a fermented vegetable product (FVP) made by natural fermentation of several fruits, plant roots, cereals, marine algae, and *kokuto*, a kind of non-centrifugal cane sugar. These materials were crushed and fermented by bacteria generated spontaneously for 3 years and 3 months. Kawai *et al.*¹⁾ reported that Manda[®] exhibits a free radical scavenging action toward reactive oxygen species, which was examined by electron spin resonance spectrometry involving spin trapping. Reactive oxygen species such as superoxide, hydrogen peroxide, and hydroxyl radical cause oxidative stress in animals, which involves peroxidation of lipids and inactivation of enzymes.

Bacterial infection of animals activates host defense systems, causes the production of reactive oxygen species such as superoxide and hydrogen peroxide, and results in oxidative stress in the organisms. In fish, it has been reported that the oxidative stress is a principal cause of jaundice^{2,3)} and hemolysis⁴⁾, and the activities of superoxide dismutase and glutathione peroxidase, which are active oxygen-scavenging enzymes, increase with the bacterial infection. Murata *et al.*⁵⁾ stated that cultured fish experience more oxidative stress than wild fish, and that the activities of active oxygen-scavenging enzymes in cultured fish are significantly higher than those in wild fish, showing the higher oxidative stress in the cultured fish. Moreover, they reported that the addition of scavengers such as α -tocopherol and ascorbic acid to the diet of yellowtail is effective for reducing oxidative stress. Murata and Yamauchi⁶⁾ reported that dietary α -tocopherol fed to cultured red sea bream protects the ordinary muscle from lipid peroxidation.

We expect that the feeding of FVP to fish may reduce

oxidative stress, and may be useful for the rearing of cultured fish. Therefore, in this study, we investigated the effects of this FVP on the growth and lipid peroxidation, and discussed the feeding effectiveness of this material in Japanese flounder.

Japanese flounder (*Paralichthys olivaceus*) were hatched and reared in our laboratory at 20°C in 200 l tanks with a recirculation water system and were fed on Super Ex diet (Nihon-nosanko Co., Ltd.). The diets contain 46% crude protein, 13% crude lipid, 3% crude fiber, and 16% crude ash.

Fish were then divided into 4 groups of 30 fish/group. One group was fed on the commercial diet as a control group, and the others were fed on the diet containing 3, 6, and 15 mg/kg body weight/day of FVP. FVP (Manda[®]) is the product of Manda Fermentation Co., Ltd. (Hiroshima, Japan). The analytical data were shown in Table 1. The experimental diets were fed at the rate of 1.5% body weight per day for 8 weeks. The rearing water temperature was 16.0 to 23.5°C (av. 19.4°C).

After feeding, fish were measured for the body weight and the tissue weight of liver, kidney, spleen and intestine. The peroxidation of lipids in livers was measured as the amount of thiobarbituric acid-reactive substance (TBA-RS) by the procedure of Uchiyama and Mihara⁷⁾. For this experiment livers were isolated and 9-fold 1.15% (w/v) potassium chloride was added to them, and then samples were homogenized. An aliquot of the sample (0.5 ml) was mixed with 3 ml of 1% (v/v) phosphoric acid and 1 ml of 0.67% (w/v) 2-thiobarbituric acid. The mixture was heated in a boiling water bath for 45 min and cooled in tap water, and then 4 ml of *n*-butanol was added. The mixture was then centrifuged at 2,000 $\times g$ for 10 min. The $A_{535nm} - A_{520nm}$ value of the supernatant was used to calculate the concentration of TBA-RS. For these calculations, standards were prepared with 1,1,3,3-tetraethoxypropane. The protein concentrations of the homogenates were determined with a BCA protein assay kit (Pierce, Illinois, U.S.A.), and a standard

Table 1. General nutrient composition of FVP supplemented to diets fed Japanese flounder

Composition	Content (/100 g)
Water	38.1 g
Protein	2.5 g
Lipid	0.2 g
Carbohydrate	57.2 g
Ash	2.0 g

Analyzed by Institute of Food Hygiene, Japan Food Hygiene Association. (No. 01-9104).

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curve was obtained with bovine serum albumin.

The significance of differences between means was determined with ANOVA.

Results of the feeding experiment for 8 weeks were shown in Table 2. The supplement of a low concentration (3mg/kg B.W./day) of FVP slightly enhanced the growth, but without significance. On the other hand a high concentration of FVP (15mg/kg B.W./day) was found to increase the feed efficiency.

Between the commercial and FVP-containing diet fed fish, no anatomical difference in the internal organs was observed. In other words, the tissue weight % of FVP fed fish were not different significantly from that of the control fish (data not shown), and the anemia and blood congestion of tissues were not observed in all fish. Then, the feeding effect of the FVP was evaluated using the TBA-RS value for malonaldehyde (MDA). The TBA-RS value in the control fish (no FVP) was 5.46 ± 0.88 nmole MDA/mg protein in liver. On the other hand, the values in FVP fed fish were significantly ($p < 0.01$) lower than that in the control fish, moreover, the effect depended on the FVP concentration (Fig. 1).

The focus of this study was to breed more healthy fish in farms. Generally, cultured fish are reared at higher density than wild fish, and the nutrition variation of food is restricted in cultured fish. These circumstances may have a profound effect as to fish disease. Moreover, it seems that cultured fish receive much higher lipid-containing diets, especially ones containing oxidized lipids, than wild fish. The TBA-RS values for the livers of farm yellowtail⁵⁾ and red sea bream⁶⁾ were reported to be higher than those for the respective wild fish. In cultured fish, the control of lipid peroxidants decreased the possibility of several diseases such as jaundice³⁾. We investigated whether the use of FVP-containing diets affects the lipid peroxidant contents of the livers of Japanese flounder. The results showed that

Table 2. Influence of supplementation with FVP to fish diet on growth performance of Japanese flounder

Experimental condition	Average body weight(g)		Weight gain (%)	Feed* efficiency (%)	Survival rate (%)
	Initial	Final			
Control	26.2±4.3	89.5±23.8	239	142	100
3 mg FVP	27.4±4.9	98.8±18.0	261	145	100
6 mg FVP	27.9±4.6	90.2±16.6	223	141	100
15 mg FVP	27.4±4.7	92.1±21.0	237	152	100

*Wet weight gain (g) × 100/dry feed intake (g).

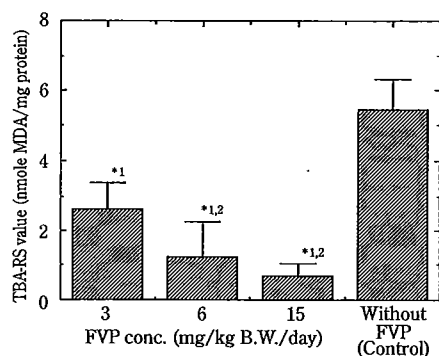


Fig. 1. TBA-RS contents in livers of Japanese flounder after feeding of experimental diets for 8 weeks. Fish were fed on commercial diets with or without FVP. Vertical bars show the standard deviation ($n=6$). *¹Significantly different from control with ANOVA ($p < 0.01$). *²Significantly different from 3mg/kg B.W. /day FVP feeding group with ANOVA ($p < 0.01$).

with increasing doses of FVP there was a decrease in the TBA-RS value in the liver (Fig. 1), indicating a decrease in the lipid peroxidation level in the liver. Therefore, this FVP may reduce not only the level of *in vivo* lipid peroxidation in the liver but also the oxidative stress caused by produced reactive oxygen species.

As an antioxidant component, Manda[®] contains 1.2 mg vitamin E per 100 g, but vitamin C was not detected⁹⁾. Sekiya *et al.*⁹⁾ and Stephan *et al.*¹⁰⁾ reported that the use of α -tocopherol containing diet resulted in protection of fish against lipid peroxidation. However, the content of vitamin E in the Manda[®] supplemented diet we used is much lower than that of α -tocopherol in the diet they used. This suggested the possibility of the existence of other antioxidant components in FVP, such as polyphenol and flavonoid substance originated from raw materials, although we have not identified the possible substance.

In conclusion, our finding that the diet containing Manda[®] reduces the lipid peroxidation level in the liver of Japanese flounder may offer protection against several diseases in fish and enhance the economic value of fish farming.

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