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NOTE

Effects of Dietary Supplementation of Ferulic Acid and γ-Oryzanol on Integument Color and Suppression of Oxidative Stress in Cultured Red Sea Bream, *Pagrus major*

Takashi Maoka[1]*, Fumio Tanimoto[1], Mitsuhiko Sano[2], Kanji Tsurukawa[2], Takuo Tsuno[3], Satomi Tsujiwaki[3], Katsuya Ishimaru[4] and Kenji Takii[5]

[1] *Research Institute for Production Development (15 Shimogamo-Morimoto-Cho, Sakyou-Ku, Kyoto 606-0805, JAPAN)*
[2] *Dainichi Corporation (1385 Yorimatsu-Ko, Uwajima, Ehime 798-0084, JAPAN)*
[3] *Tsuno Rice Fine Chemicals Co., Ltd. (94 Shinden, Katsuragi, Wakayama 649-7194, JAPAN)*
[4] *Fisheries Laboratory, Kinki University (3153 Shirahama, Wakayama 649-5145, JAPAN)*
[5] *Fisheries Laboratory, Kinki University (468-3 Uragami, Nachikatsuura, Wakayama 649-5145, JAPAN)*

Abstract: The effects of ferulic acid (FA) and γ-oryzanol (OZ) supplementation on cultured red sea bream were examined. Commercial brown fish meal diets supplemented with FA (0.01-0.5%) or OZ (0.05-0.5%) were given to zero-year, cultured red sea bream for 98 days. After the experiment, the brightness of the integument color ("L" value) of FA- and OZ-administrated fish was higher than that of control fish. Furthermore, 2-Thiobarbituric acid reactive substances (TBARS) in the liver of FA- and OZ-administrated fish was lower than in control fish. These results indicate that FA and OZ suppressed not only dark-color pigmentation but also oxidative stress in cultured red sea bream.

Key words: ferulic acid, γ-oryzanol, red sea bream, integument color, TBARS

1 INTRODUCTION

Apart from body weight and flesh quality, the integument color is one of the important factors for dictating commercial value of cultured red sea bream, *Pagrus major*. The integument color of red sea bream is caused not only by carotenoids (astaxanthin, lutein, and tunaxanthin) but also by melanin[1,2]. Compared with wild red sea bream, cultured fish are reared at a shallow depth (5-10 m) in net pens and show a dark integument color due to exposure to sunlight[2,3] and several oxidative stresses[4]. Dark-color pigmentation is caused by the excessive production of melanin caused by sunburn[2,3]. It is well-known that dark-color pigmentation of the integument is prevented by the inhibition of melanogenesis and/or presence of sunshade.

Also, astaxanthin given as a supplement to cultured red sea bream led to red-color pigmentation[1,2]. However, active oxygen species and free radicals generated by several oxidative stress decompose carotenoid and inhibit their accumulation in the fish integument. Therefore, it is necessary to administrate a melanogenesis inhibitor and/or antioxidant to cultured red sea bream to achieve fine pigmentation similar to that in wild fish.

Ferulic acid (FA) and γ-oryzanol (OZ), which is a sterol, triterpene alcohol, and higher alcohol esters of ferulic acid, both have antioxidative and melanogenesis-inhibitory activities[5-7]. Thus, we attempted to apply FA and OZ supplementation for the improvement of the integument color and suppression of oxidative stress in cultured red sea bream. This paper presents these results.

* Correspondence to: Takashi Maoka, Research Institute for Production Development, 15 Shimogamo-Morimoto-Cho, Sakyou-Ku, Kyoto 606-0805, JAPAN.
E-mail: maoka@mbox.kyoto-inet.or.jp
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2 EXPERIMENTAL

2.1 Feeding experiment

Zero-year red sea bream obtained from the Fish Nursery Center, Kinki University, Uragami, with an average body weight of about 250g, were randomly divided into eight groups of 30 fish each in $2 \times 2 \times 2.5$ m^3 floating net pens covered with sunshade netting in Uragami Bay, Nachikatuura, Wakayama Prefecture, Japan. The test fish were fed test diets twice a day, six times a week, for 98 days. On the initial and final days of the feeding trial (all cases), fish were sampled and weighed individually for each test group. On the final day of the feeding trial, hematology was assessed by the previously reported method[8].

2.2 Test diets

The commercial diet for red sea bream containing 60 ppm astaxanthin (Madai EP45, Kirin Food Co., Ltd., Japan) was used for the basal diet (diet 1). This diet was composed of brown fish meal (60%), grains (15%), plant oilcake (10%), rice bran (5%), fish oil (3%), and other components (7%) such as brewery yeast, calcium carbonate, and calcium phosphate. It contained 0.045% DL-α-tocopherol acetate. Experimental diets (diets 2-8) composed 0.01-0.5 % of FA or 0.05-0.5 % of OZ added to the basal diet, as shown in Table 1. FA and OZ were provided from Tsuno Rice Fine Chemicals Co., Ltd., Japan. OZ consisted of 24-methylcycloartanal ferulate (42%), cycloartenol ferulate (29%), campesterol ferulate (16%), β-sitosterol ferulate (9%), and some higher alcohol esters of ferulic acid (4%). All experimental diets were processed to dry pellets by the extruding method.

2.3 Estimates of integument color

On the final day of the feeding trail, integument color was estimated by the "L" value (brightness), "a" value (red color), and "b" value (yellow color) obtained by the Chroma Meter CR-300 (MINOLTA Co., Ltd., Japan)[2,3,9]. Three areas (head, lateral side, and ventral side) were assessed regarding integument color, as shown in Fig. 1.

2.4 Analysis of carotenoid content and compositions in the integument

Carotenoid contents of integuments were measured according to the methods described in previous literature[10,11]. The whole integument of each fish except for the head, fins and tail were removed. Carotenoids were extracted with acetone from the integument. After transferring to hexane/ether (1 : 1) by adding water, the total carotenoid content was calculated using an extinction coefficient of $E_{cm}^{1\%} = 2500$ at λ max. The individual carotenoid contents were estimated by column chromatography on silica gel and HPLC.

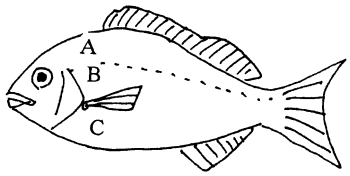

Fig. 1 Measuring Position of Integument Color in Red Sea Bream.
A: head, B: lateral side, C: ventral side.

Table 1 Content of FA and OZ in Experimental Diet and Growth Performance and Hematology of Red Sea Bream.

	Group							
	1	2	3	4	5	6	7	8
FA content (%)	0	0.01	0.05	0.1	0.5	0	0	0
OR content (%)	0	0	0	0	0	0.05	0.1	0.5
Growth performance								
Mean body weight (g)								
Initial	293	287	285	286	294	280	287	302
Final	665	659	672	658	695	673	673	668
Weight gain (kg)	9.41	10.22	10.33	9.55	10.18	10.33	9.63	9.27
Feed intake (kg)	17.42	17.21	17.05	17.31	17.09	17.69	17.10	16.62
Feed efficiency (%)	54.0	59.4	60.6	55.2	59.0	58.4	56.3	55.7
Survival	96.7	96.7	100	96.7	100	100	96.7	96.7
Hematology*								
Hematocrit value (%)	0.42	0.42	0.37	0.38	0.38	0.38	0.38	0.37
Hemoglobin concentration (g/100 ml)	9.81	9.91	9.33	9.03	9.37	9.22	9.51	9.24

* n=5.

2.5 Analysis of 2-thiobarbituric acid reactive substances (TBARS) levels

2-Thiobarbituric acid reactive substances (TBARS) values in the liver were measured according to the method described by Masugi and Nakamura[12]. Liver (1 g) was homogenized with 5 mL of 0.05 M phosphate buffer (pH 7.4). Then, 0.5 mL of homogenate was transfered to a screw-capped tube containing 0.2 mL of 7% sodium dodecyl sulfate and 2 mL of 1N HCl and mixed gently. After adding 0.3 mL of 10% tungsto phosphoric acid and 1 mL of 0.5% 2-thiobarbituric acid, the solution was mixed and heated in a water bath at 95℃ for 45 min and then cooled quickly with running tap water. After adding 5 mL of *n*-butanol to the solution, the mixture was shaken vigorously and centrifuged at 3000 rpm for 10 min. TBARS values were expressed as mg malondialdehyde (MDA) /g tissue. The MDA content was calculated by absorbance at 532 nm of the *n*-butanol layer using a coefficient of $E^{1\%}_{cm}=156000$.

2.6 Statistical analyses

The integumental color value and TBARS data are expressed as the mean ± standard deviation for the mean. Statistical comparisons were performed with Student's *t*-test.

3 RESULTS AND DISCUSSION

3.1 Effects of ferulic acid and γ-oryzanol supplementation on growth performance

Table 1 shows the growth performance of fish fed the diets for 98 days. The survival rates of each group were between 96.7 to 100%. No significant differences among the dietary treatments were found in weight gain, feed efficiency, and survival, with a slightly higher trend in feed efficiency in FA (diet 2-5 groups)- and OZ (diet 6-8 groups)-administrated groups than the control group (diet 1 group). There were no significant differences in hematology in the groups (Table 1). Therefore, it was revealed that FA and OZ dietary supplementation caused no undesirable effect on growth, but rather enhanced the growth of red sea bream.

3.2 Effects of ferulic acid and γ-oryzanol supplementation on integument color

Integument color in red sea bream was estimated by the "L", "a", and "b" values obtained by a chromatic meter[2,3]. The "L", "a", and "b" values indicated the index of brightness, red-color, and yellow-color values, respectively. It was reported that the "L" value showed a negative correlation with the melanin content, whereas "a" and "b" values showed a positive correlation with the astaxanthin and yellow xanthophyll (lutein and tunaxanthin) content, respectively[2,3]. After 98 days of feeding, the integument color of FA- and OZ-administrated fish (diet 2-8 groups) showed higher-level brightness than that of the control group (diet 1 group). Not only the "L" value but also the "a" and "b" values of FA- and OZ-administrated fish were higher than those of the control fish, as shown in Table 2. For the head integument, the "L" values of FA- and OZ-administrated groups were higher than that of the control group. Among them, the "L" value in diet groups 2, 3, 5, and 6 were significantly ($p<0.05$) higher than that of the control group (diet 1 group). In lateral and ventral areas, FA- and OZ-administrated groups also showed an increased "L" value compared to the control group. It is well-known that melanogenesis is induced by UV light and catalyzed by tyrosinase. FA and OZ are not only effective UV absorbers[6], but also tyrosinase inhibitors[5–7]. Therefore, FA and OZ suppressed dark-color pigmentation in the integument of red sea bream.

Concerning the red and yellow color, the "a" and "b" values of FA- and OZ-administrated groups (diets 2-8 groups) were slightly higher than those of the control group (diet 1 group). This result showed that FA and OZ could intensify the red and yellow coloration of the integument of red sea bream. It was reported that the red color in the integument of marine fish is due to astaxanthin and the yellow color is due to lutein and tunaxanthin[13], and that astaxanthin was converted to tunaxanthin via lutein in marine fish[10–11, 13]. The total carotenoid contents in the integuments in FA- and OZ-administrated groups were slightly higher than those of the control group, as shown in Table 3. Therefore, supplementation with FA and OZ might enhance the accumulation of carotenoid in the integument of red sea bream. This effect might be due to their antioxidative activity. Details are discussed in the next section.

3.3 Effects of ferulic acid and γ-oryzanol supplementation on oxidative stress

It was reported that oxidative stress caused some undesirable effects in marine animals[14–16]. TBARS comprise one of the useful indicators to estimate the oxidative stress[14–16]. TBARS values in the liver of FA- and OZ-administrated groups (diet 2-8 groups) were lower than that of the control group (diet 1 group), as shown in Fig. 2. Especially, TBARS values in experimental groups 4, 5, and 8 were significantly ($p<0.05$) lower than the control group. These results clearly indicated that FA and OZ suppressed oxidative stress in fish.

It was reported that astaxanthin itself was an excellent antioxidant in fish, especially for singlet oxygen and lipid peroxidation[17,18]. These activities were higher than those of β-carotene and vitamin E[17,18]. However, astaxanthin was immediately decomposed when reacted with active oxygen such as hydoxyl or superoxide radicals. On the other hand, FA and OZ showed excellent quenching activity for

Table 2 Effect of Supplementation on FA and OZ for Integument Color of Red Sea Bream.

	Group							
	1	2	3	4	5	6	7	8
Head								
L value	32.7 ± 0.8	37.1 ± 1.2**	37.9 ± 1.7*	34.2 ± 1.1	38.7 ± 1.0**	40.9 ± 1.0**	37.1 ± 0.8	38.0 ± 1.4
a value	8.0 ± 0.3	7.9 ± 0.5	8.2 ± 0.4	8.0 ± 0.2	8.1 ± 0.7	8.5 ± 0.2	8.5 ± 0.4	8.0 ± 0.4
b value	10.7 ± 0.7	14.1 ± 0.8**	13.6 ± 1.0*	12.4 ± 0.9	12.7 ± 0.4*	10.5 ± 0.6	12.2 ± 1.0	11.9 ± 0.7
Lateral side								
L value	62.5 ± 1.5	66.7 ± 1.8	66.7 ± 1.2*	68.3 ± 1.3**	64.6 ± 0.9	63.6 ± 1.4	62.5 ± 1.4	62.9 ± 1.2
a value	12.2 ± 0.6	14.3 ± 0.7*	13.7 ± 1.1	13.1 ± 0.5	14.4 ± 1.1	14.3 ± 1.4	15.7 ± 0.6**	14.4 ± 0.9
b value	20.8 ± 1.1	27.7 ± 1.0*	20.9 ± 0.6	20.5 ± 0.9	21.7 ± 1.0	18.2 ± 0.8	21.9 ± 0.7**	18.1 ± 0.8
Ventral side								
L value	82.2 ± 1.2	84.7 ± 0.5	86.1 ± 1.3*	85.3 ± 0.7*	84.0 ± 0.9	84.5 ± 0.7	85.0 ± 0.8	85.0 ± 0.8

Values are expressed as means ± SE of 20 fish par group.

The asterisk indicates significant difference from the control group within the same row (* $p < 0.05$, ** $P < 0.01$).

Table 3 Total Carotenoid Content and Composition of Carotenoid in the Integument of Red Sea Bream.

	Group							
	1	2	3	4	5	6	7	8
Total carotenoid content (mg/specimens)	0.30	0.30	0.34	0.31	0.31	0.30	0.32	0.38
Composition (%)								
Astaxanthin	65.0	65.0	66.0	65.0	65.0	65.0	66.0	68.0
Lutein	10.0	11.0	11.0	10.0	10.0	10.0	11.0	12.0
Tunaxanthin	17.0	16.0	17.0	17.0	17.0	18.0	17.0	15.0
Others	8.0	8.0	6.0	8.0	8.0	7.0	6.0	5.0

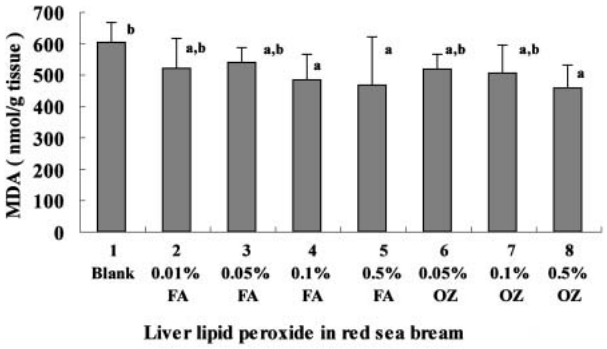

Fig. 2 TBA Values in the Liver of Red Sea Bream.
TBA values are expressed as nmol of MDA /g tissue.
The same superscript in a row indicates no significant difference among the experimental group ($p<0.05$).

hydroxyl, superoxide, and lipid peroxide radicals[6]. Thus, it was strongly suggested that FA and OZ could protect astaxanthin from decomposition by active oxygen and free radicals in fish. Therefore, supplementation with FA and OZ could enhance the accumulation of carotenoids in the integument of red sea bream.

Some antioxidants such as glutathione[9,19], cysteine[20], kojic acid[21], polyphenols from grape seeds, guava leaves and soybeans[22], and the leaf extract of *Eucommia ulmoides*[23] were administered for the improvement of pigmentation to cultured red sea bream. These compounds suppressed melanogenesis. However, the carotenoid-accumulating effect of these compounds in red sea bream is uncertain. Therefore, this is the first report to emphasize the accumulation of carotenoids in the integument of red sea bream by antioxidant presence.

FA and OR are widely used in food ingredients and cosmetics as an antioxidant and UV absorber. To our knowledge, this is the first report on the application of FA and OR in aquaculture.

4 CONCLUSION

From the present experimental results described above, FA and OZ might be valuable as integument color improvers and oxidative stress suppressors in cultured fish.

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