Improvement of Biochemical Features in Fish Health by Red Yeast and Synthetic Astaxanthin

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The biochemical characteristics of the liver and blood in rainbow trout (*Oncorhynchus mykiss*) fed a diet supplemented by red yeast (*Phaffia rhodozyma*) containing astaxanthin (Ast) as its principal carotenoid pigment or synthetic Ast were studied. Surprisingly, the hepatosomatic indices and serum glutamic-oxaloacetic transaminase activities of fish fed a diet containing red yeast or synthetic Ast were significantly lower than those of fish fed a control diet. The mean amount of serum lipid peroxide of fish fed a diet containing red yeast or synthetic Ast was also lower than that of the control fish. It was suggested that the dietary red yeast and synthetic Ast have the potential for improving not only the pigmentation of fish muscle but also the health of fish in aquaculture (e.g. improvement of liver function and increase of defensive potential level against oxidative stress).

Keywords: *Red yeast; astaxanthin; antioxidant; rainbow trout; liver; serum; lipid peroxide; Phaffia rhodozyma*

INTRODUCTION

It is well-known that fish have high levels of polyunsaturated fatty acids. Feed in fish culture usually contains fish oils or raw fish pastes, so they are susceptible to oxidation (Stansby, 1990). The oxidation of lipids is thought to produce lipid peroxides (LPO), aldehydes, and ketones (Josephson et al., 1984; Koizumi, 1992). LPO are reactive oxygen species (ROS) and are further decomposed to other oxygen-containing radicals. The resulting ROS attack almost all cell components, such as proteins, lipids, and membranes, and, finally, cause fetal damage in the cells (Asada, 1988). Hence, ROS seem to be undesirable for organisms. Against the toxicity shown by ROS, aerobic organisms have both enzymatic and nonenzymatic defensive systems (Asada, 1988). Recently, in the course of studies on such defensive systems of fish (Nakano et al., 1992a–c, 1993, 1995), it has been accepted that improvement of defensive potential of cultivated fish against oxidative stress is very important.

Astaxanthin (3,3′-dihydroxy-β,β-carotene-4,4′-dione; Ast) is reported to show potent antioxidative activity, and the activity is much stronger than that of α-tocopherol, which is a common antioxidant in animals (Miki, 1993). Ast and certain kinds of carotenoids have been suggested to show biological activities for mammals (Bendich and Olson, 1989; Matsuno and Miki, 1990; Ito, 1992; Miki, 1993). Therefore, when Ast is given to fish, dietary Ast also seems to have a possibility to show antioxidative and some biological effects on fish tissue. However, most research on dietary Ast in fish culture has been discussed from the standpoint of control of muscle pigmentation (Storebakken and No, 1992), and little information is reported on the biological activities of Ast for fish (Torrissen, 1984; Matsuno and Miki, 1990).

In this study, we examined the effects of red yeast, *Phaffia rhodozyma*, which contains Ast as its principal carotenoid pigment (Andrewes et al., 1976), and synthetic Ast on the biochemical characteristics of the liver and blood of rainbow trout *Oncorhynchus mykiss*. The results obtained are discussed in relation to the red yeast, synthetic Ast, and fish health.

Table 1. Composition of Experimental Diets

ingredient	diet 1	2	3	4	5
white fish meal (g/kg)	600	600	600	600	600
α-starch (g/kg)	200	200	200	200	200
soybean oil (g/kg)	60	60	60	60	60
pollack liver oil (g/kg)	40	40	40	40	40
mineral mixture[a] (g/kg)	40	40	40	40	40
vitamin mixture[b] (g/kg)	10.05	10.05	10.05	10.05	10.05
red yeast[c] (g/kg)	0	11.36	9.80	10.20	0
astaxanthin[d] (g/kg)	0	0	0	0	0.91
cellulose (g/kg)	49.95	38.59	40.15	39.75	49.04

[a] Minerals added to supply at the following levels (g/kg of diet): Ca lactate, 13.08; KH_2PO_4, 9.59; $MgSO_4$·7aq, 5.48; $Ca(H_2PO_4)$aq, 5.43; NaH_2PO_4·2aq, 3.49; NaCl, 1.74; Fe citrate, 1.19; $ZnSO_4$, 0.12; $MnSO_4$, 0.03; $CoCl_2$, 0.03; $AlCl_3$, 0.006; KI, 0.006; $CuCl_2$, 0.004. [b] Vitamins added to supply at the following levels (g/kg of diet): vitamin B_1, 0.05; vitamin B_2, 0.2; vitamin B_6, 0.05; vitamin B_{12}, 0.0001; nicotinic acid, 0.75; *d*-Ca pantothenate, 0.5; inositol, 2; biotin, 0.005; folic acid, 0.015; ascorbic acid, 1; vitamin K_3, 0.04; α-tocopherol, 0.4; choline chloride, 5; vitamin A, 20000 IU; vitamin D_3, 4000 IU. [c] *Phaffia rhodozyma*. See text for details. [d] Synthetic astaxanthin.

MATERIALS AND METHODS

Experimental Design, Fish, Rearing System, and Diets. About 120 unpigmented rainbow trout (approximately 7 months old), *O. mykiss*, were obtained from the Tanii Trout Farm, Zao town, Miyagi, Japan. During 2 weeks of acclimatization prior to commencement of feeding trials, the fish were kept in 10^3 L flow-through fiberglass tanks and fed a commercial dry pelleted feed free from Ast. The fish were then individually weighed and allotted to five groups of 10 healthy fish each (approximate body weight 110 g). They were then reared in 60 L flow-through glass tanks over a 2 month period at 16.5 °C. The fish were fed to satiation two times daily. The composition of the diet is presented in Table 1. The concentration of Ast in each diet was adjusted to 50 mg/kg (Storebakken and No, 1992). The red yeast, *P. rhodozyma*, and synthetic Ast were kindly donated by KI Chemical Co., Shizuoka, Japan. The red yeast was harvested and thoroughly washed with

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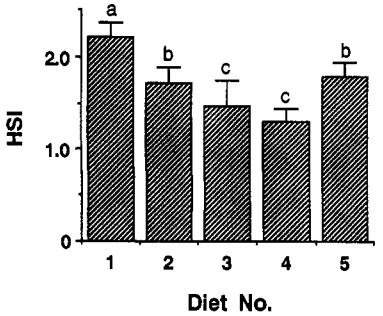

Figure 1. Effects of red yeast and synthetic astaxanthin on hepatosomatic index (HSI) of rainbow trout. Data points represent mean ± SD. There are significant differences ($p < 0.05$) among different letters.

distilled water and was then prepared in three different ways for incorporation into the diets listed in Table 1 as follows:

(*1*) The yeast cells were freeze-dried without other treatment (intact yeast, diet 2).

(*2*) The yeast cells were dipped in 0.05 N NaOH solution (pH 12.0) for 1 h at room temperature and neutralized with HCl. The treated cells were centrifuged from suspension and thoroughly washed with distilled water and then freeze-dried (alkali yeast, diet 3). The yeast cell walls were found to be partially destroyed by this procedure (confirmed by electron microscopy).

(*3*) A portion of the treated cells from diet 3 were milled and freeze-dried (milling yeast, diet 4). The yeast cell walls were found to be considerably destroyed by this procedure.

Ast contents of each freeze-dried yeast preparation and synthetic Ast are as follows: intact yeast, 4.4 mg/g of yeast; alkali yeast, 5.1 mg/g of yeast; milling yeast, 4.9 mg/g of yeast; synthetic Ast, 54 mg/g of synthetic matter. Each freeze-dried yeast preparation and synthetic Ast were thoroughly mixed with the dry ingredients before pelleting. The diets were frozen and stored in vacuo at −20 °C until used.

Blood, Liver, and Flesh Collection. At the end of the feeding trial, the fish were netted rapidly and the blood was individually collected from the caudal vessels under MS222 (*m*-aminobenzoic acid ethyl ester methanesulfonate) anesthesia. Serum from the blood was placed on ice. The fish were then gutted and skinned. The liver and flesh were collected and frozen at −80 °C for later analysis.

Serum Enzyme Activity and LPO Measurement. Serum enzyme activities, glutamic-oxaloacetic transaminase (GOT; EC 2.6.1.1) and glutamic-pyruvic transaminase (GPT; EC 2.6.1.2), were determined spectrophotometrically. Serum LPO was measured according to the method of Yagi (1975) and was expressed as the amount of total thiobarbituric acid reactive substances (TBARS). Each enzyme and TBARS assay was carried out on five samples. Then their mean values were expressed as Karmen unit and nanomoles of malondialdehyde (MDA) per milliliter of serum, respectively. The enzymatic activities and LPO levels were measured with Wako test kits (Wako Pure Chemical Industries, Japan) according to instructions.

Ast Analysis. The muscles were used for analysis of Ast content which was measured according to the methods described by Tsukuda (1974). Ast analysis was run on five samples and the mean value reported.

Statistical Analysis. All data were subjected to one-way analysis of variance (ANOVA). Significant differences between means were ranked by use of Fisher's least significant difference (lsd) test at the 5% level (Shinjo, 1986).

RESULTS

Hepatosomatic Index and Growth Performance. The percentage of liver to body weight (hepatosomatic index; HSI) is shown in Figure 1. Surprisingly, HSIs of the groups fed a diet containing yeast or synthetic Ast (diets 2−5) were significantly lower than that of the control group (diet 1). Furthermore, there were significant differences in HSI: diet 3 = diet 4 < diet 2 = diet 5 < diet 1. The ratio of decrease in HSIs of diets 2−4 appears to relate to the level of destruction of the yeast cells, with diet 4 showing the lowest, and being only about 60% in diet 1.

As shown in Table 2, the fish in all diet groups grew very well. At the end of the feeding trial, there were no significant differences among the groups (diets 1−5) in the following items (Table 2): (1) average body weight; (2) weight gain; (3) daily feed intake (DFI) (=100 × feed intake/total days/[(initial body weight + final body weight)/2]); and (4) feed conversion efficiency (FCE) (=wet weight gain/dry diet fed).

Muscle Pigmentation. The mean amounts of Ast in fish fed diets 1, 2, 3, 4, and 5 were 0.09, 0.21, 0.99, 3.02, and 3.70 mg/kg of tissue, respectively. There were significant differences in mean Ast concentration of muscle: diet 4 = diet 5 > diet 1 = diet 2 = diet 3. Thus, the most efficient accumulation of Ast in the muscle occurred when the fish were fed a diet containing milling yeast (diet 4) or synthetic Ast (diet 5).

Enzyme Activity and LPO Level of Serum. The activities of serum GOT and GPT in fish fed diets 1−5 were compared (Figure 2). Differences in mean GOT activity were observed; the activities of diets 2−5 were significantly lower than that of diet 1. Diet 2 showed the lowest GOT activity (27.39 Karmen units), followed by diet 3 (35.07 Karmen units), diet 4 (35.90 Karmen units), diet 5 (42.54 Karmen units), and diet 1 (84.04 Karmen units). That is to say, the red yeast and synthetic Ast appear to have an effect of decreasing the serum GOT. On the other hand, the differences in the activities of serum GPT of diets 1−5 cannot be regarded as significant.

Mean amounts of LPO of diets 2−5 were low compared to that of diet 1 (Figure 3). The lowest LPO contents were exhibited in diets 3 and 4 (6.7 nmol of MDA/mL of serum), followed by diet 5 (7.6 nmol of MDA/mL of serum), diet 2 (11.7 nmol of MDA/mL of serum), and diet 1 (16.3 nmol of MDA/mL of serum). Furthermore, LPO levels of diets 3 and 4 were significantly lower than those of diets 1, 2, and 5. There were no significant differences in LPO levels between diets 1 and 5; however, the mean values of diet 5 were determined to be less than 50% of that of diet 1.

DISCUSSION

The liver is the main organ in intermediary metabolism and is the site of detoxification (Ozaki, 1971). In particular, the liver stores fat and glycogen, so that its weight is affected by the ingredients of the diet. For example, the ratio of the amount of protein to carbohydrate is reported to influence HSIs of fish (Ozaki, 1971). However, the ratio mentioned above was approximately constant in each diet (Table 1). Accordingly, the decrease in HSI of experimental fish may be attributed not to the amounts of carbohydrate and protein but to the red yeast and synthetic Ast. The HSI of fish fed diet 4 was significantly lower than that of fish fed diet 5 (Figure 1), so that the red yeast seems to have unknown HSI-decreasing factors other than Ast. These unknown factors are considered to be specific for *P. rhodozyma*, because, though the cell wall of bakery yeast *Saccharomyces cerevisiae* was considerably destroyed, the HSI of rainbow trout fed a diet containing bakery yeast was not observed to decrease (data not shown).

The enzyme-abundant tissues contribute to the aspect of the circulating enzyme pattern in the serum. When

Table 2. Growth Performance of Rainbow Trout Fed Diet Containing Red Yeast or Synthetic Astaxanthin[a]

parameter	diet 1	2	3	4	5
initial wt[b] (g)	111.30 ± 5.64	111.84 ± 8.99	110.08 ± 7.25	111.75 ± 7.50	111.18 ± 6.19
final wt[c] (g)	248.75 ± 28.38	228.22 ± 44.88	237.84 ± 23.57	241.17 ± 47.76	233.05 ± 35.50
wt gain (%)	123.5	104.1	116.06	115.81	109.62
DFI[d] (%)	1.24	1.23	1.30	1.29	1.31
FCE[e]	1.06	1.03	1.04	1.05	1.00
	(8)[f]	(10)	(10)	(10)	(10)

[a] Values are mean ± SD. [b,c] Statistical significances are not detected. [d] Daily feed intake = (100 × feed intake/total days/[(initial wt + final wt)/2]). [e] Feed conversion efficiency = (wet wt gain/dry matter intake). [f] Numbers of fish in parentheses.

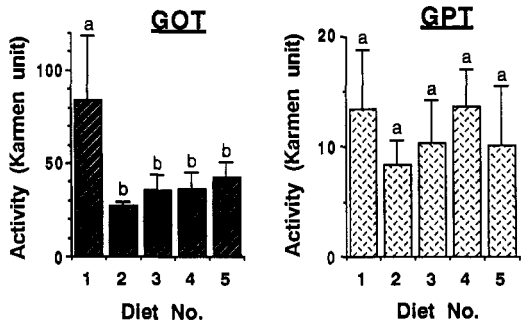

Figure 2. Effects of red yeast and synthetic astaxanthin on serum glutamic-oxaloacetic transaminase (GOT) and serum glutamic-pyruvic transaminase (GPT) activities of rainbow trout. Data points represent mean ± SD. There are significant differences ($p < 0.05$) among different letters.

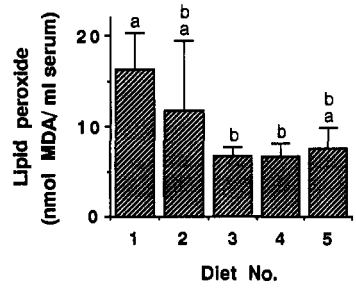

Figure 3. Effects of red yeast and synthetic astaxanthin on serum lipid peroxide level of rainbow trout. Data points represent mean ± SD. There are significant differences ($p < 0.05$) among different letters.

the damage occurs in enzyme-abundant tissue, some enzymes leak from injured cells and the activities of serum enzymes will change. Liver damage especially follows such a phenomenon (Oda, 1990). For example, GOT and GPT activities in fish serum are known to be very useful as an index for diagnosis of liver function (Ozaki, 1978; Yamamoto, 1981). In this study, the GOT activities in fish fed diets 2–5 were significantly lower than those of control fish (Figure 2). The GOT activities in all fish seem to be normal values (Hille, 1982). It is reported that Ast is safe to administer to animals without exerting any toxicity for the cells (Miki, 1993) and the dietary Ast shows an improvement in the histology of the fish liver structure (Segner et al., 1989). Hence, the data obtained suggest that any serious damage or malfunction might not occur in the liver of fish fed a diet containing red yeast or synthetic Ast. The GOT activity of fish fed diet 2 tended to be lower than that of fish fed diets 4 and 5 (Figure 2). The Ast content in the muscle from fish fed diet 2 was significantly lower than that from fish fed diets 4 and 5. Thus, the amount of Ast that is required to decrease the serum GOT activity appears to be small.

The results mentioned above suggest that the red yeast and synthetic Ast give positive metabolic effects on fish, e.g. acceleration of digestion and/or absorption. At the present time, it is not yet clear why the GOT and GPT activities do not decrease simultaneously (Figure 2). However, similar observations have been reported (Ito, 1973; Racicot et al., 1975; Williams and Wootten, 1981).

LPO in serum are thought to be metabolites derived from various organs such as the liver, kidney, blood platelets, and leukocytes (Hata, 1986). For example, certain diseases (arteriosclerosis, hepatitis, etc.) of animals are thought to relate to ROS and are observed to be accompanied by an increase in serum LPO (Hata, 1986; Saito, 1988; Furukawa and Suga, 1988; Inoue, 1992). Hence, serum LPO seems to be an indicator of damage to various tissues. In mammalian serum, most LPO are observed to exist in lipoproteins (Hata, 1986). Lipoproteins are known to contain lipids, such as triglyceride, cholesterol, and phospholipid (Chapman, 1980), that appear to be easily oxidized. The resulting oxidized lipoprotein is thought to cause great damage to the endothelial cells of blood vessels, followed by disturbance of the blood cycle system, and finally the occurrence of certain diseases in many organs (Inoue, 1992; Terao, 1992). Thus, LPO is thought to be a key factor that significantly increases the risk of several diseases. In fish serum, it has been reported that there are many kinds of circulating small molecule antioxidants, such as ascorbic acid (Sato et al., 1991), bilirubin (Tsuda et al., 1994), α-tocopherol (Tokuda, 1994), and carotenoids (Ando, 1993). Unfortunately, the ability for decompose LPO in serum is thought to be insufficient, so it seems to be important to inhibit the generation of LPO (Hata, 1986). Both tocopherol and carotenoids are observed in lipoprotein of fish serum (Ando, 1993; Tokuda, 1994) and are thought to protect the lipoprotein from oxidation with other circulating antioxidants (Machlin and Bendich, 1987; Ando, 1993). Furthermore, at low oxygen pressure in most tissues under physiological conditions, carotenoids, such as β-carotene and Ast, should exhibit radical trapping activity (Burton and Ingold, 1984; Olson, 1993). Thus, in the tissues of fish fed the red yeast or synthetic Ast, the generation of ROS is probably inhibited and lipoproteins are protected from oxidation.

In conclusion, a prime important factor affecting consumer acceptance of salmonids is the distinctive red color of their muscle; a variety of sources of dietary carotenoids, such as crustacea, algae, and the red yeast, have been tested for coloration of fish, and a great amount of data is available on this subject (Storebakken and No, 1992; Mori, 1993). Because salmonids are unable to synthesize the carotenoids de novo, they depend on the accumulation of pigments from dietary supplies (Storebakken and No, 1992). Therefore, this paper demonstrates for the first time that the dietary intake of red yeast or synthetic Ast has an effect not

only on improvement of pigmentation but also on fish health, and this work should provide significant information from the novel standpoint of usage of red yeast and synthetic Ast for aquaculture.

We plan to determine the effect of dietary red yeast or synthetic Ast supplementation to fish on fish tissues which are subjected to oxidative stress caused by oxidized oil administration. To clarify unknown factors in the red yeast, which affect biochemical features of fish health, further work is in progress.

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