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Some Antioxidants and Malondialdehyde Levels in the Flesh of Rainbow Trout, (*Oncorhynchus mykiss* W., 1792) from Various Feeding Habitats

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Abstract

The present study was aimed to find the effect of feeding habitats on the amounts of some antioxidants (vitamin A, E, C, ß-carotene and selenium) and malondialdehyde (MDA) levels in the flesh of rainbow trout (*Oncorhynchus mykiss*). For this purpose, vitamins (A, C and E), β-carotene amounts and malondialdehyde (MDA) levels were determined by HPLC and selenium amount was determined by fluorometric method in the flesh of rainbow trout obtained from various feeding habitats. The highest amounts of vitamins (A, C and E), β-carotene and selenium were found in the flesh of wild rainbow trout (WRT), followed by cage reared rainbow trout (CRRT) and pond reared rainbow trout (PRRT). However, the levels of MDA in the flesh of PRRT were the highest, followed by CRRT and the lowest in WRT.

Key words: Rainbow trout, *Oncorhynchus mykiss*, Euphrates River, Vitamins, β-carotene, Selenium.

Introduction

Fish nutrition has an important impact on several parameters directly influencing the quality of the fish, such as colour and appearance, smell and taste, texture, nutritional quality, shelf life, and level of contaminants. The need for improved knowledge of fish nutrition is therefore of great importance (1).

In normal conditions aerobic organisms are protected against oxidative damage by a variety of antioxidant systems. The antioxidant system is divided into two groups as enzymatic and non-enzymatic. Non-enzymatic system constitutes antioxidant vitamins such as vitamin A, C, E and selenium (Se) have been shown to react with organic free radicals and to protect bio-membranes from damage induced by these free radicals (2, 3).

Reactive oxygen species (ROS) such as hydroxyl radicals, superoxide anions, and hydrogen peroxide, which are produced by activated granulocytes, play an important role in many biochemical processes such as intracellular messaging in the cell differentiation, apoptosis, immunity, and defence against microorganisms (4, 5). In contrast, relative overproduction of these reactive species, which occurs in inflammation, results in oxidative stress. Main targets of ROS are the polyunsaturated fatty acids in cell membranes causing lipid peroxidation and malondialdehyde (MDA) formation, which may lead to damage of the cell structures and function (6).

Lipid peroxidation occurs in polyunsaturated fatty acids. The process is initiated by an -OH when it captures a hydrogen atom from a methylene carbon in the polyalkyl chain of the fatty acid. During lipid peroxidation, malondialdehyde (MDA), a highly reactive dialdehyde, which is an end-product of polyunsaturated fatty acid peroxidation, can be generated (7). Lipid peroxidation is a free radical chain reaction (8) which causes the degeneration of cell membranes. Most products of lipid peroxidation are known to have mutagenic and/or carcinogenic properties (9). Free radical species affect all important components of cells such as lipids, proteins, carbohydrates and nucleic acids (10).

Several reports have indicated that stress affects synaptic plasticity, dendritic morphology and neurogenesis in animals (11) and induces both clinical and anatomical features of neurotoxic damage in humans (i.e., posttraumatic stress disorders) (12).

Lipids are oxidized by free radical attack, and hence membranes are damaged (13). Lipid peroxides are disintegrated quickly and form reactive carbon compounds. Among these, MDA is an important reactive carbon compound which is used commonly as an indicator of lipid peroxidation (14).

The aim of the present study was to investigate the effect of feeding habitats on the amounts of antioxidant vitamins (A, C and E), ß-carotene, selenium and MDA level (an indicator of lipid peroxidation).

Materials and methods

Experimental animals

In this study, a total of 30 rainbow trout (*Oncorhynchus mykiss* W., 1792) (1-2 years old, 400-800 g in weight), including 10 fish from each habitat, were used. Pond reared rainbow trout (PRRT) were obtained from a commercial fish farm (Keban Alabalık Ltd.), Cage reared rainbow trout (CRRT) were obtained form a cage unit established on Euphrates River and wild rainbow trout (WRT) were caught from Euphrates River. Fish were anaesthetized with benzocaine (25 mg/l water). Nearly 5 g flesh samples from each fish without skin were removed, labelled and kept at –20ºC in a deep freezer until analyzed.

Table 1. The amounts of antioxidant vitamins (A, C and E), β-carotene, selenium and MDA levels in the flesh of pond reared rainbow trout (PRRT), cage reared rainbow trout (CRRT) and wild rainbow trout (WRT). Results are given as mean ± standard deviation (SD), n=5.

	PRRT	CRRT	WRT
Vitamin A, µg/g	3.32 ± 0.67^{a}	4.28 ± 0.62^{b}	5.96 ± 0.94^{c}
Vitamin C, µg/g	60.61 ± 5.36^{a}	57.85 ± 8.72^{a}	65.54 ± 7.16^{a}
Vitamin E, µg/g	5.85 ± 1.00^{a}	7.3 ± 0.71^{b}	9.96 ± 1.35^{c}
β-Carotene, µg/g	4.71 ± 0.52^{a}	6.82 ± 0.65^{b}	19.37 ± 2.31^{c}
Selenium, ng/g	157.4 ± 9.19^{a}	183.60 ± 11.69^{b}	224.00 ± 20.70^{c}
MDA, nmol/g	0.81 ± 0.07^{a}	0.64 ± 0.08^{b}	0.38 ± 0.11^{c}

Mean values with different superscript letter in the same row are significantly different (P<0.05; ANOVA Duncan's Multiple Range Test).

Determination of vitamin A, E and ß-carotene

Sampling procedure was as follows; rainbow trout flesh was homogenized, 1.0 g of sample was then taken to a polyethylene tube and 2.0 ml ethanol was introduced to the flesh sample to precipitate the proteins. After that, it was separated by centrifuge, followed by the addition of 0.3 ml n-hexane, and mixed well, and then vitamins were extracted. This step was repeated again, and n-hexane phase added to the first one. Under nitrogen atmosphere, n-hexane was evaporated to dryness. Then 0.2 ml methanol was added to solve the residue followed by injection to HPLC. Separations were accomplished at room temperature with a Cecil liquid chromatography system (Series 1100) consisting of a sample injection valve (Cotati 7125) with a 20 µL sample loop, an ultraviolet (UV) spectrophotometric detector (Cecil 68174) operating at 326, 296 and 436 nm for vitamin A, E and ß-carotene respectively, an integrator (HP 3395) and a Techsphere ODS-2 packed (5 µm particle and 80 °A pore size) column (250x4.6 ID) with methanol : acetonitrile : chloroform (47: 42: 11, v/v) as the mobile phase at 1.0 mL min^{-1} flow rate (15).

Determination of vitamin C and MDA

Vitamin C and free MDA were determined by the following procedure; 1.0 g flesh sample was taken in polyethylene tube and 0.5 ml of perchloric acid (0.5 M) was added (16), and then the volume of sample brought to 3.0 ml, after that it was centrifuged for ten minutes at 4500 rpm. The supernatant was filtered and the vitamin C amount was determined by using the method of (17) and MDA levels by (18). A Supelcosil LC-18-DB HPLC reversed-phase column (3 µm particle size and 250 x 3.9 ID) was utilized for the detection of vitamin C and MDA levels. While a 3.7 mM phosphate buffer, pH 4.0 mobile phase was used at 1.0 mL min^{-1} flow rate to determine the vitamin C amounts, the free MDA level was determined with a 30 mM KH_2PO_4 buffer, pH=4 with H_3PO_4 and methanol (65%-35% v/v) mobile phase at 1.5 mL min^{-1} flow rate.

Determination of selenium

The flesh samples for Se determination were treated as follows; 2.5 mL nitric acid: perchloric acid (1:5, v/v) was added to 2.0 g flesh samples and the mixture was held in an Teflon bomb at 100°C for 12 h for breaking the organic material and then cooled down to room temperature. Mixture was transferred into tubes and a 4.0 N HCl concentration was achieved by adding concentrated HCl (about 2.0 mL). The mixture was held at 90 °C for 15 min. to reduce Se (VI) to Se (IV). To this mixture 2.0 mL 2.5 M formic acid, 5.0 mL 0.1 M EDTA and 1.5 mL freshly prepared 3,3-diaminobenzidine (DAB) solution (1.0 mg/mL) was added and the pH of mixture was adjusted to 1.7 with 4 N NH_3 and let stand in dark for 1.0 h for the formation of a metal-ligand complex. Mixture was made to 50 mL by adding H_2O and 5 mL toluene and mixed for 2.0 min. The mixture was transferred into a volumetric separation funnel and let stand at room temperature for 2 min for phase separation. Se was separated in toluene phase and its amount was determined fluorometrically by a Perkin Elmer 100 fluorescence spectrophotometer at 570 nm using standard addition method (19).

All chemicals and reagents used were of analytical grade and were purchased from Merck Chemical Co. (Darmstadt, Germany). Double distilled water used to in the all studies.

Statistical analysis

The SPSS software (SPSS Inc, Chicago, IL, USA) was used for statistical analyses. Results for the groups are expressed as means ± standard deviation (*SD*). Differences between the group's means were analyzed for significance using the ANOVA Duncan's Multiple Range Test. Statistical significance was defined as P<0.05.

Results and Discussion

In this study, the recovery rates were determined to be 98.0% for vitamin A, 99.3% for vitamin E, 96.0% for vitamin C, 95.4% for β-carotene, 95.0% for Selenium and 98.2 % for MDA.

The amounts of vitamins (A, C and E), β-carotene, selenium and MDA levels in the flesh of PRRT, CRRT and WRT are given in Table 1. Apart from vitamin C, all other antioxidants and MDA levels amongst fish groups were statistically found significant (P<0.05). Antioxidants (vitamins A, C and E, β-carotene and selenium) levels were found the highest in the flesh of WRT and it followed by CRRT and PRRT. However, MDA levels in the flesh of fish samples showed a reverse state compared with the antioxidants (Table 1). According to these results, it may be said that WRT caused less stress compared with CRRT and PRRT. Antioxidant status may play a critical role in the defence against oxidative stress. Since α-tocopherol stops lipid peroxidation by trapping the free radicals. In this process, α-tocopherol is converted to α-tocopheroxyl radical. Vitamin C regenerates α-tocopherol from α-tocopheroxyl radical (20, 21). Vitamin C may have an important role in regeneration of reduced form of vitamin E (20). Malondialdehyde (MDA) is a stable end-product of peroxidation of membrane lipids by reactive oxygen species, and is widely used as an indicator of increased lipid peroxida-

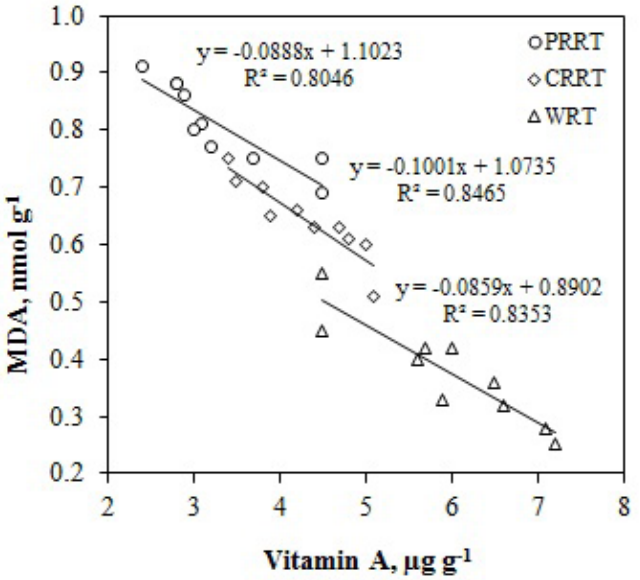

Figure 1. Correlation between vitamin A and MDA levels in rainbow trout.

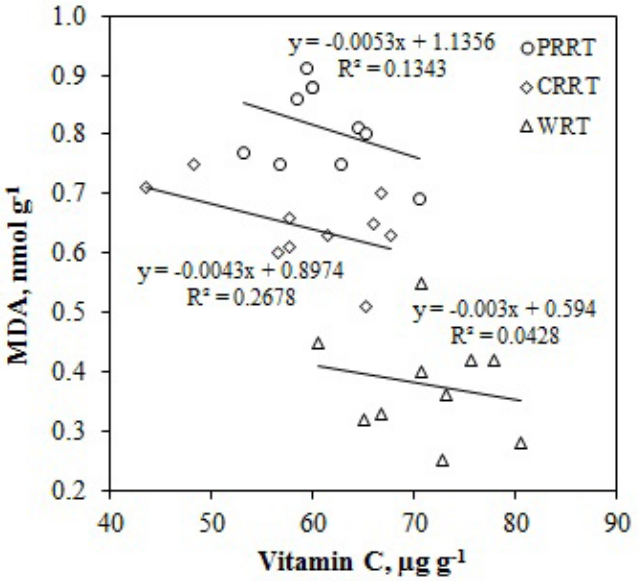

Figure 2. Correlation between vitamin C and MDA levels in rainbow trout.

tion (22,23).

The correlations of vitamins (A, C and E), β-carotene and selenium levels with MDA level are given in Figs. 1-5. These correlations were negative-linear for all antioxidants and very strong for vitamin A (Fig. 1) and vitamin E (Fig. 3). Although vitamin C is a very strong antioxidant, its correlation with MDA was found poor (Fig. 2). The reason for this may be that it is a water soluble vitamin and cannot be storage in the body of animals. The correlations of β-carotene and selenium levels with MDA level were also found poor. This may be expected result, because they are not as strong antioxidant as vitamins.

This first defence system tries to handle all free radicals, but if the oxidative stress is far greater than the capacity of the system, the second line of defence (vitamins and other antioxidants) may come into play. Vitamins scavenge and quench the free radicals, but are oxidized and inactivated in the process. Each of these antioxidant nutrients has specific activities, and they often work synergistically to enhance the overall antioxidant capacity of the body (24).

Selenium (Se) has effect on preventing decomposi-

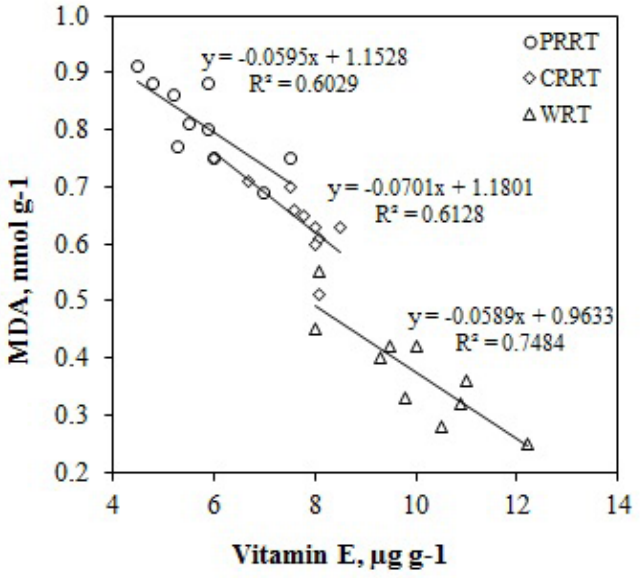

Figure 3. Correlation between vitamin E and MDA levels in rainbow trout.

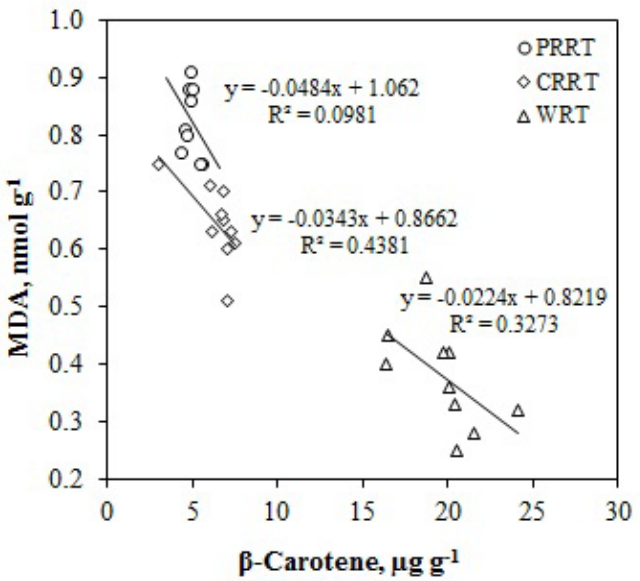

Figure 4. Correlation between ß-carotene and MDA levels in rainbow trout.

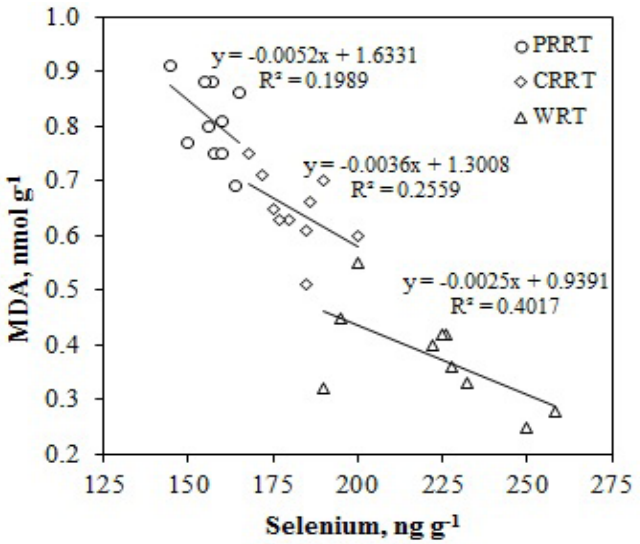

Figure 5. Correlation between selenium and MDA levels in rainbow trout.

tion, absorption and biological activity of α-tocopherol (25). At the same time, Se protects the cell by inhibiting free oxygen radical production (26). Moreover, an

important antioxidant, vitamin E is transported by selenoproteins as a free radical scavenger; ascorbate works lipid rich areas of the cell, interacting with vitamin E in the later medium. The same property of vitamin C prevents the formation of nitrosamines from nitrites and nitrates (27). Vitamin C inhibits division and growth of cell through the production of hydrogen peroxide, which damages the cells probably through an unidentified free radical(s) generation/mechanism (28).

In conclusion, the results of the present study show that PRRT have more free radicals and this state decreases the amount of antioxidants in the flesh these fishes. It was suggested to add more vitamins in the diet of PRRT in order to reduce free radical formation and to increase the quality of fish meat.

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