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Susceptibility to oxidative damage in wild and cultured Brook Trouts (Salvelinus Fontinalis Mitchill, 1815)

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Abstract
The aim of this study was to determine susceptibility to oxidative damage of wild (WBT) and cultured brook trout (CBT) (*Salvelinus fontinalis* Mitchill, 1815). The average weights of fish were 90 g and 60 g for CBT and WBT, respectively. Fishes used in the present work were randomly selected from ponds and pool. Fifteen fish were considered for each group. CBT were fed with commercial feed including 48-52% crude protein twice a day whereas WBT were fed with natural foods. At end of experiment, the levels of MDA, HDL and PON/HDL in the serum samples obtained from CBT was observed higher than the one obtained from WBT. However, these differences were found statistically insignificant. Our results showed that size, partial starvation, feeding and sex of the animal, stress in same fish species may be reason for the change of PON activity, MDA and HDL levels.

Keywords: Malondialdehyde, Paraoxonase activity, High-density lipoprotein, brook trout

1. Introduction

Fish is one of the most important aquatic organisms and they are important protein source for human nutrition [1, 2]. Biochemical and hematological blood parameters is an important tool used in following the general health status of fish as well as physiological and pathological changes of fish [3, 4]. Different investigators have shown that the values for biochemical blood parameters may vary in physiology and pathology [4].

Paraoxonase (PON) is an enzyme related to HDL containing apolipoprotein (apo) A1 that hydrolyzes organophosphate compounds such as oxidized phospholipids and lipid peroxides [5, 6]. PON hydrolyzes organophosphorus compounds such as insecticides and nerve agents; PON metabolizes toxic oxidized lipids related to HDL [7]. Serum paraoxonase (PON) is an esterase (EC 3.1.8.1) synthesized by the liver. The enzyme is found to protect HDL against peroxidation. A protective role of paraoxonase has been suggested as well as an explanation of protective role of HDL against LDL oxidation [5, 8].

Lipid peroxidation consisting by free radicals is the major mechanism of cell damage or destruction [9]. Free radicals found in mammalian tissues are occurred in physiological and pathological conditions. The excessive production of free radicals is an important factor in terms of the tissue damage [10]. Due to this excessively reactive in nature, ROS can attack almost all biomolecules including lipid membranes [11]. Lipid peroxides occur as a result the oxidation of PUFA in the membranes by a free radical chain reaction [12]. The MDA used to assess oxidative stress is a lipid peroxidation product [13]. In the other words, MDA is considered a marker of oxidant / antioxidant balance. The increase in MDA can cause a variety of diseases. In both mammals and fish, inadequate nutritional intake of antioxidants may cause reduce the ability of the defense against oxidative stress and the occurrence of some diseases [13, 14].

HDL is composed of protein, phospholipid and cholesterol, and HDL is synthesized in the liver and small intestinal wall [15]. HDL is first introduced into the bloodstream and converts to spherical HDL mature circulating cholesterol esters. Cholesterol-enriched HDL is separated from cholesterol after it is returned to liver. HDL is responsible for transporting cholesterol from the tissue to the liver.

Therefore, the increased HDL is benefit of the organism while the decreased HDL is detrimental of the organism [13].

Brook trout is an important fish species with its commercial significance.

There is no available information about whether there is any relation between HDL and serum paraoxonase in brook trouts. In this study, we compared to the levels of malondialdehyde (MDA), paraoxonase activity (PON) and high-density lipoprotein (HDL) of wild (WBT) and cultured (CBT) brook trout (*Salvelinus fontinalis* Mitchill, 1815) reared in same water conditions.

2. Materials and methods

2.1 Fish and blood serum

WBT and CBT (*Salvelinus fontinalis* Mitchill, 1815) reared in the same water conditions were obtained from the Aquaculture Faculty at Atatürk University in Erzurum (22 November 2011) [3]. The weights were 90 g and 60 g for CBT and WBT, respectively. Fish used in the present work were randomly selected from ponds and pools, and fifteen fish were considered for the each group. CBTs were fed with commercial feed including (crude protein 45-52%, fat 17%, ash 10%, Gross energy 3648 kkcal) crude protein twice a day whereas WBTs were fed with natural feeds [13]. Handling time was kept very short so that the fish were not affected by the stress. Blood samples from fish were taken from caudal veins with the help of a syringe without causing stress and then the blood was taken into the anticoagulant tubes and waited for about 20 minutes for clotting. After clotting provided, the anticoagulant tubes were centrifuged at about 3000 rpm for 10 minutes. Samples were separated to serum. HDL concentration of the separated serum samples were analyzed by autoanalyzer Cobas C501 by using commercial kits [13].

2.2 Measurement of Malondialdehyde (MDA)

Serum MDA levels were obtained according to the method described by Karatas and Kocaman [13]. A mixture of 20% acetic acid, 8.1% sodium dodecyl and 0.9% thiobarbituric acid sulfate were added to the serum of 0.2 ml. Then, mixture was added distilled water to obtain total volume of 4 ml and was incubated for 1 hour at 95 °C. After incubation, the tubes were left to cool. Afterwards 1 ml of distilled water and 5 ml of nbutanol/pyridine (15:1, v/v) were added by mixing. The samples were centrifuged for 10 min at 4000 g. The supernatants were removed and absorbance was measured at 532 nm. Malondialdehyde level was measured as µmol/l.

2.3 Measurement of serum PON concentration

Tris-HCl acid buffer (pH: 8, 100 mM) containing 1mM CaCl2 and 1 mM paraoxon was added to the serum of 10 µL to obtain

1 ml. Absorbance increase caused P-nitrophenol has been obtained at 412 nm for each subsequent 30 seconds at the ambient temperature of 25 °C. The amount of P-nitrophenol was calculated as 17000 M-1 cm-1 using the molar extinction coefficient (PH: 8). One unit paraoxonase activity constitutes one nmol p-nitrophenol per min. Paraoxonase activity, is given in U / mL [16].

2.4 Measurement of serum PON activity

PON activity was calculated by reading the absorbance change per minute (ΔA/dk) resulting from p-nitrophenol product of the hydrolysis in 100 mMTris / HCl buffer (pH = 8) at 412 nm at 37 °C through 5 minutes. Molar absorption coefficient of

p-nitrophenol is 17,600 for the current test conditions. The amount of enzyme constituted 1 nmol p-nitrophenol per minute was expressed as a unit [18].

3. Statistical Analysis

PON activity was calculated by reading the absorbance change per minute (ΔA/dk) resulting from p-nitrophenol product of the hydrolysis in 100 mMTris / HCl buffer (pH = 8) at 412 nm at 37 °C through 5 minutes. Molar absorption coefficient of p-nitrophenol is 17,600 for the current test conditions. The amount of enzyme constituted 1 nmol p-nitrophenol per minute was expressed as a unit [16, 17].

4. Results

The results obtained from the serum malondialdehyde, paraoxonase activity and HDL levels have been given in Table 1 for WBT and CBT fed by natural and commercial feeds (twice a day), respectively. The level of MDA in WBT was found to be 1.40, times higher than the one present in CBT, respectively. PON, HDL and PON/HDL levels in CBT were found to be 1.28, 1.20 and 1.06 times higher than the ones present in WBT. However, there was not significant difference between groups ($p>0.05$).

Table 1: Variations of MDA, PON, HDL and PON/HDL levels in CBT and WBT

	CBT	%	WBT	%
MDA (µmol/L)	12.86±0.44		18.09±4.61	
PON (U/ml)	17.8±1.1	100	13.9±2.1	100
HDL (mg/dl)	28.6±2.9	100	23.7±2.4	100
PON/HDL	0.62±0.68	62	0.58±0.52	58

Results were given as mean±SD and %. WBT, Wild brook trout; CBT, cultured brook trout; MDA, Malondialdehyde; PON, paraoxonase activity; HDL, high-density lipoprotein; PON/HDL, paraoxonase/high-density lipoprotein. %: rate of PON activity in HDL, and total HDL in the blood and total PON in the blood

5. Discussion

Oxidized fats are contained lipid peroxide in tissues. Lipid peroxide lead to decomposition of the oxygen-containing radicals [13]. Decomposed free radicals attack to all cell components such as proteins, lipids and initiate oxidative stress [18, 19, 20]. MDA is a sensitive indicator in terms of determination of damage in organs, cells and tissues depend on the increase in the lipid peroxidation [11]. We compared MDA levels, a consequence of lipid peroxidation; this parameter was used as biomarker of pro-oxidant situations in mammals [21]. Based on MDA levels in the present, our results clearly showed that WBT were found to be 1.40 times higher than the one present in CBT. There were not any pathological cases or anomalies detected in groups (WBT and CBT). However, the feed intake and growth of WBT were slower than the ones of CBT in our study. This

may lead to increase of MDA level. MDA was affected by many factors such as stress, source in feed, and nutrient antioxidants (A, D, E, K vitamins) [13]. Available information concerning the influence of food in fish is limited. In a study conducted with sea bream (Sparus aurata), it was reported that long-term deprivation caused a significant increase in MDA levels. [21]. Studies conducted on rainbow trout, Oncorhynchus mykiss [22] and sea bream, S. aurata [21] indicate that partial deprivation increases MDA levels.

PON protects phospholipids in HDL from further oxidation. This protection may be associated with hydrolyze of some oxidized phospholipids of paraoxonase [23]. In varioustrials, PON prevents oxidative stress by inhibiting oxidation of cell membrane lipids induced by ROS [17]. PON activity of fish is indicated as very low or close to zero [24].

Based on PON levels in the present, our results showed that the serum PON level in WBT was higher than in CBT. The levels of PON of Pacu (*Piaractus mesopotamicus*), Matrinxas (*Brycon cephalus*), Casudo (*Hypostomus punctatus*), Dourados (*Salminus brasiliensis*), Rainbow trout (*Oncorhynchus mykiss*) and Albino rainbow trout were determined as 6.6, 1.50, 6.1, 37.3, 69.8 and 58.4 U/ml by Baston [24] and Karataş and Kocaman [13]. In this case, the serum PON may be important in reducing the adverse effects of MDA. Follya [23] reported that HDL has been related to the serum paraoxonase in pacu fish. In another study, Baston [24] reported that 95% ratio of PON/HDL in dourados was recovered in the HDL fraction. In this study, these ratios were 62–58% for CBT and WBT, respectively.

HDL, a multifunctional lipolipoprotein is responsible for the cholesterol transport and metabolism. It plays defensive role against diseases in carp and trout and in teleost fish [25]. In addition, HDL has important defensive function, maintaining invariantly high concentrations in the plasma even in diseased fish [13]. In the present study, the value of serum HDL in WBT was insignificantly lower than in CBT. Feed intake of CBT was higher than the one that WBT. Therefore, adequate feed intake or size of WBT may lead to decrease of HDL level. Leger [26] reported that values of HDL in fish are directly affected by factors like size, partial starvation, feeding and sex of the animal, stress.

In result, the difference in feeding and size of the fish (WBT and CBT) may be an important reason for the change in MDA, PON and HDL levels. The high level of serum PON and HDL both fish and animal may be important in terms of preventing the damage to the tissues and the cells. Further researches should be performed in order to investigate the association of PON with HDL and MDA.

6. Acknowledgements

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