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Comparison of Oxidant and Antioxidant Status of Çoruh trout (*Salmo coruhensis*), Anatolian trout (*Salmo rizeensis*) and Rainbow trout (*Oncorynchus mykiss*) Spermatozoa

Filiz KUTLUYER[1]✉, Mehmet KOCABAŞ[2], Mine ERİŞİR[3], Fulya BENZER[4]

1. Munzur University, Fisheries Faculty, Tunceli, TURKEY.
2. Karadeniz Technical University, Faculty of Forestry, Department of Wildlife Ecology & Management, Trabzon, TURKEY.
3. Fırat University, Faculty of Veterinary, Department of Basic Sciences, Elazığ, TURKEY.
4. Munzur University, Faculty of Engineering, Department of Food Engineering, Tunceli, TURKEY.

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Absract: The aim of present study was to compare oxidant and antioxidant status of Çoruh trout (*Salmo coruhensis*), Anatolian trout (*Salmo rizeensis*) and rainbow trout (*Oncorynchus mykiss*) spermatozoa. Fish were obtained from Uzungöl. Enzymatic antioxidant activities (superoxide dismutase, catalase, glutathione peroxidase), glutathione and lipid peroxidation (malondialdehyde) were determined in spermatozoa of three trout species. Results indicated that catalase (23.36±0.36 K/g.protein), glutathione peroxidase (74.00±1.5 U/g.protein), glutathione (0.57±1.24 µmol/g.cell) and malondialdehyde levels (6.55±2.01 nmol/g cell) were highest levels in Anatolian trout (*S. rizeensis*) spermatozoa. In conclusion, differences among species caused alterations in the antioxidant and malondialdehyde levels.

Keywords: *Oncorynchus mykiss,* Oxidant and Antioxidant Status, *Salmo coruhensis*, *Salmo rizeensis*, Spermatozoa.

Çoruh Alabalığı (*Salmo coruhensis*), Anadolu Alabalığı (*Salmo rizeensis*) ve Gökkuşağı Alabalığı (*Oncorynchus mykiss*) Spermatozoasının Oksidan ve Antioksidan Durumunun Karşılaştırılması

Öz: Bu çalışmada, doğadaki Çoruh alabalığı (*Salmo coruhensis*), Anadolu alabalığı (*Salmo rizeensis*) ve gökkuşağı alabalığı (*Oncorynchus mykiss*) spermatozoanın oksidan ve antioksidan durumu üzerindeki etkilerinin belirlenmesi amaçlanmıştır. Balıklar Uzungöl'den elde edilmiştir. Üç alabalık türünün spermatozasında enzimatik antioksidan aktiviteleri (süperoksit dismutaz, katalaz, glutatyon peroksidaz), glutatyon ve lipid peroksidasyonu (malondialdehit) belirlenmiştir. Sonuçlar katalaz (23.36±0.36 K/g.protein), glutatyon (0.57±1.24 µmol/g.hücre), glutatyon peroksidaz (74.00±1.5 U/g.protein) ve malondialdehit seviyelerinin (6.55±2.01 nmol/g hücre) Anadolu alabalığının (*S. rizeensis*) spermatozoasında daha yüksek olduğunu göstermiştir. Sonuç olarak, türler arasındaki farklılıklar antioksidan ve malondialdehit seviyelerinde değişikliklere neden olmuştur.

Anahtar Kelimeler: *Oncorynchus mykiss,* Oksidan ve Antioksidan Durumu, *Salmo coruhensis*, *Salmo rizeensis*, Spermatozoa.

✉Filiz KUTLUYER
Munzur University, Fisheries Faculty, Tunceli, TURKEY.
e-mail: filizkutluyer@hotmail.com

INTRODUCTION

Oxidative stress is defined as the imbalance between the production of reactive oxygen species (ROS) and the ability of a cell or other biological systems to detoxification the reactive intermediates or to repair the damage (1). Generation of reactive oxygen species (ROS) is affected by cellular and environmental factors such as byproduct of cellular respiration, synthesized by enzyme systems, exposure to ionizing radiation, pesticides, pollution, and heavy metals. Antioxidant defense system includes antioxidant enzymes [catalase (CAT), glutathione peroxidase (GPx), glutathione-S-transferase (GST)] and other low molecular weight substances such as glutathione (GSH), vitamins and proteins located in different tissues. They can inactive the harmful effects of ROS. The body produces more antioxidant enzymes in order to eliminate of ROS damage (2).

Oncorhynchus mykiss and *Salmo trutta* are the most important Salmonid fish species owing to its aquaculture potential, wide consumer demand and economic value and, recreational fishery (3,32). Populations of *S. trutta* inhabit in the upper streams of rivers and North Africa, Europe, West Asia and Anatolia (4,5). Recently, *S. t. labrax* and *S. t. macrostigma* ecotype have been described by Turan et al. (6) as *S. coruhensis* and *S. rizeensis* (6,7). In addition, *S. coruhensis* is an endemic anadromus fish and only distributed in the rivers of Eastern Black Sea Region (9). To our knowledge, there are no reports about comparison oxidant and antioxidant status of spermatozoa of Çoruh trout (*Salmo coruhensis*), Anatolian trout (*Salmo rizeensis*) and rainbow trout (*O. mykiss*). Within this framework, this study focused on the comparison of oxidant and antioxidant status of spermatozoa in three trout species.

MATERIALS and METHODS

Collection of Spermazotozoa

This study was performed in accordance with the ethical guidelines stipulated by the ethical committee of the University of Karadeniz Technical University (Protocol No: 2016/36). Six mature endangered trout males (1665.18±0.48 g, 44.19±2.46 cm as mean±SD), Anatolian trout males (1355.01±0.23 g, 41.12±1.35 cm as mean±SD) and rainbow trout (1372.14±0.47 g, 43.82±4.32 cm as mean±SD) were captured Uzungöl Stream, Trabzon, Turkey for sperm collection between November and January. Temperature and dissolved oxygen of water were 5.1±1ºC and 8.7±0.3 mg L^{-1}, respectively. After the fish were anesthetized in 0.6 ml L^{-1} 2-phenoxyethanol, sperm samples were collected through abdominal massage and special care was taken to prevent contamination (e.g. blood, feces or urine). Sperm samples were kept on crushed ice until use. The pH of sperm samples was measured with a pH meter (Thermo Scientific Orion 5-Star Plus pH meter, USA). Spermatozoa density was evaluated using a hemocytometer.

Evaluation of Lipid Peroxidation and Antioxidant Enzyme Activity

Sperm samples were centrifuged at 3000×*g* at 4°C for 10 min and the sperm pellet in an ice bath was suspended in KCl (1.15%) at the 1:10 ratio (weight/volume) and then homogenized (28,29). For evaluation of lipid peroxidation, TBARS (thiobarbituric acid reacting substance) was measured as defined by Placer et al. (10). MDA values were calculated by absorption at 532 nm wavelength in the spectrophotometer. The superoxide dismutase (SOD) enzyme activity was assessed based on the method of Sun et al. (11). Glutathione peroxidase (GSH-Px) was evaluated according to the method of Matkovics et al. (13). Catalase activity was assessed by the method of Aebi (12). Protein concentrations were assessed according to the method of Lowry et al. (15).

Statistical Analysis

Statistical analysis were performed using SPSS 14.0 software and values were reported as mean±SD. ANOVA (one-way) with Duncan *post hoc* tests was used for assessment differences among groups. The level of significance was set as 0.05.

RESULTS

Sperm parameters (mean ± SD) are presented in Table 1. Levels of MDA, SOD, GSH, GSH-Px and CAT are shown in Figure 1. Our results indicated that statistically differences were determined among species; superoxide dismutase (SOD) (P= 0.013; P<0.05), catalase (CAT) (P= 0.006; P<0.05), glutathione peroxidase (GSH-Px) (P= 0.013; P>0.113), glutathione (GSH) (P= 0.400; P>0.05) and malondialdehyde (MDA) levels (P= 0.003; P<0.05). CAT (23.36±0.36 K/g.protein), GSH-Px (74.00±1.5 U/g.protein), GSH (0.57±1.24 µmol/g.cell) and MDA levels (6.55±2.01 nmol/g cell) were highest levels in Anatolian trout (*S. rizeensis*) spermatozoa.

Table 1. Sperm parameters (Mean±SD) of *Salmo coruhensis, Salmo rizeensis, Oncorhynchus mykiss.*
Tablo 1. *Salmo coruhensis, Salmo rizeensis, Oncorhynchus mykiss*'in sperm parametreleri (Ortalama±SD)

Species	Sperm volume (ml)	pH	Sperm density (×10^9)
Salmo coruhensis	6.76±0.23	7.70±0.12	6.24±0.22
Salmo rizeensis	7.25±0.15	7.80±0.15	9.67±0.43
Oncorhynchus mykiss	7.35 ±0.19	7.27±0.41	3.95±0.21

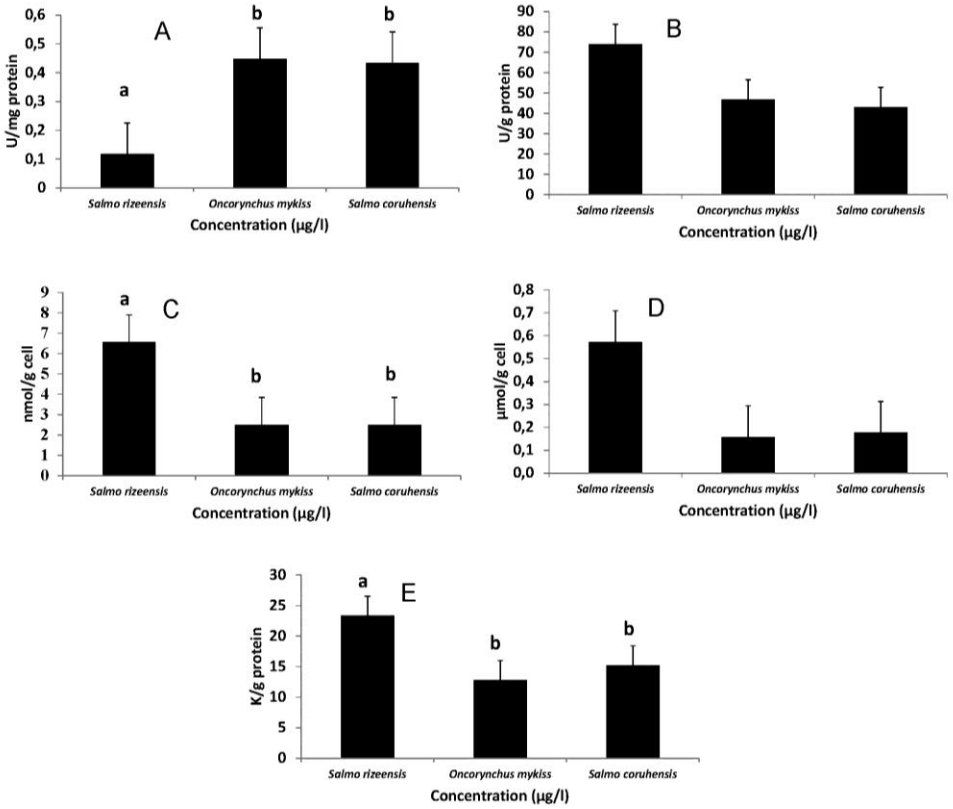

Figure 1. (A) SOD, (B) GSH-Px, (C) MDA, (D) GSH and (E) CAT levels of spermatozoa in *Salmo coruhensis*, *Salmo rizeensis* and *Oncorhynchus mykiss.*
Şekil 1. *Salmo coruhensis*, *Salmo rizeensis* ve *Oncorhynchus mykiss* spermatozoasında (A) SOD, (B) GSH-Px, (C) MDA, (D) GSH and (E) CAT seviyeleri.

DISCUSSION and CONCLUSSION

Behavior and physiology of fish are influenced by environmental factors (e.g. temperature, hardness, salinity, pH, pollutants, and habitat) (16). Habitat are important for aquatic animals due to affect each stage of life cycle, including egg, larvae, juvenile and adult and can also affect a variety of parameters including growth, improvement of feeding performance, feed intake, physiology of fish and stress (17-20). There are no comparative studies on oxidant and antioxidant status of Çoruh trout, Anatolian trout and rainbow trout spermatozoa. However, oxidant and antioxidant status of seminal plasma and spermatozoa has been compared in several fish species (21,30,31,33,34,35). In light of the above research we have examined the levels of the antioxidants SOD, CAT, GPX, GSH as well as lipid-peroxidation levels in spermatozoa of three trout species (*S. coruhensis, S. rizeensis* and *O. mykiss*) in the present study. Overall, we demonstrated differences in the physiological response of fish spermatozoa.

Malondialdehyde (MDA) is one of the oxidative damage products in lipid peroxidation and the presence in spermatozoa indicates oxidative stress (22). Our data indicated that the highest level of MDA concentration was in *S. rizeensis*, which means that differences in species affects the cellular response of fish spermatozoa facing ROS. CAT mainly found in peroxisomes and is responsible for the removal of hydrogen peroxide, which is metabolized to oxygen and water (23-25). The results showed that CAT activity was higher level in *S. rizeensis*. Reduced glutathione (GSH) is one of the most important antioxidant agents and protects cell membranes from lipid peroxidation (26). Our findings showed that glutathione (GSH) levels and GSH-Px activity were higher level in *S. rizeensis*. However, SOD activity was lower level in *S. rizeensis.* We suggested that the antioxidant response to stress can be explained by the sensitivity of high sensitivity of this endangered species (*S. rizeensis*). Since, *S. rizeensis* naturally inhabits in cold streams, rivers and lakes and, spawns in rivers and streams with swift water. Populations of this species migrate to tributaries and lake outlets, rarely spawning on stone, wave-washed lake shores. Spawning sites usually characterized by downward movement of water into gravel. Recently, populations of the species have been particularly affected by the local devastation in water sources through habitat modification and fragmentation, river damming and degradation of spawning habitats from constituted transversal structures by General Directorate of State Hydraulic Works in sampling area (Uzungöl, Trabzon) (27).

Consequently, based on the data obtained within the context of this study, the effect of each antioxidant is species-specific. The information will help to understand the effect of species on oxidant and antioxidant status of spermatozoa and provide benefit aspects related to fish farming and production.

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