

Antioxidant Enzymes and Lipid Peroxidation in *Alburnus filippii* (Kessler, 1877) and *Acanthalburnus microlepis* (Filippii, 1863): A Comparative Study

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Summary

In this study, the normal levels of various oxidative stress biomarkers in skeletal muscle in the Turkey freshwater fishes *Alburnus filippii* and *Acanthalburnus microlepis* were investigated. Fifty *A. filippii* (mean weight 5-10 g) and fifty *A. microlepis* (mean weight 5-8 g) were caught in Kura-Aras river basin in Easterian Turkey. The oxidative stress biomarkers that were analyzed included superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px) and, glutathione-S transferase (GST). Levels of reduced glutathione (GSH) and thiobarbituric acid reactive substances (TBARS) were also evaluated. The skeletal muscle antioxidant activity was generally higher in *A. filippii* than *A. microlepis* while SOD, GST activity was similar in the muscle of both species. CAT activity in skeletal muscle of *A. microlepis* was significantly higher than in the *A. filippii* ($p<0.001$) this finding becomes to this muscle in one of the most severally affected.

Keywords: Fish, Oxidative stress, Antioxidant enzymes, Lipid peroxidation

Alburnus filippii (Kessler, 1877) ve *Acanthalburnus microlepis*'te (Filippii, 1863) Lipit Peroksidasyonu ve Antioksidan Enzimler: Karşılaştırmalı Bir Çalışma

Özet

Bu çalışmada, Türkiye tatlı su balığı *Alburnus filippii* ve *Acanthalburnus microlepis*'in iskelet kaslarındaki çeşitli oksidatif stres göstergelerinin normal seviyeleri araştırıldı. Türkiye'nin Kuzey Doğu Anadolu Bölgesi, Kura-Aras nehri havzasında ortalama 5-10 g ağırlığında 50 adet *A. filippii* ve ortalama 5-8 g ağırlığında 50 adet *A. microlepis* yakalandı. Uygun koşullarda laboratuvara getirilen bu balıkların iskelet kaslarında süperoksit dismutaz (SOD), katalaz (CAT), glutatyon peroksidaz (GSH-Px) ve glutatyon S-transferaz (GST) enzimlerini içeren oksidatif stress göstergelerinin seviyeleri analiz edildi. Redükte glutatyon (GSH) ve tiyobarbitürik asit reaktif maddelerinin (TBARS) seviyeleri de değerlendirildi. Iskelet kasında antioksidan aktivite yönünden SOD ve GST enzimleri her iki türde de benzer sonuçlar verirken genel olarak antioksidan aktivite *A. filippii*'de daha yüksek düzeyde görüldü. *A. microlepis*'in iskelet kası CAT aktivitesi *A. filippii*'den önemli oranda ($p<0.001$) daha yüksek tespit edildi. Bu bulgularla balıkların iskelet kasında antioksidan aktivite yönünden özellikle CAT enzimi bakımından *A. microlepis* iskelet kasının çok daha etkili olduğu, diğer oksidatif stress göstergelerinin ise *A. filippii*'de daha fazla olduğu sonucuna varıldı.

Anahtar sözcükler: Balık, Oksidatif stres, Antioksidan enzimler, Lipit peroksidasyonu

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INTRODUCTION

Fish are particularly sensitive to environmental contamination of water, and pollutions may significantly damage certain physiological and biochemical processes when they enter the tissues of fish¹. Because metabolism of toxicant at extra hepatic stress is likely to be involved in systemic effects on reproduction, immune function, and other cellular functions^{2,4}. It is generally accepted that high level of oxygen consumption, seen in skeletal muscle compared to other tissues, results in higher concentrations of ROS⁵.

Aerobic organisms generate reactive oxygen species (ROS) such as super oxide anion radical ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($\cdot OH$) as a result of oxidative metabolism. $\cdot OH$ can initiate lipid peroxidation (LPO) in tissues⁶. The minimize the negative effects of ROS, fish, like other vertebrates, process an antioxidant defence system, which utilizes enzymatic and non-enzymatic mechanisms. ROS are highly reactive molecules, that indiscriminately interact with essential macromolecules, such as DNA, proteins and lipids, leading to the disturbance of physiological processes⁷. The enzymes that provide the first line of defence against $O_2^{\cdot-}$ and H_2O_2 include SOD (EC 1.15.1.1), CAT (EC 1.11.1.6), GST (E 2.5.1.18), and GSH-Px (EC 1.11.1.9).

Antioxidant enzymes act as scavengers of the highly reactive intermediates produced in hydrocarbon metabolism to maintain cell homeostasis. Generally, these enzymes respond differently to different chemical compounds. An individual antioxidant enzyme is unable to provide a general marker of oxidative stress because of the complexity of interactions between provident factors and antioxidants. Therefore multiple antioxidant enzyme values are often measured together to indicate the total oxyradical scavenging capacity, which has a greater indicating value^{8,9}.

Estimation of lipid peroxidation has been successfully employed in aquatic bio monitoring studies^{10,11}. The extent of lipid peroxidation is determined by balance between the production of oxidants and the removal and scavenging of those oxidants by antioxidants¹².

The family Cyprinidae is the richest and most important family of fish, and its members are distributed throughout the world¹³⁻¹⁵. The vast majority of boned fish belongs to this family in Turkey, and these are distributed widely in freshwater sources. Although this family is represented by approximately 1500 species worldwide, there are only 70 species in Turkey.

We assessed normal changes in activities of both detoxification and antioxidant enzymes as well as lipid peroxidation and reduced glutathione levels in skeletal muscle of *Alburnus filippii* and *Acanthalburnus microlepis* to determine ability tolerate the pollution of endemic fish species.

MATERIAL and METHODS

Alburnus filippii (n:50) and *Acanthalburnus microlepis* (n:50) males (mean weight 5-10 g) and *Acanthalburnus microlepis* (mean weight 5-8 g) were caught in Kura-Aras river basin in eastern Turkey.

The experimental animals, the *Alburnus filippii* and *Acanthalburnus microlepis* were obtained were transported alive to the laboratory, and kept in a well-aerated aquarium for 7 days at 20-25°C to acclimatize before analysis. Both fish species were fed the same artificial diet, with an approximate chemical composition of 28.0% of protein, 3.5% lipid, 2.0% fiber, 7.0% ashes, 7.0% water vitamin A 28 800 IE, D₃ 1800 IE, E 95 mg, L-ascorbic acid 137 mg, and 7.0% carbohydrates.

Both of groups were killed. Their skeletal muscles were removed out immediately on an ice-cold plate, washed in physiological saline solution (0.59% NaCl), weighed, frozen in liquid nitrogen and kept at -80°C until analysed.

The skeletal muscles were homogenized in 0.25 M sucrose buffer (pH 7.4) using a steel homogenizer and then centrifuged at 9500 g for 30 min at centrifuge. The entire homogenization process was carried out at 4°C. Supernatants of tissue homogenate were used to determine antioxidant enzyme activities and TBARS and protein concentrations using a spectrophotometer.

Chemical Analysis

Lipid peroxidation contents were measured with TBARS in muscle homogenate by the method of Placer et al. ¹⁶, modified by Matkovic et al. ¹⁷, and was expressed in terms of the malondialdehyde (MDA) content, which served as a standard of 1,1,3,3-tetraethoxypropone (Sigma Chemical Company, St Louis, MO, USA). The values of MDA-reactive material were expressed in terms of TBARS (nmol/mL hemolyzed nmol/g tissue). GSH-Px (EC 1.11.1.9) activities of the skeletal muscles homogenate samples were measured spectrophotometrically at 37°C and 412 nm according to Lawrence and Burk ¹⁸. Catalase levels were determined using the methods described by Aebi ¹⁹. The CAT mediated the decomposition of H₂O₂ was followed directly at 240 nm. Glutathione S- transferase (GST) and SOD activities of the skeletal muscles were assayed according to the method of Habig et al. ²⁰. Reduced glutathione (GSH) was measured by the method of Beutler et al. ²¹. The activity of the enzymes and tissue GSH concentrations were calculated for 1 g protein content from the 13000 g supernatant fraction and protein concentration, in supernatants were determined by Folin-phenol reagent with bovine serum albumin as the standard ²².

Statistical Analysis

The collected values as mean±SE were statistically analysed (student's t-test) and evaluated using the SPSS 6.0 (1993) software program P values <0.001, 0.01, 0.05 were considered significant ²³.

RESULTS

In skeletal muscle, TBARS and GSH levels in *A. filippii* was significantly higher than in *A. microlepis*. The SOD activity was similar in the muscle of both species. The CAT activity in muscle of *A. microlepis* was significantly higher than in the *A. filippii*. The GSH-Px activity in skeletal muscle of *A. filippii* was significantly higher than in the *A. microlepis*, while GST activity was very similar in both species.

Table 1. TBARS, GSH and SOD, GSH-Px, CAT, SOD activity in the skeletal muscle of *Alburnus filippii* and *Acanthalburnus microlepis* (mean±S.E.M)

Table 1. *Alburnus filippii* ve *Acanthalburnus microlepis* iskelet kasında TBARS, GSH ve SOD, GSH-Px, CAT, SOD aktivitesi (mean±S.E.M)

PARAMETERS	<i>Alburnus filippii</i> (n=50)	<i>Acanthalburnus microlepis</i> (n=50)
TBARS (nmol/g tissue)	82.9±3.4*	73.3±4.1
GSH (nmol/g tissue)	79.8±4.1*	75.6±3.7
SOD (U/mg pro)	0.038±0.002	0.037±0.003
GSH-Px (U/mg pro)	0.029±0.003*	0.024±0.002
CAT (kU/mg pro)	6.06±0.6	7.68±0.5*
GST (U/g pro)	3.95±0.18	3.89±0.12

*p<0.001

DISCUSSION

Like all aerobic organisms, fish are also susceptible to the attack of reactive oxygen species and, as a consequence, have an antioxidant defense system, as demonstrated by Works dating primarily to the 1970s. Several circumstances promote the antioxidant defense response in fish. Factors intrinsic to the fish itself, such as age, phylogenetic position, karyotype analysis, and feeding behavior, as well as environmental factors such as the type of diet supplied, daily or seasonal changes in temperature, dissolved oxygen, toxins present in the water, pothole dissolved oxygen, and toxins present in the water, can either fortify or weaken antioxidant defenses ^{24,25}.

Buet et al. have observed the changes in oxidative stress parameters in fish as response to direct uranium exposure. They have determined the dose-dependent accumulation of uranium (U) waterborne in gills of juvenile rainbow trout following short-term exposure that direct exposure of fish to natural uranium had not induced catalase and SOD activities that are involved in the protection of organisms against reactive oxygen species. Contrary to what had observed for some other heavy metals in other conditions, the response of both enzymes was significantly reduced by U exposure suggesting a possible deterioration of the protective defense system of fish ²⁶. Soyer et al. have investigated the effect of glazing and

storage time on lipid oxidation of frozen fish. Results that involved thiobarbituric acid reactive substances (TBARS) and free fatty acids (FFA) as lipid oxidation parameters have indicated that glazing with some antioxidants or water of the fish retarded lipid oxidation when compared to control samples during frozen storage ²⁷. In the study another has determined that can substantially affect both physiological status and muscle tissue quality when fish exposed to cyanobacterial water blooms are under stress. Observations have indicated that exposure of fish to toxic cyanobacterial blooms induces oxidative stress, a fundamental factor of numerous diseases and accelerated ageing in living organisms ²⁸. Avcı et al. have observed that there was significant oxidation in the muscle tissue of the fish obtained in the river downstream of the industry compared to those obtained upstream of the industry. Although there were no meaningful differences between the SOD and GSH-Px activities, CAT activities has found to be reduced in the muscle tissues from the fish obtained downstream. In the liver tissues, the SOD and GSH-Px activities were found to be increased in the fish obtained downstream but no differences were observed in the CAT values ²⁹. Lenartova et al. have observed total GST activity was higher in fish from polluted areas ³⁰. The circumstances possible in our explanations will be differently for various pathways. These may demonstrate with the scientific studies that results of biochemical parameters are consistent with reported studies.

Mitochondria are the sites where ROS are mainly produced, and red muscles are the most important source of mitochondria in endodermis. Therefore, this tissue is considered to be the main contributor to ROS generation in mammals and birds. However, in most fish muscle makes up only a small proportion of tissues, and other tissues such as liver, kidney and also gills are more important in this regard ^{3,4,31}.

It is known that vertebrate liver exhibits high metabolism and oxygen consumption, and it probably best represents the status of antioxidant defenses in organisms, and therefore, it is frequently referred to in the literature ³²⁻³⁴. There was no evidence of biochemical in *Alburnus filippii* and *Acanthalburnus microlepis*. We assessed changes in activities of both detoxification and

antioxidant enzymes as well as lipid peroxidation levels in skeletal muscle of *A. filippii* and *A. microlepis*.

The lower oxygen consumption by Cyprinidae may explain the lower activity of the antioxidant enzymes. Another possible explanation, according to some authors, is that antioxidant enzymes appear to correlate with phylogenetic position, where more ancestral species exhibit less activity ^{35,36}. Also, atmosphere with a large percentage of O₂ (80%) causes oxygenation of heme pigments present in meat when the oxygen molecule binds to the central iron atom ³⁷. The formation of deep-red oxymyoglobin stabilizes the naturally red colouring of meat. In our experiment, too, we observed colouring different from normal of muscle tissue occurred as a result of oxymyoglobin formation.

In this study skeletal muscle, TBARS and GSH levels *A. filippii* was significantly higher than *A. microlepis*. GSH has been reported as a cofactor in thiol-disulfide Exchange reactions in the protection of protein-SH groups. These groups are involved in cell division, and their oxidation results in damage to this important function ³⁸. In fact, the progress of embryonic development is delayed by oxidative stress and protein-thiol group oxidation ³⁹. Oxidation processes in muscle may be related with the length of storage ⁴⁰. The TBARS and GSH levels and levels of the other parameters determined are influenced especially by barrier properties of the packaging sheet and biochemical processes in muscle tissue that determine keeping quality of meat and its colouring.

Also, in all muscles SOD activity in *A. filippii* was greater than in *A. microlepis* (Table 1). This is also reflected in the percentage of activity in the totality of the skeletal muscle.

CAT activity, found mainly in peroxisomes, is associated with elevated concentrations of H₂O₂. We detected higher activity of this enzyme in both species. In the muscle of both species a positive relationship was found between SOD and CAT, as reported for other fish ⁴. It is possible that the function of SOD, necessary for the formation of H₂O₂ on which CAT acts, might also involve the intervention of other enzymes, such as glycolate oxidase or urate oxidase ⁴¹. On the other hand,

based on the example of the digestive tract of the fish, high values of SOD activity may be associated with a low CAT activity, which in a certain way could be of set by a high GSH-Px and GSH levels, as found in this muscle.

It is reported that glutathione system constitutes a sensitive biochemical indicator of chemical pollution⁴². Fish from the contaminated area display a tendency toward decreased GST activity. This might be related to the fact that xenobiotics are detoxicated by GST pathways, which thus enabled fish exposed to toxic pollutants to survive^{42,43}. We have demonstrated the presence of GSH S-transferase activity at very early stages of the Cyprinidae development. We have investigated the biochemical parameters will be useful to determine effects on probable toxication and oxidative stress in endemic two fish species of water pollution for Kura-Aras River but these biochemical parameters also need to be studied many times for each different species in this region. Our results we believe to highly advantage to probable studies for continuity of endemic species.

Further studies, particularly comparative ones which take into consideration different physiological situations, will shed more light on the functional complexity of the *Alburnus filippii* and *Acanthalburnus microlepis* antioxidant defences with the aim of optimizing farming conditions of both species.

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