

## Effects of Water Temperature on Catalase Activity and Malondialdehyde Level in Muscle, Mouth, and Intestine Tissues of Doctor Fish (*Garra rufa*)

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(Alınış / Received: 15.11.2018, Kabul / Accepted: 05.04.2019, Online Yayınlanma / Published Online: 24.04.2019)

### Anahtar Kelimeler

*Garra rufa*,  
Doctor fish,  
Enzyme,  
Feeding,  
Temperature

**Abstract:** In this study, the catalase (CAT) activities and malondialdehyde (MDA) levels of some tissues (muscle, mouth and intestine) of *Garra rufa* (doctor fish) fed in different water temperatures (18°C and 34°C) were studied. No fish death was observed during the experiments. Despite the different significance levels, the proportional length increase, the proportional weight increase, food conversion efficiency of doctor fishes fed at 34°C were found to be lower than in fishes fed at 18°C. This can be due to the fact that doctor fishes living in a water temperature of 34°C have a higher metabolism, are more motile, and are more ready to the therapy. The CAT activities and the MDA levels of the tissues studied were found to be significantly different. The highest CAT activities and MDA levels were observed in intestine tissues. But the CAT activities and the MDA levels of the tissues studied for the fishes fed in 34°C water temperature were not significantly different from those of fishes fed in 18°C water temperature.

## Su Sıcaklığının Doktor Balık (*Garra rufa*) Türünün Kas, Ağız ve Bağırsak Dokularındaki Katalaz Aktivitesi ve Malondialdehit Düzeyleri Üzerine Etkileri

### Keywords

*Garra rufa*,  
Doktor balık,  
Enzim,  
Besleme,  
Sıcaklık

**Özet:** Bu çalışmada farklı su sıcaklıklarında (18°C ve 34°C) beslenen *Garra rufa* (doktor balık) türü balıkların bazı dokularında (kas, ağız ve bağırsak) katalaz (CAT) aktiviteleri ve malondialdehit (MDA) düzeyleri incelenmiştir. Deney süresince hiç balık ölümü görülmemiştir. Önemlilik dereceleri farklı olmakla birlikte, 34°C de beslenen doktor balıkların oransal boy artışı, oransal ağırlık artışı ve yem dönüşüm etkinlikleri 18°C de beslenen balıklardan daha düşük bulunmuştur. Bu durum, 34°C su sıcaklığında yaşayan doktor balıkların metabolizmalarının daha yüksek, daha hareketli ve terapiye daha istekli olmalarından kaynaklanmış olabilir. İncelenen dokuların CAT aktiviteleri ve MDA seviyelerinin önemli derecede farklı olduğu tespit edilmiştir. En yüksek CAT aktiviteleri ve MDA seviyelerinin bağırsak dokularında olduğu görülmüştür. Fakat 34°C su sıcaklığında beslenen balıkların incelenen tüm dokularındaki CAT aktiviteleri ve MDA seviyelerinin 18°C su sıcaklıklarında beslenen balıklardan önemli derecede farklı olmadığı tespit edilmemiştir.

### 1. Introduction

Doctor fish (*Garra rufa*) belonging to the Cyprinidae family is an important species widely used for ichthyotherapy. It was determined that doctor fishes have a beneficial effect on psoriasis but also on different skin diseases such as purulent wounds [1]. The doctor fishes, which do not have teeth, smooth the scabs, and clean the human skin until it becomes totally smooth by removing these parts by mouth movements [2]. *Garra rufa* is also known to be beneficial for acne [3]. In ichthyotherapy performed in

thermal tourism facilities, *Garra rufa* are exposed to quite high temperatures.

The temperature is quite effective on the life activities of all living organisms. As fishes are poikilothermic, their body temperatures are really close to the temperature of water in which they are. Thus, the modification of water temperature also directly affects all the life activities of the fishes. Many important activities such as nutrient intake, living weight gain, ovarium development, and reproduction are performed under the effect of water temperature.

Another factor related to the temperature is the oxygen consumption. While the oxygen retaining capacity of water decreases with the increase of water temperature, the oxygen need of the fish also increases together with the acceleration of the metabolism [4]. When the fishes are out of the optimal temperature limits, the enzymal activities are firstly damaged and the life of living beings is negatively affected.

Enzymes are special proteins produced in living cells and permit the realization of chemical reactions within living beings. There is a proportional increase between most of the chemical reaction speed and the temperature because the temperature increases the kinetic energies of the reactants. Meantimes, the enzyme activities increase up to a threshold with the temperature increase [5, 6].

The increased demand of doctor fish put also a pressure on natural stocks. As the collection of doctor fishes from nature is prohibited, its production by aquaculture started to expand. However, the biology of doctor fishes are not as well known as the other cultured fishes. Yet, the culture of a species is directly related to the knowledge about the biology of that species. There are limited studies on doctor fishes. In this study, the nutrient intake and evaluation, the growth and development and CAT activities and the levels of MDA which is the product of lipid peroxidation of some tissues of doctor fishes in 18°C water temperature which is their natural environment temperature and in 34°C water used for therapy purpose were studied.

## 2. Material and Method

### 2.1. Fishes studied and experiment plan

In this study, a total of 120 doctor fish (*Garra rufa*) with an average weight of 0.6-0.7 g were used. The fishes were first acclimated to laboratory conditions for 30 days and then experiments were started. During the experiments, 6 aquariums of 30x40x100 cm dimensions were used. Three of these aquariums were set at 18°C, while the other three ones were set at 34°C. The experiment pursued for 60 days. During the experimentation time, the physico-chemical properties of aquarium waters were controlled regularly and it was kept at a level so that it will not affect negatively the health of fish. 2% (first group) and 3% (second group) of living weight commercial food were given to the fishes for the first month and the second month respectively. The proportional length and weight increases (PLI, PWI) and food conversion efficiency (FCE) were calculated.

### 2.2. Biochemical Analysis

The fishes collected from the aquariums were dissected on ice. The collected tissues (muscle, intestine, and mouth) were rinsed using serum physiologic, wiped and weighted and kept at -80°C

until their analysis. Before starting the biochemical analyses, the studied tissues were homogenized in cooled 50 µM, pH = 7.4 sodium-phosphate buffer at 8000 rpm for 3 minutes. In order to prevent any loss of enzymatic activity due to heating, the samples were subjected to operations within ice. The homogenates were centrifuged at +4°C, at 9500 g for 30 minutes.

All studied tissues' protein concentrations were determined by the Bradford protein assay [7], using bovine serum albumin as a standard.

MDA analysis was performed by the double heating method of Draper and Hadley [8]. This method depends on the spectrophotometric measurement of the pink color produced during the reaction of thiobarbituric acid with MDA at 532 nm. The results were compared to the protein amounts and were expressed as nmol/ml.

CAT activity was assessed using Aebi method [9]. The principle of the method is based on the determination of hydrogen peroxide decomposition by CAT enzyme. CAT activity was measured by observing the change on absorbance of sample and blank for a minute by spectrophotometrically at 240 nm. The results were compared to the protein amounts and were expressed as EU/mg protein.

### 2.3. Statistical Analyses

JMP 6 package programme was used for the evaluation of arithmetic average, standard error, and differences between groups for the data obtained. T test was used for the determination of the differences between both groups.  $p < 0.05$  was considered as statistically significant.

## 3. Results

The proportional length increases (PLI) of the fishes used in the experiments are given in Figure 1. The proportional length increase (PLI) of the first group fishes fed in 18°C and 34°C were respectively 10.72 and 2.79 on average. The PLI values of the second group fishes fed in 18°C and 34°C were respectively 8.00 and 6.85 on average. While the decrease in the first group is statistically significant ( $p < 0.05$ ), the variation observed in the second group is not significant ( $p > 0.05$ ). The proportional weight increases (PWI) of the fishes used in the experiments are given in Figure 2. The proportional weight increase (PWI) of the first group fishes fed in 18°C and 34°C were respectively 27.64 and 14.98 on average. The PWI values of the second group fishes fed in 18°C and 34°C were respectively 39.35 and 29.35 on average. On the contrary to PLI, while the PWI did not show any significant variation in the first group, the PWI levels of fishes fed at 34°C in the second group are statistically low compared to the fishes fed at 18°C ( $p < 0.05$ ).

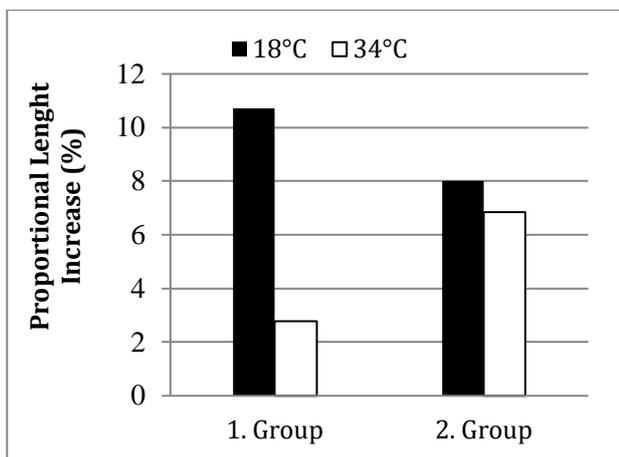


Figure 1. Proportional length increase of the fishes used in the experiments

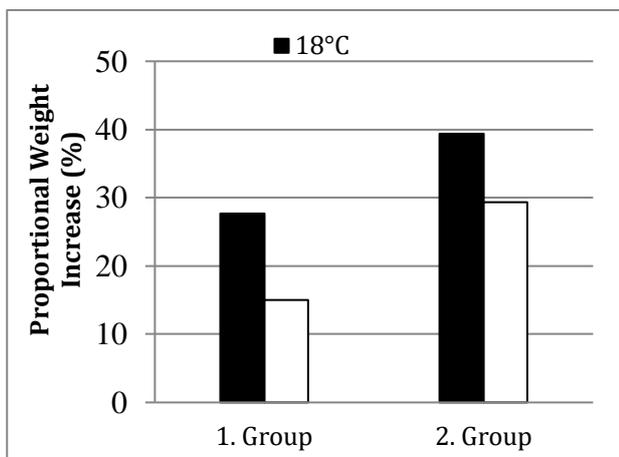


Figure 2. Proportional weight increase of the fishes used in the experiments

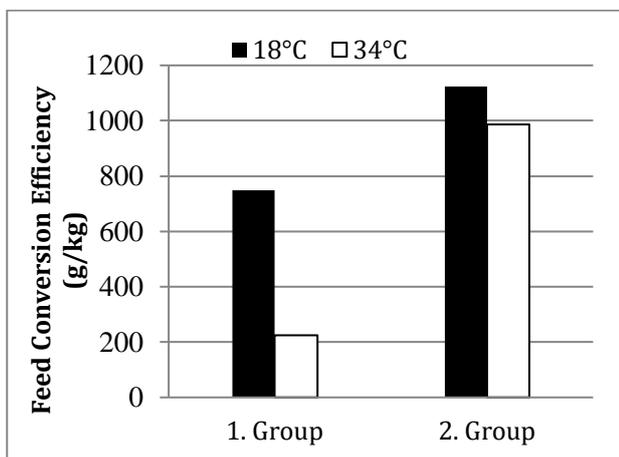


Figure 3. Feed conversion efficiency of the fishes used in the experiments

The food conversion efficiencies (FCE) of the fishes used in the experiments are given in Figure 3. The food conversion efficiency (FCE) of the first group fishes fed in 18°C and 34°C were respectively 748.72 and 224.6 on average. The FCE values of fishes fed in 18 °C water temperature in the first group were found to be significantly high compared to the fishes fed in 34 °C water temperature ( $p < 0.05$ ). The food conversion efficiency (FCE) of the second group fishes fed in 18°C and 34°C were respectively 1123.83 and 987.5 on

average. However, no statistically significant difference between the FCE values of the fishes fed in 18 °C and 34 °C in the second group was observed ( $p > 0.05$ ).

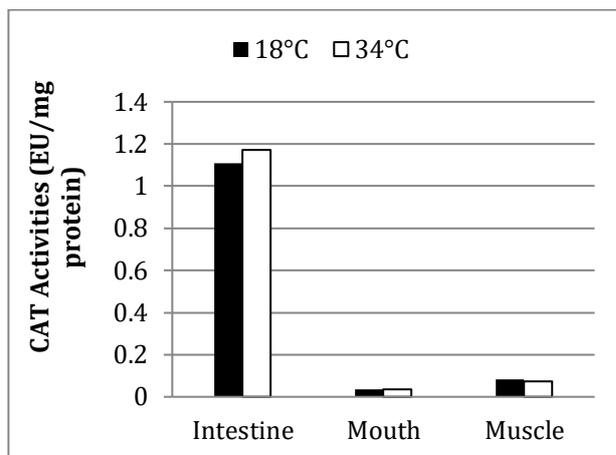


Figure 4. CAT activities of the fishes used in the experiments

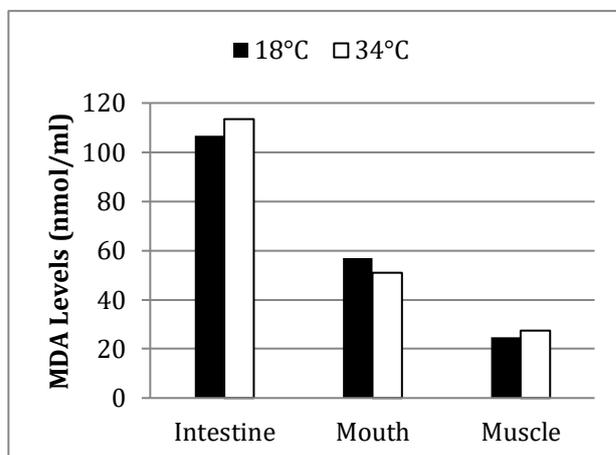


Figure 5. MDA levels of the fishes used in the experiments

The CAT activities (enzyme unit / mg protein) and the MDA values (nmol/mg protein) in muscle, intestine, and mouth tissues of the fishes used in the experiments are given in Figure 4 and 5. It was determined that CAT activities and MDA levels in muscle, intestine, and mouth tissues of *Garra rufa* are significantly different. The highest CAT activities and MDA levels were observed in intestine tissues. However, no important difference was observed for CAT activities and MDA levels in the studied tissues between the fishes fed in 18 °C and 34 °C water temperatures ( $p > 0.05$ ).

#### 4. Discussion and Conclusion

In the first group fishes (18 °C) fed daily with 2% living weight commercial feed, proportional length and weight increase and the parameters of food conversion efficiency were considered statistically different ( $p < 0.05$ ). With the same amount of feed, the fishes fed in 18 °C water temperature developed better than fishes fed in 34 °C water temperature; and the conversion rate of the food into meat is higher. In spite of this, as a result of the observations performed

during the experiments, it was determined that the fishes fed in 34 °C water temperature were more motile than fishes fed in 18°C water temperature, that their food intake were faster and shorter and that they were more open to therapy. On the contrary, the movements of the fishes fed in 18°C water temperature were slower, and their feed intake was quite long. This situation shows that as the fishes fed in 34°C water temperature are extremely motile, they use the energy taken from the food for movement, while as the fishes fed in 18°C water temperature are less motile, they use better the energy taken from the food for growth and development.

The water temperature is quite impactful on the growth and development of poikilothermic fishes. The same fish species consume more oxygen in high temperature water than in low temperature water. The high rate consumption of oxygen accelerates the metabolism and causes the fish to become more motile. Thus, it increases the energy needs [10]. The results obtained about the doctor fishes in this study were conform to the relevant results given in the literature.

No fish death was observed during the experiments. All the fishes lived in a healthy way. That is why it is possible to state that a high temperature such as 34°C used for therapy purpose will not create any trouble for the life of *Garra rufa*. Duman [11] and Karaaslan [4] also found results supporting our observations in their studies on *Garra rufa* and *Cyprinion macrostomus* species.

The increase of temperature has many effects such as the damage of the structure of proteins in the fish, the deactivation of enzymes, and the modification of cell membrane. In addition, increased temperatures cause damage to the cardiovascular system, nervous system, protein and enzymatic system in fishes. Heat stress causes harmful structural and functional modifications in enzyme and structural proteins [12]. In this study, the highest CAT activities and MDA levels were observed in intestine tissues while the lowest values were obtained in mouth tissues. However, it was established that 18 °C and 34°C water temperatures do not create an important difference in CAT activities and MDA levels in the studied tissues of *Garra rufa* ( $p>0.05$ ).

As a result, it is possible to say that even in high water temperature that cannot be reached frequently in natural environment, such as 34 °C used for therapy purpose, the life of *Garra rufa* is not affected negatively, and that the heat tolerance of these fishes is quite high.

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