BMC Veterinary Research

RESEARCH

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The effect of different oxygen concentrations on oxidative stress and some biochemical parameters in the transfer of adult rainbow trout (*Oncorhynchus mykiss*)

Utku Duran[1*] and Sena Çenesiz[2]

Abstract

Background Transfer operations are one of the stress sources that cause mortality in fish. This study investigated the effects of different dissolved oxygen concentrations on oxidative stress and several biochemical parameters during the transfer of adult rainbow trout (*Oncorhynchus mykiss*). The aim was to determine the optimum dissolved oxygen concentration to minimize stress and potential tissue damage during transfer.

Result The study analyzed GSH-Px, SOD, and LDH enzyme activities; MDA, Cp, TOS, TAS, OSI, cortisol, glucose, urea, uric acid, creatinine, Ca, Mg, and MDA levels; as well as Cp, TOS, TAS, and OSI values in serum and muscle tissue. Findings indicated that transfer and preparation for transfer induced oxidative damage in fish. Comparing different dissolved oxygen levels, it was observed that hypoxic and hyperoxic conditions increased ROS levels, suppressed the antioxidant mechanism, and caused oxidative stress.

Conclusions Among the tested conditions, transfer under normoxic conditions with a dissolved oxygen concentration of 10 mg/L was the most effective in minimizing oxidative stress and tissue damage. This suggests that maintaining adequate oxygen levels during transfer plays a crucial role in reducing physiological stress in rainbow trout.

Keywords Live fish transfer, Tissue damage, Rainbow trout (*Oncorhynchus mykiss*), Oxidative stress

Introduction

Rainbow trout (*Oncorhynchus mykiss*) has become one of the most widely cultivated fish species globally, having been introduced to all continents except Antarctica. According to 2020 data, global aquaculture production reached approximately 850,000 metric tons, representing a market value of roughly US$3.6 billion [1–3]. In rainbow trout farming, live transfer of both fry and adult fish can occur for reasons such as sale to different farms, improvement of water quality, and transfer of smolts to seawater for salmon production. Live fish transfer is a critical practice in aquaculture because it is an essential

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practice in the production process of species such as Rainbow Trout (*Oncorhynchus mykiss*) and Atlantic Salmon (*Salmo salar*), ensuring optimal growth conditions at different life stages. In practice, transport tanks utilize pressurized oxygen systems (typically 80–100% pure O_2) with diffusers or venturi injectors to maintain dissolved oxygen levels above 6 mg/L, while avoiding oxygen toxicity through continuous monitoring [4]. Additionally, it supports the economic viability of fish farms by allowing the distribution of live fish to markets or other production systems, ensuring continuous supply and meeting consumer demand. During these transport operations, it is known that environmental conditions such as the physical and chemical properties of water, as well as farm operations such as handling, live fish transfers, grading, and inoculation, cause stress in fish, leading to biochemical changes such as the release of catecholamines and corticosteroids as well as increased blood glucose levels [5–8]. Studies on stress in fish have shown that stress has consequences such as oxidative damage, suppression of the immune system, and thus increased disease susceptibility [9, 10]. To reduce the negative effects of transfer processes on fish in the aquaculture sector, applications such as the addition of sedative additives such as isoeugenol or salt to transfer water are performed [11, 12]. Sedatives like isoeugenol and salt are commonly used to reduce transport stress in fish, though their efficacy varies across species. For instance, while salinity adjustments benefit osmoregulation in Labeo victorianus [13], rainbow trout may require different approaches due to distinct physiological adaptations. The most important factor in the transfer of fish is the presence of sufficient dissolved oxygen in the transport water. It has been reported in previous studies that high oxygen levels may cause respiratory acidosis and low oxygen levels may cause death of fish due to hypoxia [14–16].However, sufficient dissolved oxygen levels alone do not necessarily indicate that fish are in good condition. In addition, factors such as stress and metabolic by-products also affect fish health. The ability of fish to utilize oxygen depends on the concentrations of metabolic products, such as stress, water temperature, pH, carbon dioxide (CO_2), and ammonia, and the tolerance of the fish to these products [17]. For this reason, it is very important to plan properly so that the metabolic waste generated during the transfer of fish does not harm the fish [18].

An increased concentration of dissolved CO_2 in water is harmful to fish and is one of the main limiting factors in transfer operations. The dissolved CO_2 in water causes the pH of the transfer water to decrease. This is one of the factors that reduces the oxygen-carrying capacity of the blood. Another factor that can cause a decrease in blood pH is lactic acid [19]. Fish exhibit great muscle activity during transfer operations. Especially on cage farms, fish must swim long distances during the transport of their cages to the transfer area. Since the muscles are actively used during long-distance swimming, there is not enough oxygen intake to meet the needs of the muscles. However, during loading into the tanks for transfer, intense muscle activity is observed with flapping. Owing to intense muscle activity and insufficient oxygen, lactic acid increases in tissues [20, 21]. The transport of lactic acid by blood to the liver for gluconeogenesis causes a decrease in blood pH, which is another factor that causes a decrease in oxygen uptake [22].

Under normal conditions, even in hyperoxic water, a resting fish consumes a minimum amount of oxygen. During transfer, fish require more than the minimum amount of oxygen because they are not at rest [23]. They may also consume close to the maximum amount of oxygen during loading or transfer due to stress. Fish consume more oxygen in the transport unit during the first 15 min than during the following 15-minute periods [24]. Therefore, during loading and the first hour of haulage, additional oxygen should be provided at twice the flow normally needed. After this acclimatization period, when the fish have settled and oxygen consumption has stabilized, the oxygen flow can be reduced to its normal level [25]. However, in aerobic cells, superoxide (O_2^-), a free radical, is formed when molecular oxygen (O_2) is reduced by taking an electron [26]. High oxygen concentrations, especially hyperoxic conditions, cause the formation of reactive oxygen species (ROS) not only in mammals but also in fish. However, fish have developed biological responses to adapt to hyperoxic and hypoxic conditions, which vary among species [27].

In the field research in our study, hyperoxic conditions were used during transfer on farms. For this purpose, in our study, oxidative damage caused by free radicals or other stress factors was measured in fish transferred at different dissolved oxygen concentrations. In our study, different oxygen saturations were created in 4 tanks with the same stocking density during transfer, and biochemical changes in the blood and muscle tissues of the fish were determined after transfer. By determining these changes, the amount of oxygen that should be used during the transfer of trout was determined. In addition, it will lead to the development of a methodology that can prevent mortality in tanks during the transfer of fish and in farms after transfer. Therefore, the aim of this study was to investigate the effects of different dissolved oxygen concentrations on oxidative stress markers and antioxidant defense responses in rainbow trout during transport and to determine the optimal oxygen level to maintain fish health and welfare.

Table 1 Groups constituting the research setup

Groups	*n*	Dissolved Oxygen (mg/L)
Control (Dam lake)	20	8 mg/L (Fig. 1.)
Group 1 (Transfer)	20	6 mg/L
Group 2 (Transfer)	20	8 mg/L
Group 3 (Transfer)	20	10 mg/L
Group 4 (Transfer)	20	Dissolved oxygen content in transport with the method routinely applied on the farm

Materials and methods

The material of the study consisted of 100 female rainbow trout (*Oncorhynchus mykiss*) aged 10–12 months with an average weight of 350–400 g. The fish were reared in the Kürtün Reservoir, and 'dam lake conditions' refer to the natural environmental parameters (e.g., temperature, dissolved oxygen) of the water in this lake. No mortality was observed in any group during the experiment. However, to adhere to ethical considerations, the initially planned sample numbers were maintained without reduction. All fish were sampled according to the original study design. The fish were starved for at least 2 days before transport. Starving fish prior to transport is a critical practice that reduces acute stress by minimizing dissolved oxygen consumption and NH_3/CO_2 accumulation in water [16].

A total of 5 groups, including the control group, were used for the experiment (Table 1).

- The control group, the group containing 8 mg/L dissolved oxygen in the dam lake conditions before the fish were placed in the transport tanks to show the values before transfer and in the dam environment;
- Group 1: Group with 6 mg/L dissolved oxygen in the tank during transport;
- Group 2 included the same dam lake conditions during transport, with 8 mg/L dissolved oxygen in the tank.
- Group 3: Group with 10 mg/L dissolved oxygen in the tank during transport;
- Group 4: Group with > 30 mg/L dissolved oxygen in the tank, which is routinely used during transfer to farms.

The physical and chemical properties of water in dams are influenced by all environmental factors, such as season, air temperature, daylight duration, the amount of metabolic residues of other aquatic organisms, and precipitation, and are constantly changing. To determine the transfer water oxygen data of Group 2, a stationary oxygen meter (OxyGuard Atlantic Single Channel Dissolved Oxygen Meter) was placed in Kürtün Reservoir. Measurements were made every 5 min with the device. The data were calculated in a computer environment. When

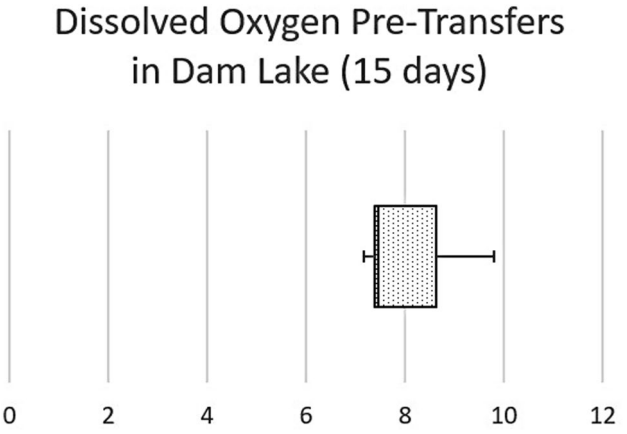

Fig. 1 Dissolved oxygen in the reservoir during the 15 days before the transfer operations

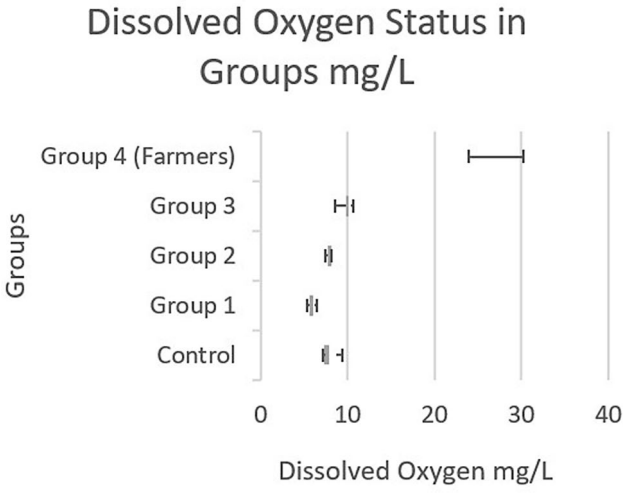

Fig. 2 Dissolved oxygen in the tanks of the control and transfer groups during the transfer period

the collected data were analyzed, the lowest dissolved oxygen content in the water was found to be 7.16 mg/L, and the highest dissolved oxygen content was found to be 8.85 mg/L. The deviations here were very short-term, and the average oxygen concentration was 8 ± 0.84 mg/L (Fig. 1). Measurements were continuously recorded over a 15-day monitoring period to accurately reflect the environmental conditions of the reservoir.

The dissolved oxygen values are given in Fig. 2 when averages of the measurements made during the transfer applications and in the dam forming the control group are taken.

The oxygen valve was kept under control manually to keep the oxygen in the transport tanks at the specified values. Environmental factors that could influence dissolved oxygen levels, such as ambient temperature and sunlight exposure, were minimized by using covered transport tanks and continuous monitoring of water

temperature and oxygen levels throughout the transfer process [28]. To control the amount of dissolved oxygen, continuous control was carried out with an oxygen meter probe in the tank. If the dissolved oxygen concentration was above the desired value, the valve was closed, and if it was below the desired value, the valve was opened to keep it at the desired level. The transfer process took a total of 4 h for the 120 km journey between Kürtün Reservoir and Trabzon Arsin port and loading/unloading operations.

Sampling

The fish from the control group before transplantation and the experimental groups after transplantation were individually placed into another container and anesthetized with phenoxyethanol (100 mL phenoxyethanol per 1 m^3 water). Blood was collected from the arteria-ven caudalis via caudal puncture with a 21 G needle tip. The blood samples were brought to the laboratory under appropriate conditions. The serum was removed via centrifugation at 3000 rpm for 10 min and stored at -20 °C until analyses were performed [29].

To obtain muscle tissue samples, after the animals were sacrificed, the 3 × 3 × 1 cm area between the lateral line and the dorsal fin was cut with a scalpel, and the samples were collected and placed in separate sample containers. This area, being away from the viscera and fins, has a lower risk of contamination and is suitable for obtaining reproducible results in terms of biochemical analyses [30]. Muscle tissue samples were brought to the laboratory under appropriate conditions and stored at -20 °C. Then, 1 g of each sample was weighed and thoroughly crushed with 0.15 M KCl solution in a glass homogenizer to prepare a homogenate.

GSH-Px, SOD and LDH enzyme activities; MDA, Cp, TOS, TAS, OSI, cortisol, glucose, urea, uric acid, creatinine, Ca, Mg, and MDA; and the Cp, TOS, TAS and OSI values in the serum and muscle tissue were investigated in the control group and groups with 4 different dissolved oxygen concentrations.

Malondialdehyde (MDA) in the serum and supernatant was analyzed to indicate lipid peroxidation via the method described by Yoshioka et al [31]. This method depends on the spectrophotometric analysis of the colored compounds produced through the reaction of MDA with TBA. The estimation of ceruloplasmin was performed spectrophotometrically via the modified Ravin method [32]. As an enzyme, ceruloplasmin oxidizes the colorless substance phenylene diamine into a blue–violet-colored product. This effect in the serum was stopped at a certain time during the experiment by the addition of sodium azide and at the beginning of the experiment in a blinded manner, after which the effects were measured spectrophotometrically. A Fish GPX ELISA Kit was used for the analysis of glutathione peroxidase (GSH-Px) activity. This kit is based on a sandwich ELISA method that measures GSH-Px enzyme activity quantitatively in samples. A Fish SOD ELISA Kit was used for superoxide dismutase (SOD) activity analysis. The SOD assay is used to determine oxidative stress by measuring the activity of the enzyme that catalyzes the dismutation of superoxide radicals. Total Oxidant Status (TOS) and Total Antioxidant Status (TAS) were analyzed with a Rel Assay Diagnostics colorimetric test kit. TOS represents the total amount of oxidant substances in the serum, whereas TAS represents the total amount of antioxidant substances. The oxidative stress index (OSI) was calculated via the formula TOS/TAS*10; this index indicates the changes in the balance between the oxidative and antioxidative systems. Cortisol levels were determined via the Fish Cortisol ELISA Kit. The kit is specific and sensitive for cortisol measurement via the ELISA method. Serum glucose, urea, uric acid, calcium (Ca), magnesium (Mg), creatinine, and lactate dehydrogenase (LDH) activity analyses were performed on a Beckman Coulter AU 5800, USA, autoanalyzer. All these analyses were carried out via enzymatic and colorimetric methods, which are applied in common practice in clinical biochemistry laboratories, ensuring accurate and reliable measurements of biochemical parameters.

For the study, "Research Permit", dated 12.03.2020 and numbered 67,852,565–145–E.868,991, was obtained from the Ministry of Agriculture and Forestry, General Directorate of Fisheries and Aquaculture. Approval from the ethics committee, dated 14.07.2020 and numbered 2020/26, dated 14.07.2020, was obtained from the Samsun Ondokuz Mayıs University Animal Experiments Local Ethics Committee to conduct a study on the fish to be used in the study.

Statistical analysis

SPSS for Windows 22.0 software was used for the statistical analysis. The data obtained from the study are presented as the mean ± standard deviation (X ± SD). In addition, the standard error values of the data are presented in the tables, and P values less than 0.05 were considered statistically significant.

The groups were evaluated by the Shapiro–Wilk normality test. For the parameters found to be normally distributed in the groups, the groups were compared via one-way ANOVA, which is a parametric test. The homogeneity of variance was tested by Levene's test. For the parameters that provided homogeneity, the Tukey test was used as a post hoc test in the comparison of the groups. For the parameters that did not provide homogeneity, Dunnett's T3 test was used as a post hoc test in the comparison of the groups. For the parameters for which a normal distribution was not found in the groups, the

groups were compared via the Kruskal–Wallis test, which is a nonparametric test. For the parameters for which a significant difference was found according to the results of the test, the groups were compared with the Mann–Whitney U test in pairs to determine which groups were significantly different.

Since both parametric and nonparametric tests were used in the comparison of the groups in the study, the graphic representations of the parameters were presented both with a column graph based on the mean of the parameters and with a box plot graph showing the intragroup distribution of the parameters.

Result

The mean and standard deviation values of the oxidant-antioxidant parameters of the serum samples obtained from the subjects in the five groups in the study are given in Table 2.

The means and standard deviations of the oxidant-antioxidant parameters of the serum samples obtained from the five groups of rainbow trout were analyzed. Malondialdehyde (MDA) levels were not significantly different between the groups ($p > 0.05$). Compared with those in the control group, glutathione peroxidase (GPx) activity was significantly lower in Group 1 and Group 2 and greater in Group 3 ($p < 0.05$). The superoxide dismutase (SOD) levels were low in Group 2 and high in Group 3, and significant differences were found between the groups ($p < 0.05$). The TAS levels were low in Group 4 and high in the other groups ($p < 0.05$). The TOS level was greater in all the treatment groups than in the control group, and significant differences were found between the groups ($p < 0.05$). The oxidative stress index (OSI) was significantly greater in Group 4 than in the other groups ($p < 0.05$). Ceruloplasmin levels did not significantly differ between the groups ($p > 0.05$). Cortisol levels were significantly lower in Group 1 and Group 2 than in the control group, and significant differences were observed between the groups ($p < 0.05$).

The mean and standard deviation values of the oxidant-antioxidant parameters of the muscle samples obtained from the subjects belonging to the five groups in the study are given in Table 3.

The means and standard deviations of some of the biochemical parameters of the serum samples obtained from the subjects in the five groups in the study are given in Table 4.

In this study, the means and standard deviations of several biochemical parameters in serum samples obtained from five groups of rainbow trout were analyzed. Malondialdehyde (MDA) levels were significantly lower in Group 3 (10 mg/L) (29.26 ± 7.45), and there was no significant difference between Group 1 (6 mg/L) and Group 2 (8 mg/L) ($p < 0.05$). The TAS level was the lowest in Group 2 (8 mg/L) (0.26 ± 0.16), and significant differences were detected in this group compared with the other groups ($p < 0.05$). The TOS level was low in Group 1 (6 mg/L) and Group 2 (8 mg/L), whereas it was high in the other groups ($p < 0.05$). The oxidative stress index (OSI) was lowest in Group 2 (8 mg/L) (138.30 ± 93.33) and higher in the other groups ($p < 0.05$). The ceruloplasmin levels were highest in Group 1 (6 mg/L) (9.48 ± 5.42) and significantly different from those in the other groups ($p < 0.05$).

Discussion

Stress factors in fish can cause an increase in ROS in serum and tissue, thereby inducing lipid peroxidation [33, 34]. MDA is produced as the final product of lipid peroxidation and an increase in MDA levels in tissue and serum

Table 2 Mean and standard deviation values of the serum oxidant-antioxidant parameters

Parameters	Control (8 mg/L)	Group 1 (6 mg/L)	Group 2 (8 mg/L)	Group 3 (10 mg/L)	Group 4 (> 30 mg/L)	
	n = 20	*n* = 20	*n* = 20	*n* = 20	*n* = 20	
MDA (µmol/ml)	21.53± 6.66[a]	24.08± 4.14 [a]	25.90± 4.49 [a]	26.05± 9.20 [a]	24.85± 8.00 [a]	$p > 0.05$
GPx (µU/ml)	756.13± 287.40[a, b]	362.81± 244.68[c]	451.86± 171.74[c]	890.02± 279.00[a]	574.32± 236.43[b, c]	$p < 0.05$
SOD (ng/ml)	1.87± 0.56[a, b]	1.64± 0.26 [a, b]	1.59± 0.33[b]	1.97± 0.42[a]	1.61± 0.56 [a, b]	$p < 0.05$
TAS (mmol/L)	0.57± 0.34[a, b]	0.80± 0.60[a]	0.96± 0.79[a]	0.77± 0.4 [a]	0.47± 0.42[b]	$p < 0.05$
TOS (µmol/L)	11.69± 2.83[b]	14.60± 6.37[a, b]	17.03± 16.42[a]	17.33± 8.17[a]	19.77± 15.88[a]	$p < 0.05$
OSI	0.05± 0.12[b]	0.02± 0.01[b]	0.03± 0.02[b]	0.03± 0.02[b]	0.21± 0.50[a]	$p < 0.05$
Ceruloplasmin (mg/dl)	1057.24± 847.59[a]	450.30± 351.00[a]	776.18± 724.11[a]	1149.05± 1679.68[a]	708.04± 337.63[a]	$p > 0.05$
Cortisol (ng/ml)	42.34± 10.08[a]	28.64± 8.02[b]	21.14± 7.91[c]	30.58± 12.32[b, c]	23.81± 11.68[b, c]	$p < 0.05$

Table 3 Mean and standard deviation values of the muscle oxidant-antioxidant parameters

Parameters	Control (8 mg/L)	Group 1 (6 mg/L)	Group 2 (8 mg/L)	Group 3 (10 mg/L)	Group 4 (>30 mg/L)	
	n = 20	*n* = 20	*n* = 20	*n* = 20	*n* = 20	
MDA (µmol/gr)	76.95± 77.63^{ab}	95.35± 160.51^{a}	51.38± 20.94^{a}	29.26± 7.45^{c}	66.75± 27.67^{b}	*p* < 0,05
TAS (µmol/ml)	0.46± 0.10^{a}	0.34± 0.21^{ab}	0.26± 0.16^{b}	0.48± 0.05^{a}	0.48± 0.08^{a}	*p* < 0,05
TOS (µmol/ml)	106.39± 29.62^{a}	45.33± 31.51^{b}	25.94± 10.48^{b}	111.71± 21.41^{a}	118.74± 20.18^{a}	*p* < 0,05
OSI	250.75± 128.46^{a}	204.70± 206.21^{b}	138.30± 93.33^{b}	238.15± 50.30^{a}	252.42± 61.24^{a}	*p* < 0,05
Ceruloplasmin (% mg)	1.59± 0.75^{e}	9.48± 5.42^{a}	3.70± 1.28^{c}	3.18± 0.83^{d}	6.11± 1.70^{b}	*p* < 0,05

Table 4 Mean and standard deviation values of several biochemical parameters

Parameter	Control (8 mg/L)	Group 1 (6 mg/L)	Group 2 (8 mg/L)	Group 3 (10 mg/L)	Group 4 (>30 mg/L)	
	n = 20	*n* = 20	*n* = 20	*n* = 20	*n* = 20	
Glucose (mg/dl)	84.65± 22.03^{c}	160.95± 34.11^{b}	158.75± 31.23^{b}	155.10± 27.50^{b}	198.80± 26.79^{a}	*p* < 0,05
Urea (mg/dl)	2.40± 0.82$^{a, c}$	3.70± 0.98$^{a, b}$	3.35± 1.04$^{a, b}$	3.95± 3.19^{a}	3.55± 0.94$^{a, b}$	*p* < 0,05
Uric Acid (mg/dl)	0.14± 0.06^{e}	0.40± 0.12^{b}	0.20± 0.03^{d}	0.37± 0.26^{c}	0.72± 0.33^{a}	*p* < 0,05
Creatinin (mg/dl)	0.14± 0.10^{d}	0.44± 0.19$^{a, b}$	0.58± 0.24^{a}	0.39± 0.41^{b}	0.17± 0.08^{c}	*p* < 0,05
Calcium (mg/dl)	10.45± 1.46^{b}	12.62± 1.11^{a}	11.82± 1.04^{a}	10.67± 1.36^{b}	12.87± 1.04^{a}	*p* < 0,05
Magnesium (mg/dl)	2.38± 0.32^{c}	2.30± 0.30^{c}	2.71± 0.35^{b}	2.31± 0.52$^{b, c}$	3.43± 0.56^{a}	*p* < 0,05
LDH (mg/dl)	422.95± 298.42^{e}	765.80± 360.49^{d}	932.95± 349.81^{c}	1404.5± 922.29^{b}	4589.30± 2250.18^{a}	*p* < 0,05

indicates the toxic activity of free radicals [35]. The most important feature of the antioxidant defense system is that all components of the system act synergistically with reactive oxygen species [36]. Therefore, all antioxidants are vital for maintaining homeostasis in living organisms [35]. The increased activity of both SOD and GSH-Px in our study is indicative that these two important antioxidant enzymes work together to combat oxidative stress caused by fluctuating oxygen levels. Antioxidants are broadly classified into natural and synthetic antioxidants, and natural antioxidants further divided into endogenous and exogenous antioxidants. Endogenous antioxidants can be categorized into enzymatic and nonenzymatic, which helps in better understanding their roles in the body. Notably, interacting antioxidant enzymes such as GSH-Px, SOD, catalase (CAT) and glutathione reductase (GRd) have the highest antioxidant defense activity [37].

In addition to antioxidant enzymes, Low-molecular-weight antioxidants such as vitamins C and E, coenzyme Q, carotenes, glutathione and trace elements also play a key role in neutralizing reactive radicals [38]. Due to the combined action of oxidants and antioxidants, the overall oxidative and antioxidative effects in the blood are greater than the sum of individual effects. Therefore, it has been reported that TOS and TAS measurements may be more useful for determining the total oxidant/antioxidant balance than for determining the oxidants or antioxidants separately [39].

To better understand the relationship between serum ceruloplasmin variation and its antioxidant effect, it is important to evaluate it alongside other oxidant and antioxidant values. This approach helps in understanding the role of ceruloplasmin in regulating oxidative stress [40]. Among the most important antioxidant defense enzymes, SOD and GSH-Px play a crucial role in neutralizing reactive oxygen species and superoxide radicals. SOD reduces superoxide radicals and transforms them into hydrogen peroxide. Glutathione peroxidase enzyme (GSH-Px) neutralizes H_2O_2 formed by superoxide radicals reduced by SOD by converting them into H_2O and O_2 [41]. A decrease in GSH-Px activity causes an increase in H_2O_2 levels and thus oxidative stress [42].

Various biomarkers are used to assess fish health. Several factors influence the levels of biomarkers such

as cortisol and glucose, with some linked to metabolic adjustments and others to stress induced during blood collection [43]. Measuring cortisol levels is particularly critical since this hormone is highly sensitive to stress. Wu et al. (2017) suggested that the steady increase in plasma cortisol could be considered a nonspecific response to increased stress levels in freshwater [44]. Therefore, glucose can also serve as a biomarker in stress-related conditions. In addition to serum findings, muscle tissue parameters were evaluated to provide a more comprehensive assessment.

Dissolved oxygen in water, in particular, is highly important both physiologically and in terms of animal welfare. Well-designed transport conditions and appropriate environmental factors help maintain the health and welfare of fish. This reduces fish stress levels and minimizes negative impacts during transport. Ensuring animal welfare is an essential element for obtaining a quality product and a sustainable fishing industry. During current transfer operations in aquaculture, the dissolved oxygen level in tanks is maintained at a maximum, and transfer operations are carried out under hyperoxic conditions. In our study, we aimed to investigate the metabolic values and oxidative stress changes associated with different oxygen levels in serum and muscle tissue during the transfer of rainbow trout and to determine the most appropriate dissolved oxygen value during transfer in light of these results. In this study, the GSH-Px, SOD, and LDH enzyme activities; the MDA, Cp, TOS, TAS, OSI, cortisol, glucose, urea, uric acid, creatinine, Ca, and Mg values in the serum; and the MDA, Cp, TOS, TAS and OSI values in muscle tissue were investigated.

In our study, no statistically significant difference was found when the serum MDA levels of the control groups and the transfer groups with different dissolved O_2 levels were compared. Although there was an increase in MDA levels in both studies investigating the effects of stress induced by dissolved oxygen levels and transfer-related stress on serum MDA levels, there was no change in our study.

In a study investigating the effects of transfer on serum MDA levels in rainbow trout, samples were taken from juvenile trout before, during and after a 30-minute transfer process (at 6 and 12 h), and it was reported that the serum MDA values significantly increased at 6 h and decreased to the pretransfer level at 12 h [45]. In a study investigating the effects of hypoxia exposure, it was reported that plasma MDA levels increased in fish exposed to hypoxic shock in tanks containing 2–5 mg/L dissolved oxygen for 10 min and that hypoxia caused lipid peroxidation [46].

In contrast to the other two studies, since the transfer periods were longer in our study, the adaptation process to the new conditions started in the fish, and therefore, the serum MDA values returned to the control levels.

In our study, when the muscle tissue MDA levels of the control and transfer groups at different dissolved oxygen concentrations were compared, the MDA value of group 3 decreased compared with that of the control group, whereas no significant change was observed in the other transfer groups. In addition, when the transfer groups at different dissolved oxygen concentrations were compared, the MDA value of group 3 was lower than that of the other transfer groups.

In our study, the lack of increase in the MDA level in the transfer groups compared with the control group may be related to the transfer time. We believe that the decrease in the MDA value in group 3 indicates that the antioxidant defense system works effectively against lipid peroxidation. However, despite the lower MDA levels, the elevated OSI value in Group 3 could be attributed to a relative decrease in TAS, rather than an increase in oxidant production. Since OSI reflects the balance between total oxidants and antioxidants, a reduction in antioxidant defenses alone could lead to an increased OSI. In addition, the increase in GSH-Px and SOD enzyme activities and the decrease in the MDA level in group 3 among the transfer groups containing different amounts of dissolved oxygen in our study are similar. In addition, the decrease in GSH-Px and SOD enzyme activities in group 1, where the MDA level increased, was also compatible with the increase in the MDA level.

In a previous study, the transfer process in catfish caused an increase in the muscle MDA level of the fish, and there were obvious changes in the antioxidant capacity [47]. In another study, an increase in MDA levels was reported in rainbow trout exposed to hyperoxic stress with oxygen and ozone (O_3).

According to the results of our study, lipid peroxidation is greater under hypoxic and hyperoxic conditions than under normoxic conditions. Our results are consistent with those of previous studies. We believe that the antioxidant defense mechanism against lipid peroxidation becomes more active and decreases lipid peroxidation under normoxic conditions, especially in transfer processes performed at a DO concentration of 10 mg/L.

In our study, while there was no statistically significant difference between the control and transfer groups, the SOD activity value of group 3 under normoxic conditions was greater than the SOD activity value of group 2 in the comparison between the transfer groups with different dissolved O_2 concentrations. Under normoxic conditions, as in groups 2 and 3, 2 mg/L dissolved oxygen exchange was an effective factor in the production of reactive oxygen radicals. Compared with that in group 2, the amount of SOD in group 3 is thought to increase with increasing oxygen radical content.

In addition, in our study, when the transfer and control groups were compared, the GSH-Px activity of group 1, group 2 and group 4 decreased compared with that of the control group. Compared with those of all the transfer groups containing different amounts of dissolved O_2, the GSH-Px activity of group 3 increased.

Ritola et al. (2002) reported an increase in plasma SOD activity in fish exposed to supersaturation (140%) under normoxic conditions [48]. Our results are consistent with those of Ritola et al. and show that antioxidant defense mechanisms, including SOD, are activated to detoxify the increased formation of superoxide radicals caused by oxygen.

In our study, compared with that in group 2, the GSH-Px activity in group 3 was significantly greater. Another study reported that liver SOD and GSH-Px activities decreased in fish exposed to hyperoxygen for a long period (7 weeks) [49]. Radi et al. (1988) reported that hepatic antioxidant enzyme activities decreased or did not change in fish exposed to hyperoxic conditions for 12 weeks [50].

Another study reported that there was no change in SOD activity in the liver or brain tissues of rainbow trout exposed to short-term hyperoxia [51]. In our study, no statistically significant difference was found between the control group and the hyperoxic group. This suggests that the reason for this may be the duration of exposure to hyperoxia. However, it is important to note that prolonged hyperoxic exposure may lead to oxidative damage to cellular membranes, mitochondrial dysfunction, and impairments in respiratory and ion-regulatory functions.

In our study, no statistically significant difference was found in the comparison of the serum TAS values between the control and transfer groups with different dissolved O_2 concentrations. When the groups transferred to tanks containing different amounts of dissolved oxygen were compared, the lowest serum TAS value was observed in group 4, which had hyperoxic conditions.

According to the study conducted by Fazio et al. (2014), while TAS levels increased in the 24th hour of restriction in fish whose movement was restricted, they reported that there was no significant increase in TAS levels in the control group as the restriction time increased (at 48 and 72 h) [52].

In our study, we found that the highest OSI value was observed under hyperoxic conditions. Transfer procedures in rainbow trout cause an increase in serum TOS levels. In addition, a decrease in antioxidant levels and, consequently, an increase in the OSI value were observed in fish exposed to hyperoxygen during the transfer process.

According to our findings, we suggest that there is an increase in TOS levels in fish during transportation under normoxic conditions during transfer but also an increase in TAS levels and that normoxic conditions during transfer may reduce the effects of oxidative stress in fish.

In addition, when the muscle tissue TAS levels of the groups transferred to tanks containing different amounts of dissolved oxygen were compared, although the results were similar to those of the control group in groups 1, 3 and 4, a decrease was detected in group 2.

On the other hand, when muscle tissue TOS levels were compared, the TOS values of the control group and groups 3 and 4 were similar, whereas they were lower in groups 1 and 2. This situation revealed that the increase in the amount of dissolved oxygen in the transfer groups and the procedures performed before transfer increased the TOC value in the muscles, and the antioxidant defense system was more active in the groups with 10 mg/L or more dissolved oxygen.

In addition, in our study, we found that the highest OSI value was associated with hyperoxic conditions. When the oxidative stress index was evaluated, a decrease in the antioxidant levels was detected in the fish exposed to hyperoxygen, and accordingly, an increase in the OSI value was detected in group 4.

In our study, no statistically significant difference was found when the serum ceruloplasmin values were compared among all the groups. We believe that SOD acts as the primary mechanism for the reduction of superoxide radicals in the cell when the dissolved oxygen concentration is high and that feroxidase activity is initiated secondarily with increasing exposure time. The fact that there was no change in the ceruloplasmin value despite the changes in SOD and GSH-Px activity supports this view. The length of the transfer time is a common situation in aquaculture. In our study, the transfer process took approximately 240 min. Therefore, further studies are needed to investigate the effects of longer transfer times on serum ceruloplasmin levels in rainbow trout. These studies may help us to better understand how ceruloplasmin levels change and the sustainability of the antioxidant effect in fish under long-term transportation conditions. In our study, an increase in ceruloplasmin levels in muscle tissue was observed in all the transfer groups compared with those in the control group. When the transfer groups with different dissolved oxygen contents were compared among themselves, they were ranked as groups 1, 4, 2 and 3 in order from high to low, and all of them were statistically significant. The group under hypoxic conditions presented the highest ceruloplasmin level. In one study, tissue ceruloplasmin values were compared in yellow perch fish taken from different parts of a river contaminated with various pollutants, and it was reported that the differences between the regions were affected by the environmental conditions [9]. During hypoxia, fish not only try to compensate by increasing red blood cell counts through the spleen but also

increase erythropoiesis [53]. Increased ceruloplasmin production during hypoxia may be required to facilitate iron release from the spleen, liver and reticuloendothelial cells for use by red cell precursors for erythropoiesis [54]. In our study, the greater increase in ceruloplasmin values in hypoxic conditions than in all other groups was explained by the desire to use low dissolved oxygen effectively. Ceruloplasmin plays a key role in iron metabolism by oxidizing Fe^{2+} to Fe^{3+}, thereby facilitating iron mobilization for erythropoiesis under hypoxic stress [54].

In our study, cortisol levels were high in the fish prepared for transfer but decreased during transfer, which we believe was due to both the transfer time of approximately 4 h and the fact that daylight did not penetrate into the transfer tanks. In many studies in different fish species, which are not in agreement with our findings, it has been reported that plasma cortisol levels increase during the night, peak before the onset of light, decrease during daylight and decrease to the lowest levels before the onset of darkness [55–57]. In addition, under natural conditions, the source of dissolved oxygen in water is photosynthesizing phytoplankton, algae, and mosses. In the absence of daylight, the dissolved O_2 level in water decreases, and the dissolved CO_2 level increases. This may cause an increase in serum cortisol levels as a cause of stress in aquatic organisms. During transfer, the greater amount of dissolved oxygen in the water in the dark than in the natural environment may cause a decrease in the level of cortisol, which is a stress marker in fish. In a previous study, rainbow trout were subjected to a 24-hour dark photoperiod for 7 days under constant dissolved oxygen conditions, plasma cortisol levels were monitored at 4-hour intervals, and it was observed that plasma cortisol levels increased during the night. It has been reported that plasma cortisol concentrations in rainbow trout exhibit a circadian rhythm, with higher levels at night than during light hours [58]. When we evaluated the transfer groups containing different amounts of dissolved oxygen, the serum cortisol value of group 1 under hypoxic conditions was greater than the serum cortisol value of group 2. It is expected that cortisol levels would increase due to the lack of daylight and low dissolved oxygen concentration in the tanks, similar to natural conditions. Therefore, our results are consistent with studies on daylight. Cortisol is activated in the first stage of the stress response and increases glucose levels by stimulating gluconeogenesis and glycogenolysis processes [44]. The cortisol level in serum shows diurnal variation and has a half-life of 80–120 min [59]. This allows glucose to be used as a marker of the secondary stress response [60]. In a study conducted to determine the time-dependent effects of acute stress in rainbow trout, serum glucose levels were measured at certain time intervals after stress; the serum glucose levels peaked and remained constant after 60–120 min, and longer periods were reported to be needed for glucose levels to return to normal [61]. In our study, when we evaluated serum glucose levels, we found that the serum glucose levels of all the transferred groups were greater than those of the control group. We believe that this increase in glucose levels may be due to increased gluconeogenesis and glycogenolysis due to increased energy requirements as well as transfer-induced stress. In addition, among the transfer groups with different dissolved O_2 concentrations, group 4, which was exposed to hyperoxic conditions, presented significantly higher glucose levels than did the other transfer groups. We believe that the effects of stress may last longer under hyperoxic conditions than under normoxic conditions. Therefore, we believe that glucose levels do not return to normal even if cortisol levels decrease. In addition, the fact that the serum OSI value was the highest among all the groups under hyperoxic conditions supports this view.

In our study, the serum urea levels in groups 1, 2 and 4 were greater than those in the control group when the control and transfer groups containing different dissolved O_2 concentrations were compared. No significant difference was found when the urea levels of the transfer groups containing different amounts of dissolved O_2 were compared. These results revealed that different oxygen levels had no effect on the urea values of the fish but that transfer affected the serum urea values. The higher urea values of groups 1, 2 and 4 than those of the control group indicate that the fish in these groups were under more stress and used protein catabolism through gluconeogenesis for energy needs. The increase in the serum glucose levels in all the transfer groups supports this view. These findings suggest that the fish consumed more energy during transfer, made greater efforts in environments with low oxygen levels and obtained energy through gluconeogenesis.

When uric acid levels were compared in our study, the highest level was found in group 4, and the lowest was found in group 2. Compared with that of the other groups, the oxidative stress index (OSI) value of group 4 was the highest, suggesting that the antioxidant effect of uric acid may be effective. In our study, when the serum creatinine levels of the control and transfer groups containing different dissolved O_2 concentrations were compared, an increase was found in all the transfer groups. In addition, when the transfer groups containing different dissolved O_2 concentrations were compared, the highest creatinine value was found in groups 1 and 2, and the lowest creatinine value was found in group 4, where hyperoxic conditions were present. In our study, the urea and uric acid values increased in all the groups, whereas the creatinine values increased in groups 1 and 2 but decreased in groups 3 and 4. This may be related to renal

damage in groups 1 and 2 and may be due to increased muscle activity in groups 3 and 4. The increase in serum LDH activity in groups 3 and 4 was due to increased muscle activity. In addition, elevated serum creatinine levels in groups 1 and 2 support our view that kidney damage was present in these groups.

When the serum calcium levels of the transfer groups and the control group were compared, all the transfer groups except group 3 presented increased serum calcium levels compared with those of the control group. When the groups transferred at different dissolved oxygen concentrations were compared, the serum calcium level in group 3 was lower than that in the other groups. In our study, we found that the serum calcium levels increased in all the transfer groups except for group 3, which was normoxic. In a study on hypoxia, abnormal swimming behavior, poor growth performance, and increased serum calcium levels were reported in fish exposed to hypoxic conditions [62]. Hou et al. (2020) suggested that the SCaMC-1 gene is involved in buffering calcium homeostasis in the mitochondrial matrix and in buffering intracellular calcium levels by creating a feedback mechanism between increased serum calcium and oxidative stress [62]. We believe that the reason for the increase in serum calcium levels is feedback against oxidative stress, as suggested by Hou et al. (2020).

In our study, when the serum magnesium levels of the control group and the transfer groups containing different dissolved oxygen concentrations were compared, a significant increase was detected in groups 2 and 4 compared with the control group. When the transfer groups containing different dissolved oxygen concentrations were compared, the highest serum magnesium value was found in group 4, where hyperoxic conditions were present. In addition, the serum magnesium level of group 2 was greater than that of group 1. Blood and electrolyte concentrations are regulated by interactions of processes such as the absorption of electrolytes from the surrounding environment, control of water permeability and selective reabsorption of electrolytes from urine, predominantly through active mechanisms by the gill [63]. We believe that the increase in the serum calcium concentration in almost all of the transfer groups and the increase in the serum magnesium concentration, especially under hyperoxic conditions, is due to the disruption of homeostasis.

Our study observed a statistically significant increase in LDH activity when the control and transfer groups were compared. In addition, when the LDH activity increased as the dissolved oxygen concentration increased and LDH activity increased 6-fold under hyperoxic conditions compared with hypoxic conditions. The effects of hypoxic conditions on serum LDH activity were reported to increase serum LDH activity according to studies conducted in Neumayer barbus, goldfish and clayfish [64–66]. It has been reported that serum LDH activity is increased in Akita mice exposed to hyperoxic conditions, which is associated with increased energy demand and cell damage [67]. Although few studies have investigated LDH activity under hyperoxic conditions in fish, it has been reported that various stressors increase serum LDH activity, which may be a result of cell damage [20, 68, 69]. In our study, since there was no concordance in the parameters related to cell damage, the increase in LDH activity was likely related to the increased energy requirement. We believe that the increased energy requirement during transfer is met by glycolysis, and therefore, LDH activity is increased. The low ATP production efficiency of glycolysis compared with that of oxidative phosphorylation suggests that glycolysis increases and that LDH activity increases for the conversion of lactate to pyruvate.

Conclusion

Our study is important for the development of aquaculture, ensuring food sustainability and meeting people's need for high-quality protein, as well as maintaining animal welfare at the best levels in this process. Our study was conducted to determine the most suitable conditions for transfer by investigating the effects of transfer processes on fish under different dissolved oxygen concentrations and conditions applied on farms. For this purpose, biochemical changes were observed in fish transferred under hypoxic, normoxic, and hyperoxic conditions.

Despite the increased reactive oxygen species in the transfer procedures performed under hypoxic conditions, a decrease in GSH-Px activity was detected compared with that in the control group. In addition, lipid peroxidation was found to occur due to the increase in MDA values in muscle tissue compared with those under hyperoxic and normoxic conditions. The increase in urea, uric acid, and creatinine values compared with those of the control group suggests the possibility of kidney damage, whereas the increase in LDH activity indicates that the increased energy requirement is met through glycolysis and that the glucose needed is obtained through gluconeogenesis.

Owing to superoxide radicals formed under hyperoxic conditions, a significant increase in the serum TOS value and a decrease in the serum TAS value were observed. For this reason, the OSI value increased significantly in all the groups. Under hyperoxic conditions, oxidative damage is greater, and the antioxidant system is suppressed. It was observed that protein catabolism started as a result of the energy demand due to increased muscle activity, and this deficit was closed by gluconeogenesis.

Under normoxic conditions, TAS increased against these negativities and decreased the OSI value. In addition, the level of MDA, the end product of lipid peroxidation, decreased, which was due to increased GSH-Px and SOD activity. Among the transfer groups under two different normoxic conditions, the 10 mg/L group presented a more positive response in terms of lipid peroxidation, the antioxidant defense mechanism, and the ion concentration.

This study revealed that it is important to check the oxygen level regularly during transfer and to examine different transfer conditions to ensure that the fish grow healthier and more efficiently in the environment where they are transferred. In light of these findings, we recommend the use of 10 mg/L dissolved O_2 to reduce the effects of oxidative damage and stress in rainbow trout transfers. We believe that the present study contributes to new studies to improve the transfer process in fish.

Acknowledgements
This study is derived from the Ph.D. thesis of Utku DURAN in the Department of Veterinary Biochemistry, Institute of Graduate Studies, Ondokuz Mayıs University. We acknowledge Ondokuz Mayıs University Scientific Research Projects (BAP) Unit for their financial and institutional support.The authors would like to express their gratitude to Dr. Recai Aci and Dr. Busra Sahin for their valuable contributions.

Author contributions
U.D. and S.Ç. designed the study and developed the research methodology. U.D. conducted the experiments, collected data, and performed the statistical analyses. S.Ç. provided supervision and critical guidance throughout the study. U.D. wrote the main manuscript text, and S.Ç. reviewed and revised the manuscript for important intellectual content. Both authors approved the final version of the manuscript.

Funding
This study was supported by Ondokuz Mayıs University (Project Number: PYO. VET.1904.21.013).

Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
The present study was approved by the Local Animal Ethics Committee of Ondokuz Mayis University, Faculty of Veterinary Medicine (Ondokuz Mayis University Animal Experiments Local Ethics Committee) under the protocol number (approval code: 68489742-604.01.03-E.12020 and acceptance no. 2020/26). All methods were performed in accordance with the Basel Declaration and relevant guidelines and regulations of the International Council for Laboratory Animal Science (ICLAS). The study was conducted by local legislation and institutional requirements. The animals used in this study were obtained from Yomra Aquaculture LTD and duly informed consent was obtained from their owners. All methods were reported in accordance with ARRIVE guidelines.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Received: 6 February 2025 / Accepted: 18 April 2025
Published online: 30 April 2025

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