

Original Research

**REDOX BIOMARKERS IN SEMINAL PLASMA OF CULTURED RAINBOW
TROUT: TOS, TAS, AND OSI**

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Abstract

*This study aimed to evaluate seminal plasma levels of total oxidant status (TOS), total antioxidant status (TAS), and oxidative stress index (OSI) in cultured rainbow trout (*Oncorhynchus mykiss*) as biomarkers of potential oxidative stress. Seminal plasma redox status, determined by measuring TOS and TAS to calculate OSI, can indicate the fertility potential of fish semen more efficiently than assessing individual antioxidant enzymes and non-enzymatic antioxidants separately. Screening these three parameters provides a rapid and reliable test for evaluating the redox status and fertility of fish semen, which is particularly valuable for artificial insemination protocols.*

Keywords: Rainbow trout, seminal plasma, redox status, fertility.

INTRODUCTION

Aquaculture plays an essential role in meeting the increasing global demand for high-quality animal protein, with freshwater fish contributing significantly to human nutrition [1]. Among cultured species, *Oncorhynchus mykiss* is one of the most economically important, valued for its rapid growth, adaptability, and nutritional quality. In intensive aquaculture systems, reproductive efficiency is a key factor influencing production success, particularly in artificial fertilization protocols [2]. In externally fertilizing fish, spermatozoa exhibit short-lived motility following activation, making them highly sensitive to environmental and physiological conditions. One of the major factors affecting sperm function is oxidative stress, which occurs when the production of reactive oxygen species (ROS) exceeds the antioxidant capacity of seminal plasma [3]. Elevated ROS levels can induce lipid peroxidation, reduce sperm motility, and impair DNA integrity, ultimately compromising fertilization success and early embryonic development [4,5].

Seminal plasma contains both enzymatic (e.g., superoxide dismutase, catalase, glutathione peroxidase) and non-enzymatic antioxidants that protect spermatozoa from oxidative damage [6]. In this context, total oxidant status (TOS) and total antioxidant status (TAS) are widely

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used to assess overall redox balance, while their ratio, the oxidative stress index (OSI), provides an integrative indicator of oxidative stress. Compared with individual antioxidant markers, these parameters offer a rapid and comprehensive assessment of oxidative status in biological systems [7–9].

Despite their potential relevance, the relationship between oxidative stress indicators and reproductive performance in rainbow trout remains insufficiently understood, particularly at the level of seminal plasma under aquaculture conditions.

Therefore, the aim of this study was to evaluate oxidative status in the seminal plasma of cultured rainbow trout using TOS, TAS, and OSI, and to provide baseline data for their potential application as indicators of seminal physiological condition.

MATERIALS AND METHODS

Rearing conditions

Rainbow trout (*Oncorhynchus mykiss*) were maintained at the "Little Danube" Centre for Fisheries and Applied Hydrobiology (University of Belgrade - Faculty of Agriculture, Serbia) in indoor recirculating aquaculture systems (RAS) optimized for commercial trout production. Water quality parameters, including temperature (T), pH, and dissolved oxygen (DO), were monitored daily using a Multi 340i multi-parameter meter (WTW, Weilheim, Germany), with electrodes submerged 0.05 m below the surface. Mean values ($\pm$ SD) are presented in Table 1.

Table 1 Some physicochemical parameters of rearing water

Parameter	T (°C)	DO (mg/L)	pH
Mean $\pm$ SD	10.5 $\pm$ 1.00	9.07 $\pm$ 0.59	7.49 $\pm$ 0.04

Milt sampling

Milt was collected from five mature male rainbow trout via gentle abdominal massage, stripping directly into sterile 15 mL glass tubes to avoid contamination with urine, feces, or water (Figure 1). Samples were immediately transported to the laboratory in a portable cooler at 4°C and processed within 1 hour. Seminal plasma was isolated by centrifugation (Eppendorf centrifuge, 3000 $\times$ g, 10 min, 4°C), and the supernatant was aliquoted and stored at -80°C until analysis.

TOS, TAS, and OSI measurements

Total oxidant status and TAS were quantified using commercial colorimetric assay kits: TOS Colorimetric Assay Kit (Elabsience E-BC-K802-M) and TAS Colorimetric Assay Kit (Elabsience E-BC-K801-M), following manufacturer protocols. TOS and TAS absorbance were determined at 560 nm and 660 nm, respectively, using an ELISA microplate reader (BioTek Instruments, USA) in accordance with the assay instructions. TOS results were expressed as $\mu\text{mol H}_2\text{O}_2$ equivalents/L, and TAS as mmol Trolox equivalents/L.

The oxidative stress index (OSI) was calculated according to the formula:

$$\text{OSI (arbitrary unit)} = [\text{TOS } (\mu\text{mol H}_2\text{O}_2 \text{ eq/L}) / \text{TAS (mmol Trolox eq/L} \times 1000)]$$

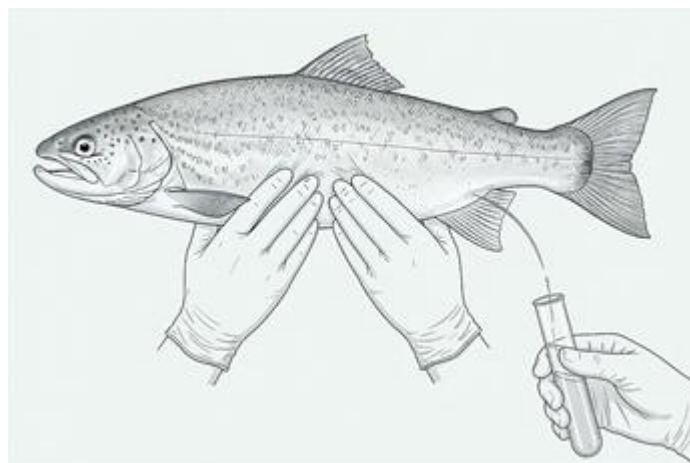


Figure 1 Schematic representation of semen collection from male rainbow trout by gentle abdominal stripping

RESULTS AND DISCUSSION

The present study demonstrated measurable variation in TOS, TAS, and OSI in the seminal plasma of cultured male rainbow trout, indicating inter-individual differences in seminal redox balance (Table 2). TOS values ranged from 65.58 to 121.89 $\mu\text{mol H}_2\text{O}_2 \text{ eq/L}$, whereas TAS varied from 0.85 to 1.11 mmol Trolox eq/L, suggesting that although the seminal plasma of the examined males retained a considerable antioxidant capacity, the oxidant burden differed among individuals. The calculated OSI values point to a generally moderate oxidative state across samples, without pronounced outliers, which may reflect the combined influence of broodstock condition, rearing history, and individual physiological variability (Figure 2).

Table 2 Total oxidant status (TOS) and total antioxidant status (TAS) in seminal plasma of cultured male rainbow trout (*Oncorhynchus mykiss*)

Sample	TOS ($\mu\text{mol H}_2\text{O}_2 \text{ eq/L}$)	TOS ($\mu\text{mol H}_2\text{O}_2 \text{ eq/g ww}^*$)	TAS (mmol Trolox eq/L)	TAS (mmol Trolox eq/g ww*)
1	78.21	469.26	1.11	6.63
2	65.58	393.47	0.85	5.10
3	76.11	456.63	1.10	6.61
4	121.89	731.37	0.91	5.47
5	84.25	603.23	0.93	5.62

*ww = wet weight. Values represent individual male samples (n=5).

TAS represents an integrative measure of antioxidant capacity, reflecting the combined contribution of enzymatic and non-enzymatic components. Compared with single-parameter assays, it provides a broader estimate of the antioxidant defense system in seminal plasma and may better reflect the capacity of the sample to neutralize ROS [10]. This is particularly relevant in fish semen, where oxidative stability plays an important role in maintaining sperm functionality and fertilization potential [11].

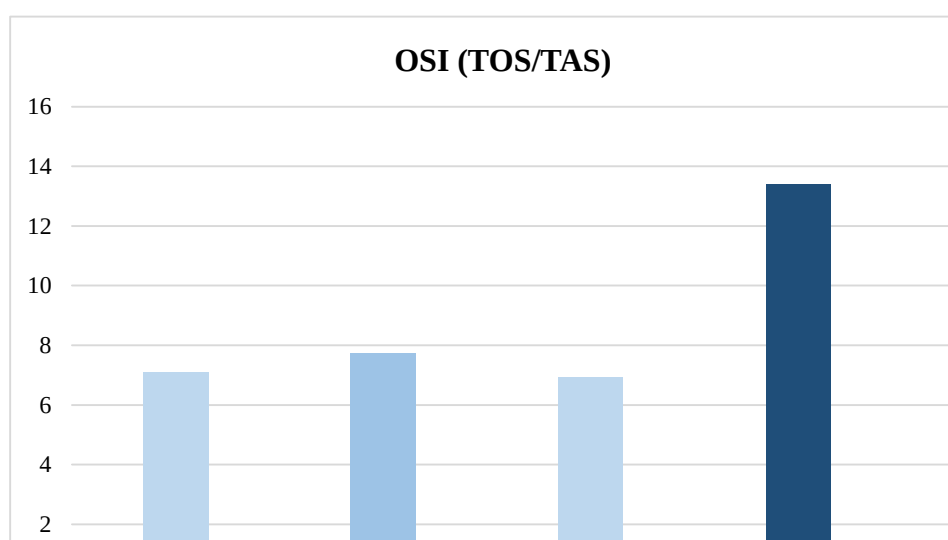


Figure 2 Oxidative stress index (OSI) calculated as $TOS/TAS \times 10$ ratio in seminal plasma samples from individual rainbow trout males

In aquaculture of *Oncorhynchus mykiss*, TOS, TAS, and OSI have been reported as useful indicators of redox status under varying environmental and nutritional conditions. Previous studies suggest that these markers are sensitive to factors such as diet, rearing conditions, and exposure to stressors, indicating that they are context-dependent rather than fixed species-specific values [12]. Accordingly, the values obtained in the present study should be interpreted as indicative of the physiological status of the examined broodstock population rather than as universal reference ranges. The observed inter-individual variability may therefore be associated with differences in diet, metabolic status, age, or environmental conditions.

The OSI values obtained in this study are particularly informative, as they reflect the balance between oxidant load and antioxidant protection in a single parameter. Lower OSI values are generally associated with reduced oxidative stress and improved preservation of sperm structural integrity, especially under storage or cryopreservation conditions [13–15]. In the present study, the redox profile suggests the absence of pronounced oxidative imbalance in the analysed samples. However, since functional sperm parameters were not assessed, these findings should be interpreted with caution and cannot be directly linked to sperm performance.

Taken together, the results indicate that seminal plasma of cultured rainbow trout exhibits measurable but variable antioxidant capacity and oxidant load, and that OSI may serve as a useful indicator of seminal redox status. Future studies should integrate these parameters with sperm motility, membrane integrity, DNA stability, and fertilization success in order to better understand the functional significance of redox variation. Such an approach would strengthen the application of TAS, TOS, and OSI as biomarkers in reproductive management and broodstock assessment.

CONCLUSION

This study provides one of the first assessments of TOS, TAS, and OSI in the seminal plasma of cultured *Oncorhynchus mykiss*, establishing baseline redox values under controlled RAS conditions. The obtained results indicate moderate TOS and TAS levels, resulting in OSI values consistent with a balanced redox status in the analysed samples. These findings contribute to the understanding of seminal redox homeostasis in aquaculture and suggest that TOS, TAS, and OSI may serve as useful indicators of seminal physiological condition. However, given the absence of direct functional sperm parameters, these results should be interpreted with caution. Future research should aim to relate redox parameters to sperm motility, membrane integrity, and fertilization success, and to validate OSI thresholds across seasonal and genetic variability in order to refine their application in commercial hatchery management.

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