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ONE-MONTH ALTERATION IN THE ANTIOXIDANT DEFENSE IN THE HEART OF RAINBOW TROUT (*ONCORHYNCHUS MYKISS* WALBAUM) VACCINATED AGAINST *YERSINIA RUCKERI*

The present work aimed to assess the effects of oral vaccination against Yersinia ruckeri at first month after immunization in the cardiac tissue of rainbow trout (Oncorhynchus mykiss Walbaum). Concentrated vaccine with inactivated by formalin Y. ruckeri strains was enclosed by fish feed, and was administered three times every other day. Our results suggest that antioxidant reaction rates, characteristic of immunization, may be sufficient to protect against at least some of the physiological changes that might promote oxidative stress. In addition, our observation that products of lipid peroxidation are higher in the cardiac tissue from vaccinated animals (but not oxidatively modified proteins) indicates that antioxidant defenses is important in setting the balance in oxidative stress during immunization. Correlations between aldehydic derivatives of oxidatively modified proteins and antioxidant defenses confirmed that increased superoxide dismutase activity caused to decreased level of protein damage in the cardiac tissue of the trout vaccinated against Y. ruckeri at first month after immunization. Discrepancies observed for antioxidant enzymes among vaccinated fishes following oral vaccination against Y. ruckeri may represent tissue-specific variation required to support tissue-specific responses to immunization, including tissue-specific differences in oxidative stress and antioxidant defenses.

Key words**:** *yersiniosis, rainbow trout (Oncorhynchus mykiss), antioxidant defenses, immunization, cardiac tissue, total antioxidant capacity.*

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ИЗМЕНЕНИЯ В АНТИОКСИДАНТНОЙ ЗАЩИТЕ СЕРДЦА РАДУЖНОЙ ФОРЕЛИ (*ONCORHYNCHUS MYKISS* WALBAUM) В ПЕРВЫЙ МЕСЯЦ ПОСЛЕ ВАКЦИНАЦИИ ПРОТИВ *YERSINIA RUCKERI*

Целью нашего исследования была оценка влияния пероральной вакцинации против Yersinia ruckeri в первый месяц после иммунизации в сердечной ткани радужной форели (Oncorhynchus mykiss Walbaum). Рыбу перорально иммунизировали кормом с инкорпорированным штаммом Y. ruckeri инактивированным формалином. Наши результаты показывают, что уровень антиоксидантных реакций, характерный для иммунизации, может быть достаточным для защиты от, по меньшей мере, некоторых физиологических изменений, которые могут способствовать окислительному стрессу при вакцинации рыб. Кроме того, наше наблюдение показало, что антиоксидантная защита важна для установления баланса в процессах окислительного стресса во время иммунизации. Корреляции между альдегидными производными окислительно-модифицированных белков и маркерами антиоксидантной защиты подтвердили, что повышенная активность супероксиддисмутазы приводит к снижению уровня повреждения белков в сердечной ткани форели,

вакцинированной против Y. ruckeri в первый месяц после иммунизации. Расхождения, наблюдаемые для антиоксидантных ферментов среди вакцинированных рыб, могут представлять собой специфические изменения, необходимые для поддержки тканеспецифических ответов на иммунизацию, включая различия в окислительном стрессе и антиоксидантной защите.

Ключевые слова: *иерсиниоз, радужная форель (Oncorhynchus mykiss), антиоксидантная защита, иммунизация, сердечная ткань, общая антиоксидантная активность.*

Introduction

Yersinia ruckeri, a Gram-negative rod-shaped enterobacterium, is the causative agent of enteric redmouth (ERM) disease or yersiniosis, which affects mainly salmonids [4]. The disease gets its name from the subcutaneous hemorrhages, it can cause at the corners of the mouth and in gums and tongue. Other clinical signs include exophthalmia, darkening of the skin, splenomegaly and inflammation of the lower intestine with accumulation of thick yellow fluid. The bacterium enters the fish via the secondary gill lamellae and from there it spreads to the blood and internal organs [10].

Vaccination plays an important role in large-scale commercial fish farming and has been a key reason for the success of salmon cultivation. Developed vaccines based on inactivated bacterial pathogens have proven to be very efficacious in fish [14]. The predictive indicators of vaccine success include: non-protective/pathological response genes (cathelicidin, C-type lection and collagenase), vaccine-induced protective genes (immunoglobulin heavy chain, selenoprotein, 60S ribosomal protein L37 and unknown) and transcriptional biosignature of predominantly immune-relevant genes including hepcidin, immunoglobulin mu heavy chain, mylelin and lymphocyte protein. Detection of these bioindicators demonstrates that there is a range of potential targets for future vaccine development [3, 10].

The first commercially available fish vaccine was an immersion vaccine against ERM consisting of *Y. ruckeri* bacterin. The ERM immersion vaccine has been successfully used in aquaculture farming of salmonids for more than 35 years [12]. Two of the most predominant groups of *Y. ruckeri* belong to serovar type 1 (Hagerman) which is more commonly isolated from rainbow trout, and serovar type II (O'Leary) first isolated from chinook salmon (*Oncorhynchus kisutch*) [16]. Serovar 1 Hagerman strains are the basis for most commercial bacterins. Lipopolysaccharide (LPS) of serovar 1 *Y. ruckeri* elicits negligible or weak antibody responses in fish and low cell-proliferation memory responses compared with serovar 2 strains [15].

T lymphocytes play a main role in the adaptive immune response, which both coordinate other immune cells and destroy infected cells. Reactive oxygen species (ROS) have been extensively implicated in T-cell hyporesponsiveness, apoptosis, and activation [1]. Moreover, ROS can kill pathogens directly by causing oxidative damage to biocompounds or indirectly by stimulating pathogen elimination by various nonoxidative mechanisms, including pattern recognition receptors signaling, autophagy, neutrophil extracellular trap formation, and T-lymphocyte responses [13].

The antioxidant defense system that constitutes antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), and glutathione-S-transferase (GST) as well as nonenzymatic antioxidants [e.g., glutathione (GSH)] serve as an important biological defense against the toxicity of ROS. All of these markers have been extensively used in numerous field studies and are known to be involved in pathology and the etiology of fish diseases [7]. The present work aimed to assess the effects of oral vaccination against *Y. ruckeri* at first month after immunization in the cardiac tissue of rainbow trout (*Oncorhynchus mykiss* Walbaum).

Materials and methods

Experimental fish and design. Clinically healthy rainbow trout were used in the experiments. The experiments were performed in water at 14.5 ± 0.5°C and pH 7.2-7.4. The dissolved oxygen level was about 9 ppm with additional oxygen supply, with a water flow of 25 L/min, and a photoperiod of 12 h per day. The same experimental conditions were used during the whole research. The water parameters were maintained under constant surveillance. The fish were held in square tanks (150 fish per tank) and fed commercial pelleted diet.

The fish were divided into two groups: untreated control and immunized against ERM. The vaccine against ERM (Department of Fish Diseases, National Veterinary Research Institute, Pulawy, Poland) contained three inactivated *Y. ruckeri* strains originating from rainbow trout cultured at different farms, in which fish were exhibiting clinical signs of ERM. The bacteria isolates belonged to O1 serotype and showed some differences in their biochemical properties. Concentrated vaccine was enclosed by fish feed, and was administered three times every other day. Fifteen rainbow trout from each group were euthanized 31 days after the immunization, and then cardiac tissue samples were collected.

The samples were homogenized in ice-cold buffer (100 mM Tris-HCl, pH 7.2) using a glass homogenizer immersed in ice water bath. Homogenates were centrifuged at 3,000 g for 15 min at 4°C. After centrifugation, the supernatant was collected and frozen at −20°C until analyzed. Protein contents were determined with the method described by Bradford (1976) with bovine serum albumin as a standard [2]. Absorbance was recorded at 595 nm. All enzymatic assays were carried out at 22 ± 0.5°C using a Specol 11 spectrophotometer (Carl Zeiss Jena, Germany) in duplicate. The enzymatic reactions were started by adding the tissue supernatant.

Antioxidant defense assays. Superoxide dismutase (SOD, E.C. 1.15.1.1) activity was assessed by its ability to dismutate superoxide produced during quercetin auto-oxidation in an alkaline medium (pH 10.0) [9]. Catalase (CAT, E.C. 1.11.1.6) activity was determined by measuring the decrease of H_2O_2 in the reaction mixture using a spectrophotometer at the wavelength of 410 nm [8]. Glutathione reductase (GR, EC 1.6.4.2) activity in the sample was measured according to Glatzle and co-workers (1974) [6] with some modifications. Glutathione peroxidase (GPx, EC 1.11.1.9) activity was determined by detecting the nonenzymatic utilization of GSH (the reacting substrate) at an absorbance of 412 nm after incubation with 5,5-dithiobis-2-nitrobenzoic acid (DTNB) [11]. The total antioxidant capacity (TAC) level in the sample was estimated by measuring the TBARS level after Tween 80 oxidation. This level was determined spectrophotometrically at 532 nm [5].

Statistical analysis. The mean ± S.E.M. values was calculated for each group to determine the significance of inter group difference. Significance of differences between the antioxidant defense biomarkers (significance level, $p<0.05$) was examined using Mann-Whitney *U* test. Correlations between parameters at the set significance level were evaluated using Spearman's correlation analysis [23]. All statistical calculation was performed on separate data from each individual with STATISTICA 8.0 (StatSoft, Krakow, Poland).

Results. Antioxidant defense in the cardiac tissue of the trout vaccinated orally against *Y. ruckeri* at first month after immunization are shown in Figs 1 and 2. There were no statistically significant alterations in the activities of antioxidant defenses except SOD activity (Fig. 1). The SOD activity was significantly increased by 9% ($p<0.05$) after immunization, while CAT, GR and GPx was non-significantly decreased by 29%, 5%, and 9% ($p>0.05$) compared to the controls (Fig. 1).

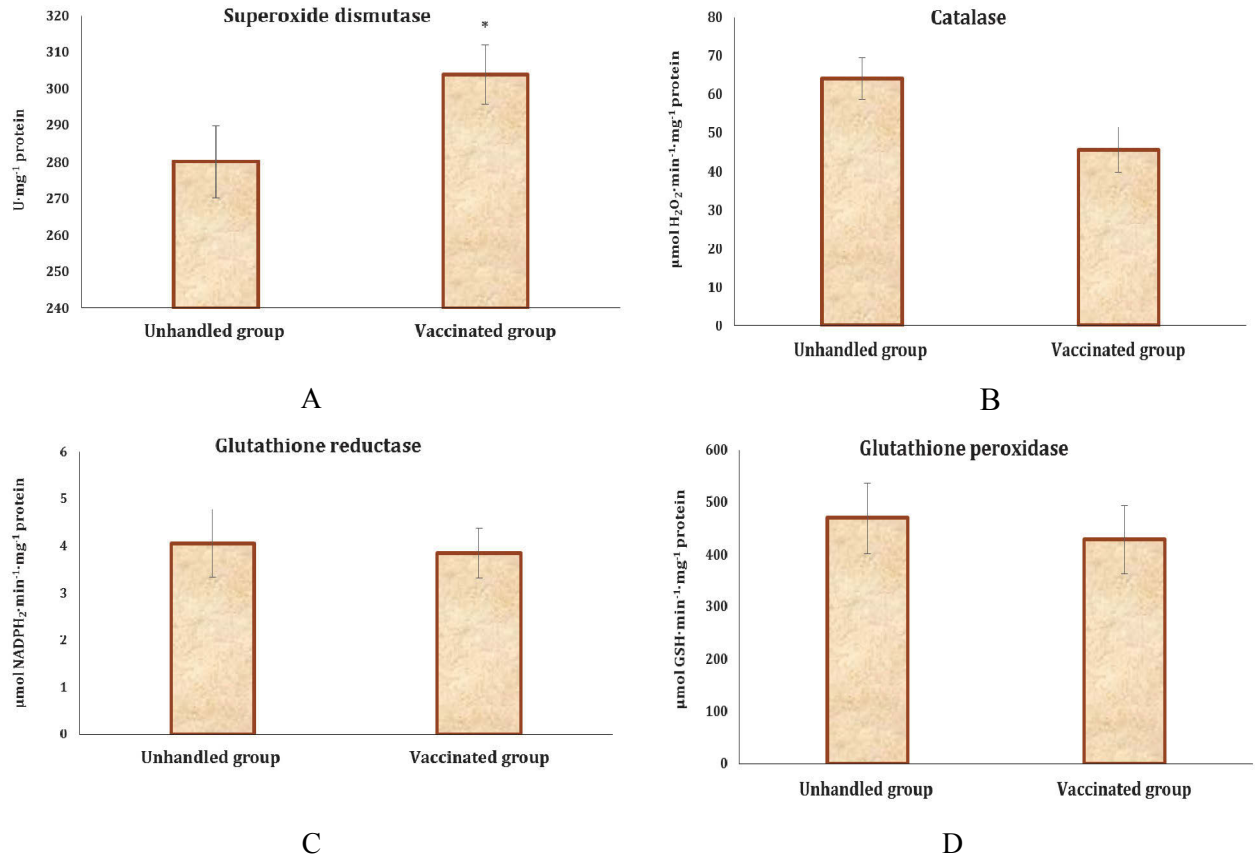

Fig. 1. Superoxide dismutase (A), catalase (B), glutathione reductase (C), and glutathione peroxidase (D) activities in the cardiac tissue of the trout vaccinated against Y. ruckeri at first month after immunization. Data are represented as mean ± S.E.M. (n=15). * – the significant change was shown as $p<0.05$ when compared values of unhandled and vaccinated groups

Non-significant increase of TAC level in the cardiac tissue of the trout vaccinated against *Y. ruckeri* at first month after immunization was found (Fig. 2).

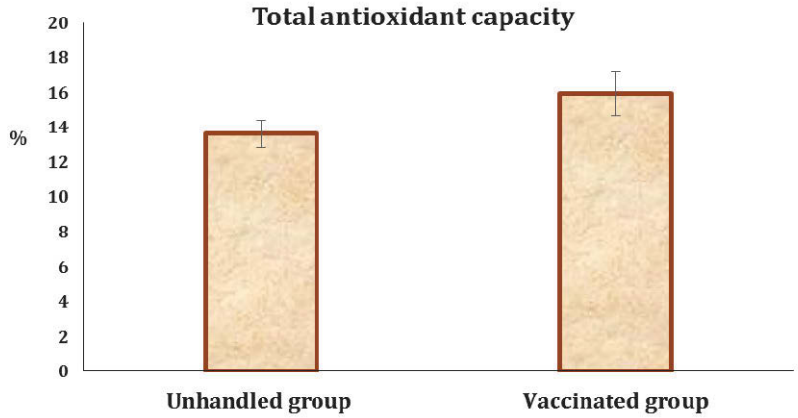

Fig. 2. The total antioxidant capacity in the cardiac tissue of the trout vaccinated against Y. ruckeri at first month after immunization. Data are represented as mean ± S.E.M. (n=15)

In vaccinated group, the aldehydic derivatives of oxidatively modified proteins correlated positively with CAT activity (r=0.833, p=0.000) (Fig. 3) and correlated inversely with SOD activity (r=-0.695, p=0.006) (Fig. 3).

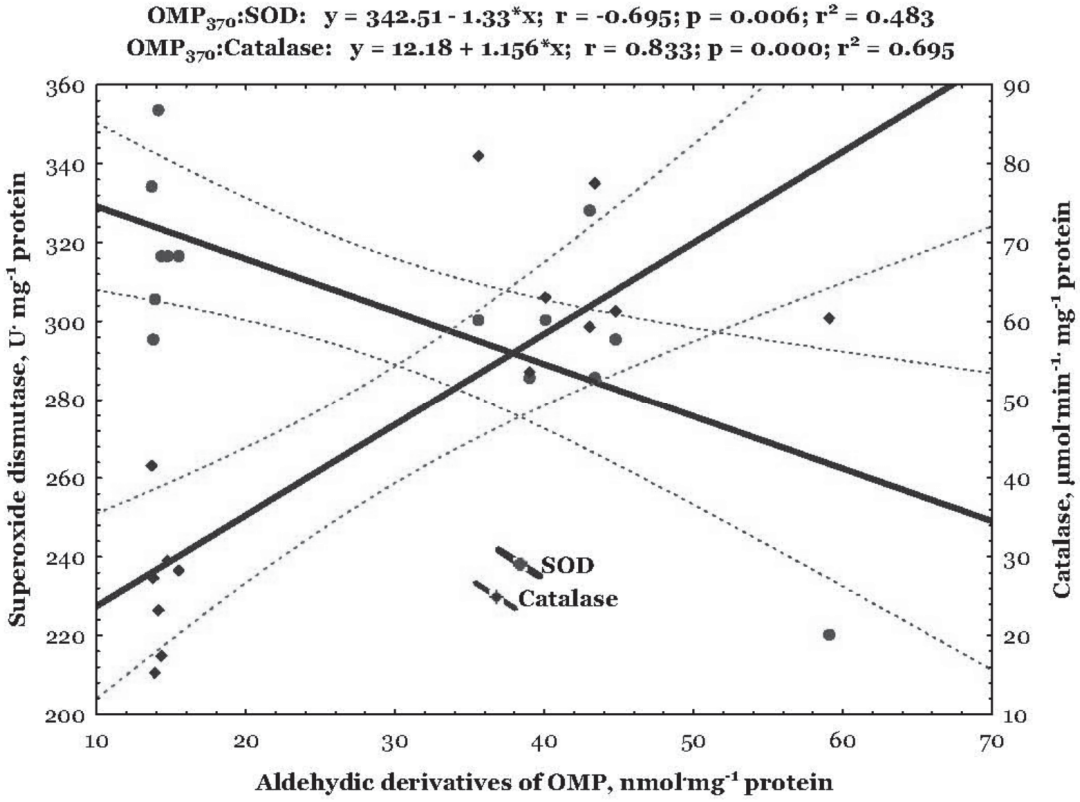

Fig. 3

Discussion

We report a general lack of response after oral vaccination against *Y. ruckeri* among the components of the enzymatic system of antioxidant defense except SOD activity in the cardiac tissue of the trout. Even with a small boost in oxidative capacities, and a greater tissue content of lipid peroxidation products, these physiological changes after immunization are not accompanied by an upregulation of the enzymatic antioxidant defense and the total antioxidant capacity in the cardiac tissue of vaccinated trout (Figs 1 and 2). Our results suggest that antioxidant reaction rates, characteristic of immunization, may be sufficient to protect against at least some of the physiological changes that might promote oxidative stress. In addition, our observation that products of lipid peroxidation are higher in the cardiac tissue from vaccinated animals (but not oxidatively modified proteins) indicates that antioxidant defenses is important in setting the balance in oxidative stress during immunization. Correlations between aldehydic derivatives of oxidatively modified proteins and antioxidant defenses confirmed that increased SOD activity caused to decreased level of protein damage in the cardiac tissue of the trout vaccinated against *Y. ruckeri* at first month after immunization (Fig. 3). The presence of such a relationship between SOD and CAT is expected because the two enzymes are linked in function: SOD converts superoxide anions to H_2O_2, which is decomposed by CAT.

Data from the current study add to a growing body of literature addressing the central question of how, and to what extent, enzymatic antioxidant defenses of salmonids are altered in response to immunization against *Y. ruckeri*. Fish exposed to vaccination exhibit a variety of

physiological responses, including oxidative metabolism imbalances [17-22]. In our previous study [22], we have analyzed the levels of oxidative stress biomarkers [2-thiobarbituric acid substances (TBARS), aldehydic and ketonic derivatives of oxidatively modified proteins (OMP), TAC] and metabolic alterations in the liver of juvenile rainbow trout determining the effectiveness of the vaccine against Y. ruckeri. A statistically significant reduction in lipid peroxidation between the mean in groups immunized after first and second months after vaccination indicated an effective adaptive antioxidant defense mechanisms of fish for the immunity against Y. ruckeri [22].

To determine the effects of vaccination against *Y. ruckeri* on health condition of rainbow trout in general, and oxidative stress biomarkers and metabolic parameters specifically, as well as to identify mechanisms that underpin the susceptibility of fish to vaccination, we compared the liver and heart function, and the oxidative mechanism underlying those effects, by detecting relevant lipid peroxidation and protein oxidation biomarkers, as well as aerobic-anaerobic metabolism in trout immunized against *Y. ruckeri* at 30 and 60 days compared to healthy individuals [17, 20]. Decreased aldehydic and ketonic derivatives of OMP and the reduction of aminotransferases and lactate dehydrogenase activities were sensitive to vaccination of trout against *Y. ruckeri* and may potentially be used as biomarkers in evaluating vaccine effects in the liver of rainbow trout [20]. The level of lipid peroxidation in the liver and heart on the 61st day after immunization of rainbow trout does not significantly differ from that in the control [17]. Vaccination caused a slight decrease of the aldehydic and ketonic derivatives level in the heart and liver against the backdrop of a significant TAC decrease in the cardiac tissue of the trout treated by the vaccine against *Y. ruckeri* on the 61st day after immunization. This is possibly a result of a long-term adaptation to immunization [17]. No significant difference was noted in lipid peroxidation level in the muscle tissue of rainbow trout in either the first or second month after vaccination, while aldehydic and ketonic derivatives of oxidatively modified proteins OMB in the vaccinated group were significantly lower in the second month compared to those in the first month after vaccination ($P<0.05$) [21]. The content of ketonic derivatives of OMB in muscles in the first month after immunization was higher compared to untreated control. All these culminated in a depletion of GPx activity and decreased TAC level. Correlations between CAT activity and lipid peroxidation and TAC confirmed the pivotal role of CAT in antioxidant defense in the muscle tissue during immunization. From a broader perspective, it is suggested that immunization of fish with anty-*Yersinia* vaccine is associated with induced ROS formation and oxidative stress. ROS would therefore be at least partially responsible for the induction of both humoral and cellular elements of the immunity and increased protective immunity against *Y. ruckeri* infection [21].

When interpreted in the context of previous works, results reported herein (Figs 1-3) indicate that the discrepancies observed for antioxidant enzymes among vaccinated fishes following oral vaccination against *Y. ruckeri* may represent tissue-specific variation required to support tissue-specific responses to immunization, including tissue-specific differences in oxidative stress and antioxidant defenses.

Conclusions

In this study, superoxide dismutase activity in the cardiac tissue of vaccinated trout was increased. Oral vaccination against *Y. ruckeri* not led to oxidative damage on tissue at first month after vaccination. Correlations between aldehydic derivatives of oxidatively modified proteins and antioxidant defenses confirmed that increased SOD activity caused to decreased level of protein damage in the cardiac tissue of the trout vaccinated against *Y. ruckeri* at first month after immunization. SOD is a metalloenzymes that play a key role in the defense against oxidative stress by transforming superoxide anions into hydrogen peroxide, which is detoxified by both GPx and CAT activities. The elevated levels of SOD in cardiac tissue shows a possible shift toward a detoxification mechanism under immunization which indicated that the rainbow trout

could protect itself against the toxic effect of superoxide anion radical by increasing its activity. The higher SOD activity than in other antioxidant enzymes could be an indicator of compensatory cardiac tissue response to vaccination.

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