УДК 636.1:591.111

SEASONAL ALTERATIONS IN EXERCISE-INDUCED RESISTANCE OF ERYTHROCYTES IN HORSES INVOLVED IN RECREATIONAL HORSEBACK RIDING (OVERVIEW)

Pażontka-Lipiński P., student
Witaszek M., student
Tkachenko H., PhD, assistant Professor
Institute of Biology and Environmental Protection, Pomeranian University in Slupsk, Poland

Erythrocytes are the best indicators of increased generation of reactive oxygen species and activation of oxidative stress. Training session led to decreased the maximum percentage of hemolyzed erythrocytes in horses by 16 % (spring), 26.9 % (summer), and 20.9 % (autumn). In winter, same value of the maximum percentage of hemolyzed erythrocytes in horses before and after training session was observed. Percentage of hemolysis during first 30 sec. as well as the maximum percentage of hemolyzed erythrocytes were significantly higher in summer season both before and after training. The later time of maximum hemolysis was noted in spring season before and after training session (5.5 and 6.5 min, respectively). Both before and after training session, increase of time of hemolysis in horses in spring was observed. The least resistant erythrocytes to hydrochloric acid were in summer, more resistance – in autumn and winter, respectively. We can assume that seasonal alterations may occur due to the greater muscle activity of the horses, as well as higher environmental temperature, especially in the summer period. The results showed that training in spring and autumn might exert beneficial effects by enhancing the erythrocytes' resistance in horses involved in recreational horseback riding.

Keywords: **recreational horseback riding, Pomeranian region, Poland, altitude, acid-induced hemolysis, resistance of erythrocytes.**

Some geographic areas are more closely associated with the use of horses than others, i.e. Pomeranian region in Poland. In general, any horse can be a trail riding horse. Some breeds, however, are more common on the trails than others. These breeds usually have a gentle disposition, tend not to spook easily, are fairly surefooted and are smooth enough that they are comfortable to ride for extended periods [1]. Common trail riding breeds include horses such as the Quarter Horse and Appaloosa and pleasure saddle breeds such as the Tennessee Walking Horse, Missouri Fox Trotter and various Mountain Saddle Horses (Kentucky, Spotted, Rocky, etc). For riding, often use horses of local breeds, which a bred directly in recreational areas. For example, Hucul horses which widespread in Carpathians – in Poland and Ukraine.

Moreover, recreational horseback riding helps develop a health and promotes to improve physical fitness, as a tourists horse-riders and horses too. Although recreational riding is a non-competitive activity, but physical fitness is necessary for a horses, especially if these horses used for long or difficult ride [2]. Proper conditioning and feeding helps are horses meet the physical demands of recreational riding, but training and mental readiness also play important roles in preparing a horse for ride.

Exercises remains a key aspect of improving of endurance and performance for horses involved to recreational riding [3]. The athletic capacity of horses is attributable to a number of physiologic adaptations. In some cases these adaptations are not affected by training – for example, lung size, whereas others change in response to training – for example, blood volume. The superior athletic ability of horses is attributable to their high maximal aerobic capacity, large intramuscular stores of energy substrates and in particular glycogen, high mitochondrial volume in muscle, the ability to increase oxygen-carrying capacity of blood at the onset of exercise through splenic contraction, efficiency of gait, and efficient thermoregulation. However, strenuous physical exercise results in an enhanced uptake of oxygen leading to increased metabolism, which may increase the production of reactive oxygen species (ROS), potentially leading to oxidative stress [4]. Oxidative stress has been defined as a disturbance in the pro-oxidant-antioxidant balance in favour of the former, leading to potential damage.

Physical activity increases the generation of ROS in several ways. Two to 5 % of oxygen used in the mitochondria forms free radicals. As oxidative phosphorylation increases in response to exercise, there will be a concomitant increase in free radicals. Catecholamines that are released during exercise can lead to free radical production. Other sources of free radical increase with exercise include prostanoid metabolism, xanthine oxidase, NAD(P)H oxidase, and several secondary sources, such as the release of radicals by macrophages recruited to repair damaged tissue [5]. The relationship between physical exercise and oxidative tissue damage has been investigated in numerous studies in men and animals [6, 7]. Exercise has been shown to induce cellular and tissue damage by oxidation of cellular components, such as membrane lipids, proteins, carbohydrates and desoxy-ribonucleic acids [8]. Exercise-induced oxidative stress is believed to contribute to accelerated muscle fatigue and muscle fibre damage, leading to exercise intolerance and poor performance in different animal species including horses [9, 10].

Despite the increased ROS production during exercise, growing evidence derived from several studies strongly indicates that habitual, moderate physical activity reduces the incidence of oxidative stress-based diseases and retard the aging process [11]. This apparent paradox can be explained taking into account that ROS produced during repeated exercise bouts could serve as mild stimulating stressors able to adaptation to exercises [12]. Regular physical exercise has well-documented health benefits and can prolong mean life span in animals. Adaptive mechanisms seem to decrease oxidative stress and they encompass increased antioxidant defenses, reduced basal production of oxidants, and reduction of radical leak during oxidative phosphorylation [13]. Moderate exercise significantly decreases the age-associated development of oxidative stress in mice, increases life span, prevents decay of mitochondrial function, and even improves behavioral performance [11]. In addition, cells have developed different antioxidant systems and various antioxidant enzymes to defend themselves against free radical attacks. These free radicals are neutralized by an elaborate antioxidant defense system consisting of enzymes such as catalase, superoxide dismutase, glutathione peroxidase, and numerous non-enzymatic antioxidants, including vitamins A, E and C, glutathione, ubiquinone, and flavonoids [5].

Several studies reported, that acute and chronic exercise, have been associated with changes of hematological, biochemical, immune and hormonal parameters [14]. Exercise has variable effects on the hematological parameters, depending on exercise duration and intensity (short-term high intensity or maximal exercise and long-term low intensity or submaximal prolonged exercise), fitness and training levels and environmental conditions [15]. Our previous study has shown significant differences of hematological parameters in mares of different breeds [16]. Values of hematological parameters determined in our previous study can be used for health control and diagnosis of diseases and allow the evaluating the level of performance potential, the accuracy of training and physiological condition of horses.

Erythrocytes are the best indicators of increased generation of ROS and activation of oxidative stress. The seasonal rhythms reflect the ability of endogenous adaptive mechanisms of animals to react in advance to environmental changes associated with seasons. Season is an exogenous factor that modulates the dynamic of blood components in horses [15]. Recent studies of equine exercise physiology have focused mainly on determining the usefulness of biochemical and haematological parameters for evaluating physiological capacity and adaptation to increasing loads in horses of different breeds. Therefore, the aim of this study was to analyze the effects of training session on acid-induced resistance of erythrocytes in horses involved in recreational riding from Pomeranian regions.

Materials and methods. Thirteen healthy adult horses from central Pomeranian region in Poland (village Strzelinko, N54º30’48.0” E16º57’44.9”, Fig. 1), aged 9.5±2.4 years old, including 5 Hucul pony, 2 Thoroughbred horses, 2 Anglo-Arabian horses and 4 horses of unknown breed, were used in this study. All horses participated in recreational horseback riding. Horses were housed in individual boxes, with feeding (hay and oat) provided twice a day, at 08.00 and 18.00 h, and water available *ad libitum*. All horses were thoroughly examined clinically and screened for hematological, biochemical and vital parameters, which were within reference ranges. The females were non-pregnant.

Exercise test. Training started at 10:00 AM, lasted 1 hour and consisted from a ride of cross country by walking (5 min), trotting (15 min), walking (10 min), trotting (10 min), walking (5 min), galloping (5 min), and walking (10 min).

Blood samples. Blood was drawn from jugular veins of the animals in the morning, 90 minutes after feeding, while the horses were in the stables (between 8:30 and 10 AM), and immediately after exercise test (between 11 AM and 2 PM). Blood samples were taken once per a season for one year: spring (April), summer (July), autumn (October), winter (February). Blood was stored into tubes with K-EDTA and held on the ice until centrifugation at 3,000 g for 15 minutes. The plasma was removed. The erythrocytes' suspension (one volume) was washed with five volumes of saline solution three times and centrifuged at 3,000 g for 15 minutes. Plasma aliquots were frozen and stored at – 25 °C until assays.

Fig. 1. Map of Pomeranian voivodeship, northern Poland. Marked village Strzelinko (N54º30’48.0” E16º57’44.9”) where blood samples of horses were collected

Assay of Acid Resistance of Erythrocytes. The acid resistance of erythrocytes was measured spectrophotometrically with 0.1N HCl [17]. The method is based on the measuring of the dynamics of erythrocytes disintegration tohaemolytic reagent action. The time of haemolytic reagent action serves as the measure of erythrocytes resistance. Freshly collected blood samples was centrifuged at 3,000 rpm for 10 minutes. The sedimented cells was washed with saline solution. The process was repeated three times. Washed erythrocytes was dissolved with saline solution to prepare 1 % erythrocytes solution. The assay mixture contained 10 mL of 1 % erythrocytes solution and 0.1 mL HCl. The absorbance was read at 540 nm every 30 second after addition of HCl till the end of haemolysis. Difference of absorbance at the beginning and at the end of haemolysis was determined as 100 %. Disintegration of erythrocytes (%) for every period of time was expressed as curve.

Statistical analysis. Results are expressed as mean ± S.E.M. All variables were tested for normal distribution using the Kolmogorov-Smirnov test ($p>0.05$). In order to find significant differences (significance level, $p<0.05$) between states at before and after exercise, Wilcoxon signed-rank test was applied to the data (Zar 1999). Statistical significance between mean in groups of horses in spring and summer both before and after exercise was evaluated by Mann-Whitney *U* test [18]. All statistical analyses were performed using STATISTICA 8.0 software (StatSoft, Poland).

Results and discussion. Acid-induced resistance of erythrocytes of horses involved in recreational horseback riding from Pomeranian regions before and after training session in spring, summer, autumn, and winter are presented in Fig.2.

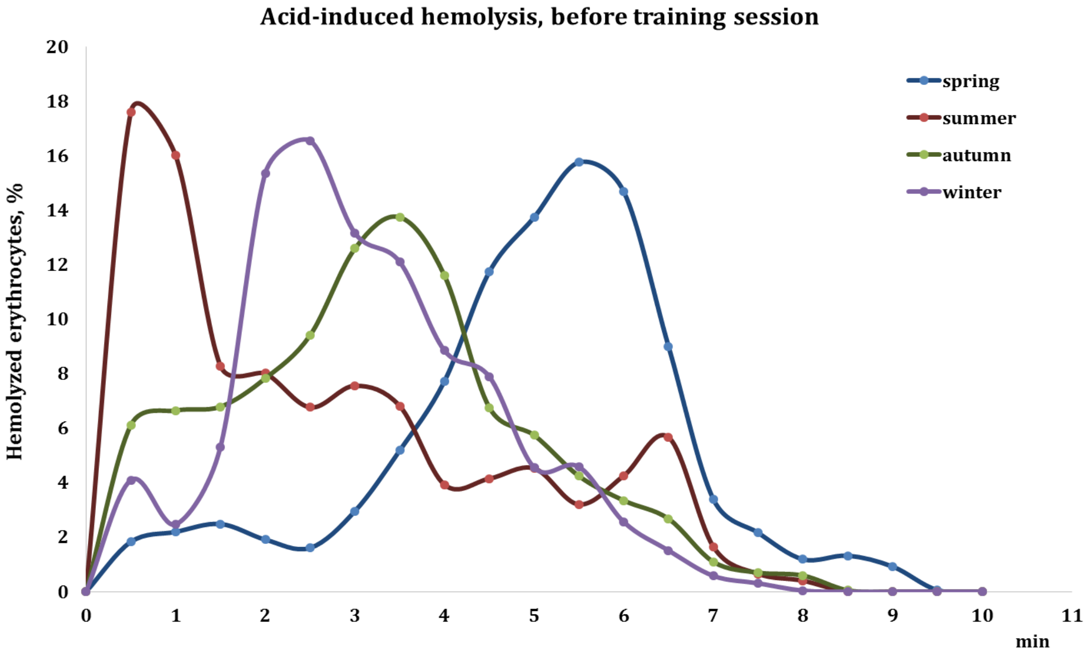
Training session led to decreased the maximum percentage of hemolyzed erythrocytes in horses by 16 % (spring), 26.9 % (summer), and 20.9 % (autumn). In winter, same value of the maximum percentage of hemolyzed erythrocytes in horses before and after training session was observed. Percentage of hemolysis during first 30 sec. as well as the maximum percentage of hemolyzed erythrocytes were significantly higher in summer season both before and after training. The later time of maximum hemolysis was noted in spring season before and after training session (5.5 and 6.5 min, respectively) (Fig. 2).

Both before and after training session, increase of time of hemolysis in horses in spring was observed. The least resistant erythrocytes to hydrochloric acid were in summer, more resistance – in autumn and winter, respectively (Fig. 2A).

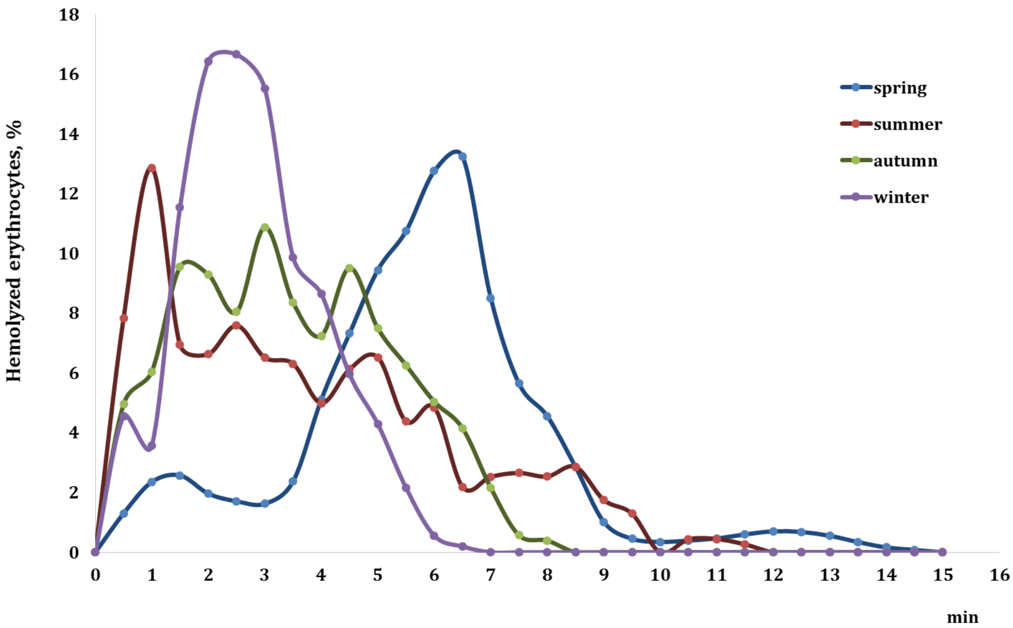
The acid resistance of erythrocytes was increased in horses after training session in spring, summer, and autumn (Fig. 2B).

We suggest that alterations in erythrocytes' resistance to hemolytic agents might be the result of two various modifications occurring in the horse body system following training session in different seasons. The first cause consists in blood lactate changes that directly influenced blood pH during exercises [3].

Although a reduction in percentage of hemolysis before and after training session was observed in blood of horses in spring, summer, and autumn, no significant difference was found in winter (Figs 2 A, B). This finding might be due to the presence of erythrocytes released by the spleen following the physical effort in winter. Effectively, the stagnantly pooled erythrocytes in the spleen could accelerate membrane lipid alteration [20] that could mask the influence on hemolysis.



A



B

Fig. 2. Acid-induced resistance of erythrocytes of horses involved in recreational horseback riding from Pomeranian regions before (A) and after training session (B) in various seasons.

Conclusions.

1. In conclusion, we found significant seasonal alterations in acid-induced hemolysis of erythrocytes of horses involved in recreational horseback riding from Pomeranian regions before and after training session. Training session led to decreased the maximum percentage of hemolyzed erythrocytes in horses by 16 % (spring), 26.9 % (summer), and 20.9 % (autumn). In winter, same value of the maximum percentage of hemolyzed erythrocytes in horses before and after training session was observed.

2. Percentage of hemolysis during first 30 sec. as well as the maximum percentage of hemolyzed erythrocytes were significantly higher in summer season both before and after training. The later time of maximum hemolysis was noted in spring season before and after training session (5.5 and 6.5 min, respectively). Both before and after training session, increase of time of hemolysis in horses in spring was observed. The least resistant erythrocytes to hydrochloric acid were in summer, more resistance - in autumn and winter, respectively.

3. We can assume that seasonal alterations may occur due to the greater muscle activity of the horses, as well as higher environmental temperature, especially in the summer period. The results showed that training in spring and autumn might exert beneficial effects by enhancing the erythrocytes' resistance in horses involved in recreational horseback riding.

References

1. Pagoulatos, A., Blackwell, M., Brown, R., Hu, W., Auchter, K. (2008*). A spatial analysis of equine trail riding* – Final Report. http://www2.ca.uky.edu/

2. Kimball, C. (2005). *The everything horseback riding book: step-by-step instruction to riding like a pro*. Adams Media, USA.

3. Hinchcliff, K. W., Geor, R. J. (2008). The horse as an athlete: a physiological overview. *Equine Exercise Physiology. The Science of Exercise in the Athletic Horse.* Eds. K. W. Hinchcliff, R. J. Geor, A. J. Kaneps. Elsevier, P. 2–11.

4. Deaton, C. M., Marlin, D. J. (2003). *Exercise-associated oxidative stress. Clinical Techniques in Equine Practice*, 2: 278–291.

5. Urso, M. L., Clarkson, P. M. (2003). Oxidative stress, exercise, and antioxidant supplementation. *Toxicology*, 189, 41–54.

6. De Moffarts, B., Portier, K., Kirschvink, N., Coudert, J., Fellmann, N., van Erck, E., Letellier, C., Motta, C., Pincemail, J., Art, T., Lekeux, P. (2007). Effects of exercise and oral antioxidant supplementation enriched in (n-3) fatty acids on blood oxidant markers and erythrocyte membrane fluidity in horses. *Vet. J.*, 174 (1), 113–121.

7. FarucUrgas, A. (2013). Effect of high intensity interval training on elite athletes' oxidant status. Sciences & Sports, 28, 253–259.

8. Clarkson, P. M., Thompson, H. S. (2000). Antioxidants: what role do they play in physical activity and health? *American J. of Clinical Nutrition*, 72 (Suppl. 2), 637–646.

9. Chiaradia, E., Avellini, L., Rueca, F., Spaterna, A., Porciello, F., Antonioni, M. T., Gaiti, A. (1998). Physical exercise, oxidative stress and muscle damage in racehorses. *Comp. Biochem Physiol. B Biochem. Mol. Biol*., 119 (4), 833–836.

10. Sen, C. K., Packer, L. (2000). Thiol homeostasis and supplements in physical exercise. *American J. of Clinical Nutrition*, 72, Suppl. 2, 653–669.

11. Radák, Z., Naito, H., Kaneko, T., Tahara, S., Nakamotoo, H., Takahashi, H., Cardozo-Pelaez, F., Goto, S. (2002). Exercise training decreases DNA damage and increases DNA repair and resistance against oxidative stress of proteins in aged rat skeletal muscle. *European Journal of Physiology*, 445 (2), 273–278.

12. Packer, L., Cadenas, L., Davies, K. J. (2008). Free radicals and exercise: an introduction. *Free Radic. Biol. Med*., 44 (2), 123–125.

13. Leeuwenburgh, C., Heinecke, J. (2001). Oxidative stress and antioxidants in exercises. *Current Medicinal Chemistry*, 8 (7), 829–838.

14. Hyyppä, S. (2005). Endocrinal responses in exercising horses. *Livestock Production Science,* 92, 113–121.

15. Satué, K., Gardón, J. C., Muñoz, A. (2014). Season of the year should be considered in the interpretation of hematology in carthusian. *J. of Hematology Research*, 1, 63–68.

16. Andriichuk, A., Tkachenko, H., Kurhaluk, N., Tkachova, I., Kleczkowska, A. (2012). Wybrane wskaźniki hematologiczne klaczy różnych ras. *Słupskie Prace Biologiczne*, 9: 21-34.

17. Terskov, I. A., Gitelson, I. I. (1957). Method of chemical (acid) erythrograms. *Biofizika,* 2, 259–266.

18. Zar, J. H. (1999). *Biostatistical Analysis*, 4th ed., Prentice Hall Inc., New Jersey.

19. Carlson, G. P. (1995). Interrelationships between fluid, electrolyte and acid–base balance during maximal exercise. *Equine Vet. J.*, 18, 261–265.

20. Hanzawa, K., Kubo, K., Kai, M., Hiraga, A., Watanabe, S. (1999). Effects of exercise on density and osmotic fragility of circulating red cells in normal and splenectomised Thoroughbred horses. *J. of Equine Sci*., 10, 67–72.

СЕЗОННЫЕ ИЗМЕНЕНИЯ В ТРЕНИНГ-ИНДУЦИРОВАННОЙ УСТОЙЧИВОСТИ ЭРИТРОЦИТОВ У ЛОШАДЕЙ, ИСПОЛЬЗУЮЩИХСЯ В РЕКРЕАЦИОННОЙ ВЕРХОВОЙ ЕЗДЫ (ОБЗОРНАЯ)

Пазонтка-Липински П., Виташек М., Ткаченко Г., Інститут біології та охорони середовища Поморского университета (Слупск, Польща)

Лучшими показателями увеличения генерации реактивных видов кислорода и активации окислительного стресса являются эритроциты. Тренинг лошадей привел к снижению максимального процента гемолизированных эритроцитов на 16 % (весна), 26,9 % (лето) и 20,9 % (осень). Зимой наблюдается такое же значение максимального процента гемолизированных эритроцитов у лошадей до и после тренинга. Процент гемолиза в течение первых 30 с, а также максимальный процент гемолизированных эритроцитов были значительно выше в летний сезон как до, так и после тренинга. Максимальный гемолиз отмечали в весенне-летний период до и после тренинга (5,5 и 6,5 мин. соответственно). Как до, так и после тренинга наблюдали увеличение времени гемолиза у лошадей весной. Наименее устойчивые к соляной кислоте эритроциты наблюдали летом, более высокую их резистентность – осенью и зимой. Можно предположить, что сезонные изменения могут возникать из-за большей мышечной активности лошадей, а также более высокой температуры окружающей среды, особенно в летний период. Результаты показали, что тренировка весной и осенью может оказать благотворное воздействие за счет повышения устойчивости эритроцитов к лошадям, использующимся для рекреационной верховой езды.

Ключевые слова: рекреационная верховая езда, Поморское воеводство (Польша), высота, кислотно-индуцированный гемолиз, резистентность эритроцитов.

СЕЗОННІ ЗМІНИ В ТРЕНІНГ-ІНДУКОВАНІЙ СТІЙКОСТІ ЕРИТРОЦИТІВ У КОНЕЙ, ЯКІ ВИКОРИСТОВУЮТЬСЯ В РЕКРЕАЦІЙНІЙ ВЕРХОВІЙ ЇЗДІ (ОГЛЯДОВА)

Пазонтка-Ліпінскі П., Віташек М., Ткаченко Г., Інститут біології та охорони середовища Поморского университета (Слупск, Польща)

Кращими показниками збільшення генерації реактивних видів кисню і активації оксидативного стресу є еритроцити. Тренінг коней привів до зниження максимального відсотка гемолізованих еритроцитів на 16 % (весна), 26,9 % (літо) і 20,9 % (осінь). Взимку спостерігається таке ж значення максимального ві-

дсотка гемолізованих еритроцитів у коней до і після тренінгу. Відсоток гемолізу протягом перших 30 с, а також максимальний відсоток гемолізованих еритроцитів були значно вищими у літній сезон як до, так і після тренінгу. Максимальний гемоліз відзначали у весняно-літній період до і після тренінгу (5,5 та 6,5 хв. відповідно). Як до, так і після тренінгу спостерігали збільшення часу гемолізу у коней навесні. Найменш стійкі до соляної кислоти еритроцити спостерігали влітку, більш високу їх резистентність – восени і взимку. Можна припустити, що сезонні зміни можуть виникати з-за більшої м'язової активності коней, а також більш високої температури навколишнього середовища, особливо в літній період. Результати показали, що тренування навесні і восени може зробити благотворний вплив за рахунок підвищення стійкості еритроцитів до коней, що використовується для рекреаційної верхової їзди.

Ключові слова: рекреаційна верхова їзда, Поморське воєводство (Польща), висота, кислотно-індукований гемоліз, резистентність еритроцитів.

УДК 57.044:577.3:639.3:612.062

ANTIOXIDANT DEFENSES IN THE HEPATIC TISSUE OF GRAYLING (*THYMALLUS THYMALLUS* LINCK) AFTER CHLORAMINE-T DISINFECTION (OVERVIEW)

Tkachenko H., PhD, assistant Professor
Department of Zoology and Animal Physiology, Institute of Biology and Environmental Protection, Pomeranian University in Slupsk, Poland
Grudniewska J., PhD, assistant Professor
Department of Salmonid Research, Stanislaw Sakowicz Inland Fisheries Institute, Poland

The aim of the current study was to examine the effects of disinfection by Chloramine-T on the hepatic tissue of grayling (Thymallus thymallus Linck) using antioxidant defense (superoxide dismutase, catalase, glutathione reductase, glutathione peroxidase, total antioxidant capacity) to observe the its toxic effects. The endpoints obtained from this study will be useful to monitor the effects of disinfective procedure with Chloramine-T for this species of fish. Our results showed that the catalase activity was increased after Chloramine-T influence. In contrary, the glutathione peroxidase activity in the hepatic tissue of grayling disinfected by Chloramine-T was decreased compared to the controls. Non-significant decrease of total antioxidant capacity level in the hepatic tissue of grayling as a consequence of disinfection with Chloramine-T was found. The antioxidant enzymatic defenses are considered an important control point for the homeostatic adjustments to disinfection-induced stress. The biomarkers of antioxidant defense in this study will therefore be suitable for the monitoring in safety of disinfected procedures. Preliminary results are highly promising and practical implications for a more robust and suitable evaluation of disinfected procedures are possible using these biomarkers.

Keywords: **Chloramine-T, disinfection, grayling *Thymallus thymallus*, hepatic tissue, antioxidant defense, total antioxidant capacity.**