

SELECTED BIOMARKERS OF ANTIOXIDANT STATUS IN HORSES

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ABSTRACT

Oxidative stress represents an important factor affecting health status, reproductive performance, physical performance, and adaptive mechanisms in horses. The aim of this study was to evaluate selected biomarkers of antioxidant status in clinically healthy American Quarter Horses and to assess their usefulness in the evaluation of oxidative balance. The study included 13 clinically healthy horses consisting of seven mares and six geldings aged between 4 and 15 years, used for breeding and recreational purposes.

Blood samples were collected from the *vena jugularis*. Serum samples were used for the determination of total antioxidant status and malondialdehyde concentration, whereas glutathione peroxidase activity was determined from heparinized whole blood.

The mean total antioxidant status reached 2.42 ± 0.53 mmol/L, glutathione peroxidase activity 862 ± 265 U/L, and the mean malondialdehyde concentration 0.131 ± 0.022 μ mol/L. The obtained results indicated a favorable antioxidant profile in the evaluated horses.

The results suggest that clinically healthy American Quarter Horses maintained under the conditions of the studied farm in eastern Slovakia do not exhibit signs of increased oxidative stress. The obtained data provide preliminary information on the antioxidant status of this breed and may serve as a basis for further studies focused on the influence of age, reproductive status, nutrition, and physical activity on the oxidative balance of the equine organism.

Keywords: American Quarter Horse; malondialdehyde; glutathione peroxidase; oxidative stress

INTRODUCTION

Oxidative stress represents a state of imbalance between the production of reactive oxygen species (ROS) and the antioxidant defense mechanisms of the organism. Excessive production of free radicals may result in oxidative damage to lipids, proteins, and nucleic acids, thereby affecting cellular homeostasis and physiological functions. In horses, oxidative stress is considered an important factor influencing health status, athletic performance, and adaptive responses of the organism (Shono et al., 2020; Arfuso et al., 2022).

The antioxidant defense system consists of both enzymatic and non-enzymatic mechanisms. Among the most important enzymatic antioxidants is glutathione peroxidase (GPx), which participates in the detoxification of peroxides and protects cellular membranes against oxidative damage. Total antioxidant status (TAS) reflects the overall antioxidant capacity of the organism, whereas malondialdehyde (MDA), a final product of lipid peroxidation, is widely recognized as a reliable biomarker of oxidative damage in horses (Brkljača Bottegaro et al. 2018; Yildirim and Apaydin Yildirim, 2023).

Several studies have demonstrated that physical exercise significantly influences the oxidative balance in horses. Increased oxygen consumption during muscular activity leads to enhanced production of reactive oxygen species and subsequent alterations in oxidative and antioxidant biomarkers. Sport and endurance horses frequently exhibit changes in oxidative stress parameters and antioxidant responses, indicating the importance of adaptive mechanisms in maintaining redox homeostasis during exercise (Brkljača Bottegaro et al., 2018; Arfuso et al., 2022).

Nutrition and trace mineral intake also play an important role in the regulation of antioxidant defense mechanisms. Latham et al. (2021) demonstrated that supplementation with complexed trace minerals influenced antioxidant enzyme activity and improved the physiological response to transportation stress in young horses. Adequate antioxidant capacity may contribute to the maintenance of physiological functions and reduce the risk of oxidative damage to tissues.

The American Quarter Horse is one of the most popular horse breeds worldwide and has become increasingly common in Central Europe, including Slovakia. However, information on the antioxidant status of clinically healthy American Quarter Horses maintained under Slovak breeding conditions remains limited. The present study aimed to evaluate selected biomarkers of antioxidant status, including total antioxidant status, glutathione peroxidase activity, and malondialdehyde concentration, and to provide preliminary reference data for horses maintained under breeding conditions in eastern Slovakia.

MATERIALS AND METHODS

Experimental Animals

A total of 13 clinically healthy American Quarter Horses were included in the study. The American Quarter Horse is a breed originating from the United States, which began to spread more significantly in Central European countries following the social changes that occurred at the turn of the 1980s and 1990s. Despite the increasing popularity of this breed, information regarding the antioxidant status of American Quarter Horses kept under the conditions of the Slovak Republic remains limited.

The experimental group consisted of seven mares and six geldings aged between 4 and 15 years, used for breeding and recreational purposes. The body weight of the horses ranged from 470 to 540 kg. The body condition of all animals was assessed using the 9-point Body Condition Score (BCS) system and corresponded to normal body condition (BCS = 5) (Potter et al., 2024).

The horses originated from a breeding farm located in the Spiš region of eastern Slovakia and were kept under outdoor housing conditions. The study was conducted during the winter period when the animals were not grazing. The diet consisted primarily of meadow hay obtained from a local supplier, which represented the main component of the ration. Throughout the study period, the horses had unrestricted access to drinking water.

All animals were regularly dewormed according to the recommended veterinary program. Prior to inclusion in the study, all horses underwent a clinical examination and showed no signs of disease or other health disorders that could have influenced the evaluated parameters. During the study period, all animals were maintained under comparable management and husbandry conditions.

Blood Sampling and Sample Processing

Blood samples were collected from the *vena jugularis* of all examined horses under standard conditions, with emphasis on minimizing animal stress during sampling. Blood intended for biochemical and antioxidant analyses was collected into serum separator tubes (SST). Additional blood samples for the determination of glutathione peroxidase (GPx) activity were collected into heparinized tubes. Following collection, the samples were transported to the laboratory and processed as soon as possible under standard laboratory conditions. Blood samples collected into SST tubes were subsequently centrifuged at 3500 rpm for 10 minutes to obtain serum. The separated serum was used for the determination of selected antioxidant parameters, whereas heparinized whole blood was used for the determination of GPx activity.



Fig. 1: Laboratory workflow for the determination of antioxidant parameters
Source: Own photographs.

Determination of Total Antioxidant Status (TAS)

Total antioxidant status was determined using the Randox Total Antioxidant Status kit NX2332, Randox Laboratories Ltd., United Kingdom. The method is based on the inhibition of the formation of the ABTS® radical cation. The absorbance was measured spectrophotometrically at 600 nm using a UV-VIS spectrophotometer ONDA V-10 PLUS (Giorgio Bormac S.r.l., Italy). The TAS values were calculated according to the manufacturer's instructions using calibration procedures and expressed in mmol/L.

Determination of Glutathione Peroxidase Activity (GPx)

Glutathione peroxidase activity was determined using the Ransel kit RS504, Randox Laboratories Ltd., United Kingdom. The assay is based on the oxidation of reduced glutathione by cumene hydroperoxide in the presence of glutathione reductase and NADPH.

The decrease in NADPH absorbance was monitored at 340 nm using a UV-VIS spectrophotometer ONDA V-10 PLUS (Giorgio Bormac S.r.l., Italy). The average change in absorbance per minute was calculated according to the following equation:

$$\Delta A/\text{min} = (A1 - A3) / 2$$

GPx activity was calculated using the following equation:

$$\text{GPx (U/L)} = 8412 \times (-\Delta A/\text{min})$$

The results were expressed as U/L of hemolysate.

Determination of Malondialdehyde Concentration (MDA)

Malondialdehyde concentration was determined using the thiobarbituric acid reactive substances method according to Ohkawa et al. (1979). Malondialdehyde reacts with thiobarbituric acid to form a colored MDA-TBA complex, which was measured spectrophotometrically at 532 nm using a UV-VIS spectrophotometer ONDA V-10 PLUS (Giorgio Bormac S.r.l., Italy), (Ohkawa et al., 1979).

MDA concentration was calculated according to the Beer-Lambert law using the following equation:

$$\text{MDA } (\mu\text{mol/L}) = A \times 6.41$$

where A represents the absorbance measured at 532 nm.

Statistical Analysis

The obtained data were statistically evaluated using basic descriptive statistics. Mean values, standard deviations, minimum values, and maximum values were calculated for all evaluated parameters.

RESULTS AND DISCUSSION

The total antioxidant status values obtained in the evaluated horses ranged from 1.81 to 3.85 mmol/L, with a mean value of 2.42 ± 0.53 mmol/L (Table 1). The highest TAS value was observed in horse No. 12, whereas the lowest value was recorded in horse No. 11. The relatively narrow range of TAS values suggests homogeneous antioxidant capacity within the studied group. Higher TAS values indicate

Tab. 1: Individual values of antioxidant parameters in American Quarter Horses

Horse	TAS (mmol/L)	GPX (U/L)	MDA ($\mu\text{mol/L}$)
1	2.31	770	0.115
2	2.12	1392	0.115
3	2.23	1178	0.122
4	2.77	967	0.115
5	2.04	1102	0.109
6	2.69	833	0.141
7	2.46	736	0.122
8	2.73	652	0.109
9	1.92	538	0.135
10	2.23	648	0.128
11	1.81	551	0.160
12	3.85	1106	0.135
13	2.27	736	0.192

Source: Own results

Tab. 2: Summary statistics of selected antioxidant biomarkers

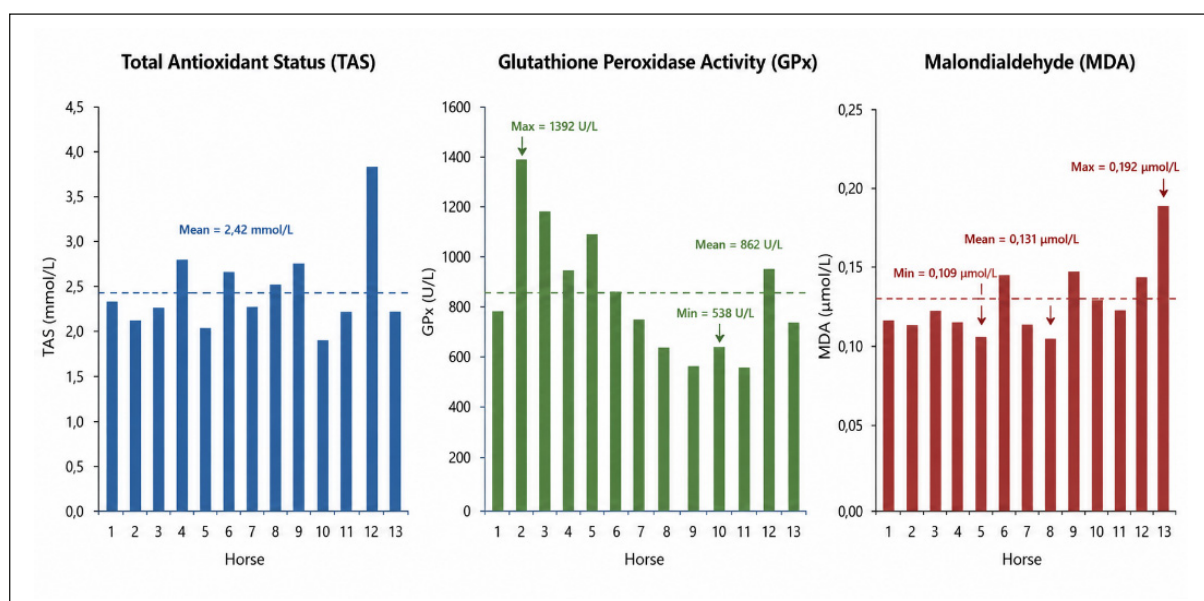
Parameter	Mean $\pm$ SD	Minimum	Maximum
TAS (mmol/L)	2.42 $\pm$ 0.53	1.81	3.85
GPx (U/L)	862 $\pm$ 265	538	1392
MDA (μ mol/L)	0.131 $\pm$ 0.022	0.109	0.192

Source: Own results

greater total antioxidant capacity and a more effective defense against oxidative stress. The obtained results correspond with findings reported by Brkljača Bottegare et al. (2018), who demonstrated that antioxidant status in horses is influenced by metabolic activity and exercise intensity.

Glutathione peroxidase activity ranged from 538 to 1392 U/L, with a mean value of 862 ± 265 U/L. The lowest activity was observed in horse No. 9, while the highest activity was detected in horse No. 2. Higher GPx activity may indicate more efficient enzymatic antioxidant defense mechanisms. The observed variability among individual animals indicates differences in enzymatic antioxidant defense. GPx plays an important role in the detoxification of hydrogen peroxide and lipid hydroperoxides, thereby protecting cellular structures from oxidative damage. Latham et al. (2021) demonstrated that antioxidant enzyme activities in Quarter Horses may be affected by nutritional status and environmental conditions. Similar observations were reported by Niedźwiedź and Jaworski (2014), who identified changes in antioxidant enzymes in horses experiencing oxidative stress.

The mean MDA concentration reached 0.131 ± 0.022 μ mol/L, with values ranging from 0.109 to 0.192 μ mol/L. The highest concentration was observed in horse No. 13, whereas the lowest values were detected in horses No. 5 and No. 8.

**Fig. 2:** Individual values of selected antioxidant biomarkers in American Quarter Horses

Abbreviations: TAS – total antioxidant status; GPx – glutathione peroxidase; MDA – malondialdehyde.

MDA is considered an important marker of lipid peroxidation and oxidative damage. The relatively low concentrations observed in the present study may indicate a low degree of oxidative stress in the examined horses. Similar findings were reported by Yildirim and Apaydin Yildirim (2023), who observed lower MDA concentrations in horses exhibiting a favorable antioxidant status. Increased MDA concentrations have been associated with various pathological conditions and elevated oxidative stress in horses.

Overall, the obtained results suggest a favorable antioxidant profile characterized by relatively high TAS values, adequate GPx activity, and low MDA concentrations, indicating effective antioxidant defense mechanisms in the evaluated horses. The observed values may serve as preliminary reference data for clinically healthy American Quarter Horses maintained under comparable breeding conditions. Low malondialdehyde concentrations suggested a low degree of lipid peroxidation, whereas adequate glutathione peroxidase activity and relatively high total antioxidant status values indicated a well-functioning antioxidant defense system.

CONCLUSION

In clinically healthy American Quarter Horses, the mean TAS value was 2.42 ± 0.53 mmol/L, GPx activity reached 862 ± 265 U/L, and the MDA concentration was 0.131 ± 0.022 μ mol/L. Low MDA concentrations together with adequate GPx activity and higher TAS values indicate preserved antioxidant protection and a low level of oxidative stress. The obtained results may serve as preliminary reference values for the antioxidant status of American Quarter Horses kept under conditions of the Slovak Republic. Considering the increasing popularity of western riding and the growing interest in breeding American Quarter Horses in Slovakia, the present findings contribute to expanding the knowledge of the antioxidant status of this breed, for which data under local conditions remain limited.

ACKNOWLEDGEMENT

The project was supported by the VEGA Grant Agency under projects No. 1/0698/24 and No. 1/0427/26.

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