THE INFLUENCE OF FOLIAR APPLICATION OF SELENIUM ON CONTENT OF GLUTATHIONE IN THE FORAGE OF PERENNIAL RYEGRASS (*LOLIUM PERENNE* L.)

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Abstract: Selenium (Se) as part of the enzyme and non-enzyme antioxidants (e.g. glutathione) has many antioxidant and detoxification functions in the cells. Its content in plants depends on its content in the soil. Its adequate intake may decide about the health, production and reproduction of the livestock. One of the possible ways to enrich feed ration of this element may be the foliar application. The aim of this study was to determine the effect of foliar application of selenium in different forms and doses on the antioxidant glutathione content in the forage of perennial ryegrass. In the experiment, perennial ryegrass (Ahoj variety) was included. The experiment took place in climabox. For foliar application, the solutions of selenium at the doses of 2; 4 and 20 mg · m^{-2} of Se were used. As a source of selenium, selenite sodium or selenate was applied. After the application, the samples of green mass of each group were sampled at a regular 14 day intervals (14^{th} day, 28^{th} day and 42^{nd} day after the application). The determinations of GSH and GSSG were performed by HPLC-ED. The foliar application of selenate and selenite increased the content of glutathione (GSH and GSSG) in aboveground mass of perennial ryegrass. The increase ($P<0.05$) of GSH content after foliar application of selenate was observed after all doses of selenium throughout the experiment. Between the doses there were no differences ($P<0.05$). The application of selenate caused the increase of GSSG ($P<0.05$), but it was evident especially in the first 28 days after application. After application of selenite the content of GSH increased ($P<0.05$). It was observed after application of the doses 4 and 20 mg · m^{-2} in every term of sampling. The application of selenite increased ($P<0.05$) the content of GSSG. It increased after each used dose and term of sampling except 42^{nd} day, when it decreased on the level of the control group. Due to the increase of both forms of glutathione can be assumed that the application of selenium on plants acts as a stress factor.

Key Words: pot experiment, forage, GSH, GSSG, oxidative stress

INTRODUCTION

The micronutrients have a significant effect on the health status of animals and humans. Although their requirement is a very small (of the order of micrograms) may be a key factor that can decide on the health, production and reproduction of livestock. One of the possible ways to enrich the feed ration of these elements can be foliar application (Gupta, Gupta 2002). The built micronutrient in chelate form in plant tissues is more effectively usable for animals (Meyer et al. 2014) and may even bring benefits also grown plant (Tang et al. 2015, Diao et al. 2014). Enrichment of various crops of micronutrients after foliar application has been performed by many authors (Nawaz et al. 2015, Fofana et al. 2014, Smoleń et al. 2014), but the efficiency for forage crops has not been given sufficient attention.

Selenium (Se) is an essential element significantly influencing health status of animals and humans. The insufficient supply of organism with this element leads to many disorders. Conversely,

higher intake can be toxic (Kaur et al. 2014, Wu et al. 2015). As a part of selenoproteins (e.g. glutathione), it regulates the antioxidant system and thus prevents the oxidative destruction of biological membranes and prevents the damage of the body by heavy metals. Consequently, its deficiency disrupts the overall health of animals and humans because of involvement of selenium compounds in many biological functions. The deficiency causes the reproductive and immune system disorders, muscular dystrophy and heart disease (Surai, Fisinin 2015, El-Ramady et al. 2015, Steinbrenner et al. 2015). Selenium concentration of plant biomass is derived from its content in the soil and may considerably vary depending on the region (Guerrero et al. 2014, Zhu et al. 2009).

The tripeptide glutathione (GSH, γ-Glu-Cys-Gly) is synthesized by the specific enzymes. It is in the animal and plant cells present in relatively high concentration. The reduced form of glutathione (GSH) participates in cell on the rows protective and detoxification processes. Glutathione as the main intracellular antioxidant contributes to the elimination of free radicals and other reactive oxygen species (Wünschiers 2012, Fajt et al. 2009). In these reactions, the oxidized form (GSSG) creates, which is again reduced by the enzyme glutathione reductase (Bender 2012). Glutathione is also involved in redox state stabilization of peptides and proteins, cell transport of amino acids into the γ-glutamyl cycle, neutralization of xenobiotics and phytochelatins synthesis in plants (Wünschiers 2012, Hopkins 1999). This prevents damage to DNA, RNA and cellular proteins (Murray 2012).

The perennial ryegrass belongs to the family *Poaceae*, it is one of the most frequently used forage crops. This is a typical grazing species, but some varieties are well also applied to meadows, temporary grasslands and lawns. It provides high-quality forage (Skládanka et al. 2014).

The aim of study was determine the effect of foliar application of selenium in different forms and doses of the antioxidant glutathione content in the forage of perennial ryegrass.

MATERIAL AND METHODS

In the experiment, perennial ryegrass (Ahoj variety) was included. Into each prepared pot with soil was seeds planted. Subsequently, the pots were stored in climabox. The pots with plants were located there throughout the experiment. In climabox, daily temperature was set at 24°C and 20°C overnight, 65% of humidity and the length of day light lasted for 12 hours (light intensity of 380 μmol · $m^{-1} \cdot s^{-1}$). Within the first 20 days after sowing, the plants were periodically watered. For the rest of the experiment, they were automatically watered. For foliar application, the solutions of selenium at doses of 2; 4 and 20 mg · m^{-2} of Se were used. As a source of selenium, selenite sodium or selenate were applied. Two experimental groups (selenium as selenite or selenate) and one control group (without treatment) were created. Selenium doses in the above mentioned forms were sprayed on 25th day on the leaf after sowing. The control samples were not affected by selenium during the whole experiment. After the application, the samples of green mass of each group were taken at a regular 14 day intervals (14th day, 28th day and 42nd day after the application).

The sampled leaves were immediately weighed and frozen to -20°C prior to detection of total content of GSH and GSSH. The chromatographic analysis was performed using high performance liquid chromatography with electrochemical detection (HPLC-ED).

The results were processed in STATISTICA CZ program version 10 (Czech Republic) using a multifactor ANOVA. The differences were considered as significant with $P<0.05$.

RESULTS AND DISCUSSION

After application of selenate was observed a significant ($P<0.05$) increase in GSH content in comparison to the control group (without treatment). But in our experiment no difference was found ($P<0.05$) between individual variants (doses) of selenate (Figure 1). Doses selenite 4 and 20 mg · m^{-2} are led to an increase ($P<0.05$) of the content of GSH in comparison with the control group. After application selenite at a dose of 4 mg · m^{-2} Se the content of GSH increased ($P<0.05$) in compared to the dose of 20 mg · m^{-2} Se. The increase ($P<0.05$) of content of GSH compared with control group after application dose 2 mg · m^{-2} Se was found in only 42nd day after application (Figure 2). In experiments by other authors (Hermosillo-Cereceres et al. 2014), high doses of selenium increased the activity of antioxidant enzymes. The high doses therefore had toxic effect and led to creation

of reactive oxygen species (ROS). This reaction was more pronounced after application selenite. Excessive use of selenium leads to an increase in the content of inorganic selenium in plants and deterioration of antioxidant capacity (Han et al. 2013).

Figure 1 The content of GSH in forage after foliar application of selenate

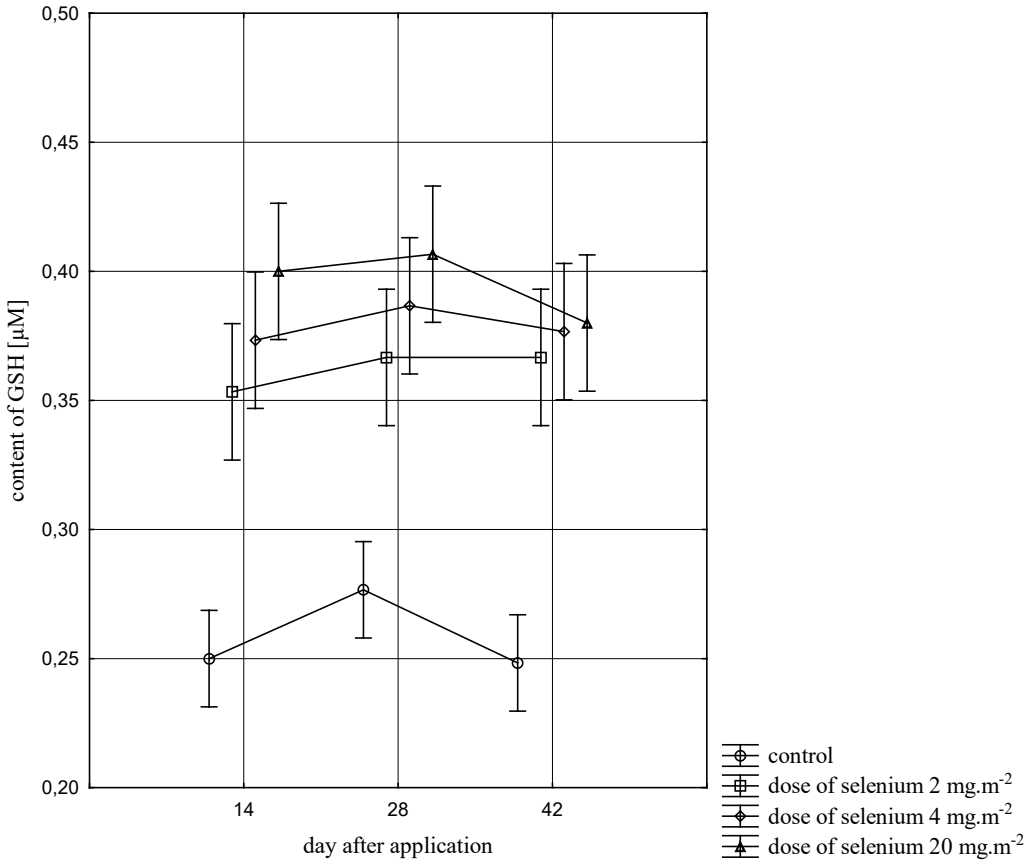
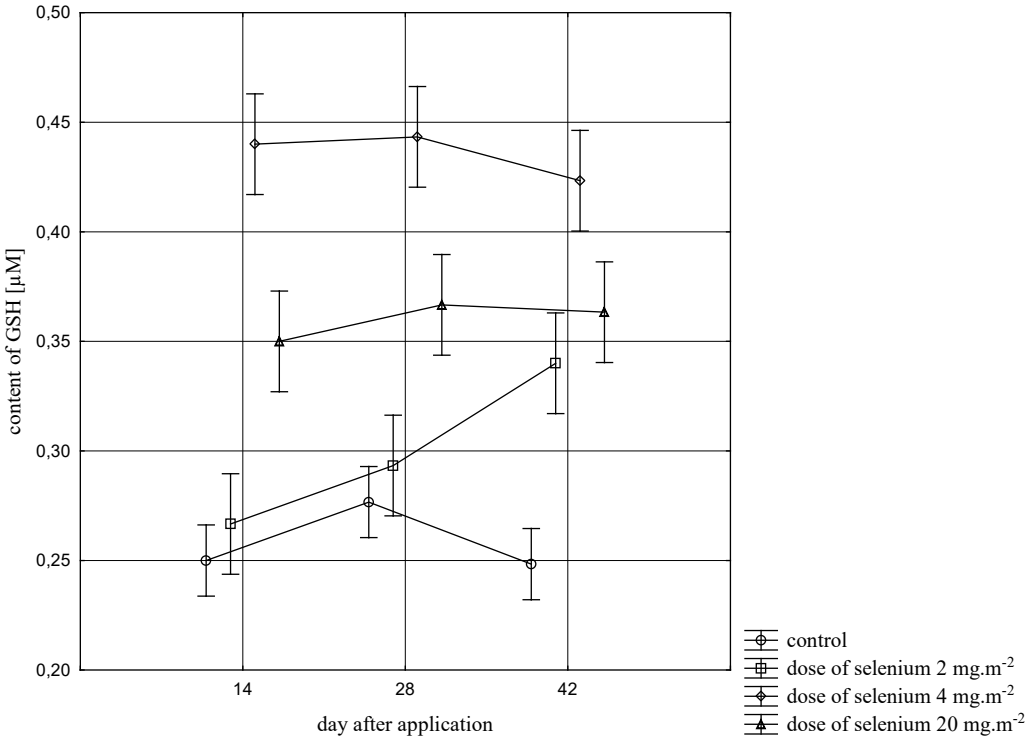


Figure 2 The content of GSH in forage after foliar application selenite



We note the difference between used doses, but the amount of GSH did not increase proportionately with increasing dose of selenium. Madhava Rao (2006) considers the content of glutathione in the cells of a marker of oxidative stress. This view but cannot be interpreted unambiguously. According to other authors (Fitter, Hay 2002) it is just necessary to take into account the plant species. Some species had to respond on exposure to stress factors by reducing and others by increasing the amount of antioxidants. By contrast, according to Hasanuzzaman et al. (2012) is to increase the content of GSH after adequate supplementation of selenium result of increased antioxidant capacity and detoxifying abilities of plants. A similar view has also Diao et al. (2014), which argues that selenium regulating antioxidant defense systems in the cells and increases the resistance of plants. This is especially apparent for plants which are exposed to stress conditions.

Antioxidants are produced in plant tissues throughout the life of the individual. Among the plant species, however, there are huge differences at current levels of these substances (Fitter, Hay 2002). Selenium helps plants to cope with a number of stresses: cold, drought, high light, water, salinity and heavy metals (metalloids). However, the mechanisms associated with this are very complicated and still not completely understood (Feng et al. 2013).

Wang et al. (2011) followed plant white clover (*Trifolium repens* L.) under drought stress increase the content of GSH and reduction GSSG after application of selenium. In our case, however, it increased content of GSSG. At all sampling terms (14th, 28th, and 42nd day) showed an increase ($P<0.05$) of the contents GSSG after application dose selenate 2 mg · m^{-2} Se. The increase ($P<0.05$) of content of GSSG was recorded on the 14th and 28th day after application the dose 4 mg · m^{-2} Se and on the 28th day after application dose 20 mg · m^{-2} Se (Figure 3). Application selenite led to an increase ($P<0.05$) the content of GSSG 14th and 28th day in all experimental variants. However, on 42nd day after application was not observed difference ($P<0.05$) between the experimental group and the control group (Figure 4).

Plant response to selenium supplementation is not fully understood. On the one hand it is an element that is part of a series of enzymatic and non-enzymatic antioxidants that help protect and detoxification plant tissues. On the other hand, when exceeding a relatively thin boundary it has toxic effect on plants. Due to differences in the data reported by other authors cannot evaluate the results obtained clearly. Increase the content of GSH could be seen as a response to stress or favorable increase in antioxidant capacity. However, the increase in the content GSSG almost all treated variants can be considered as a result of exposure to oxidative stress of plants.

Figure 3 The content of GSSG in forage after foliar application of selenite

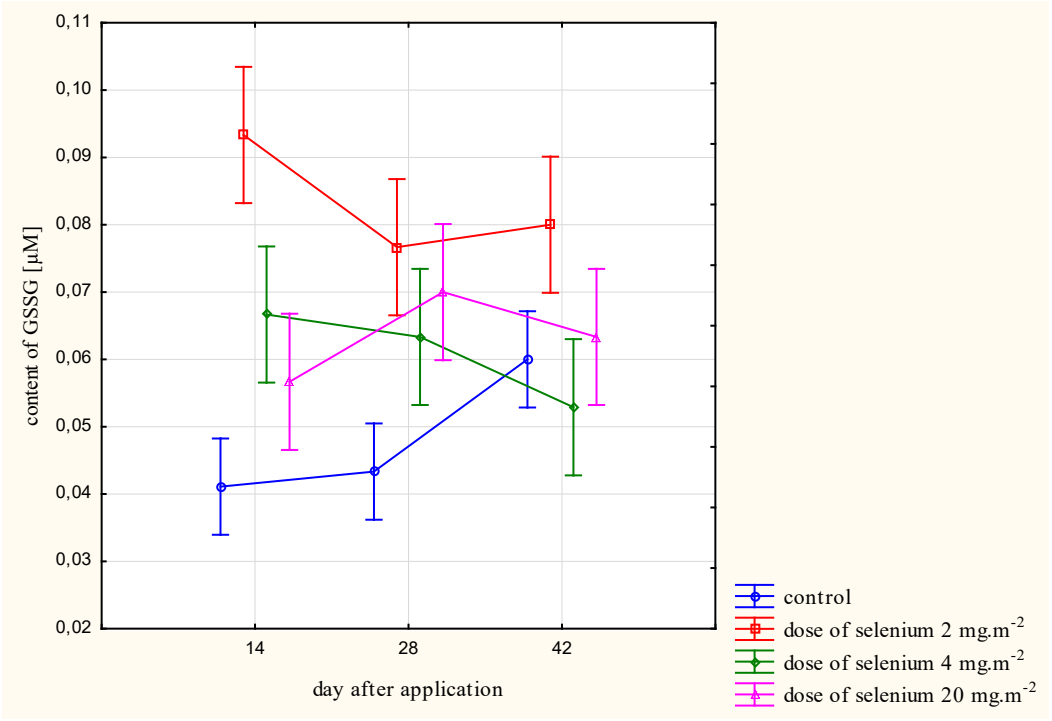
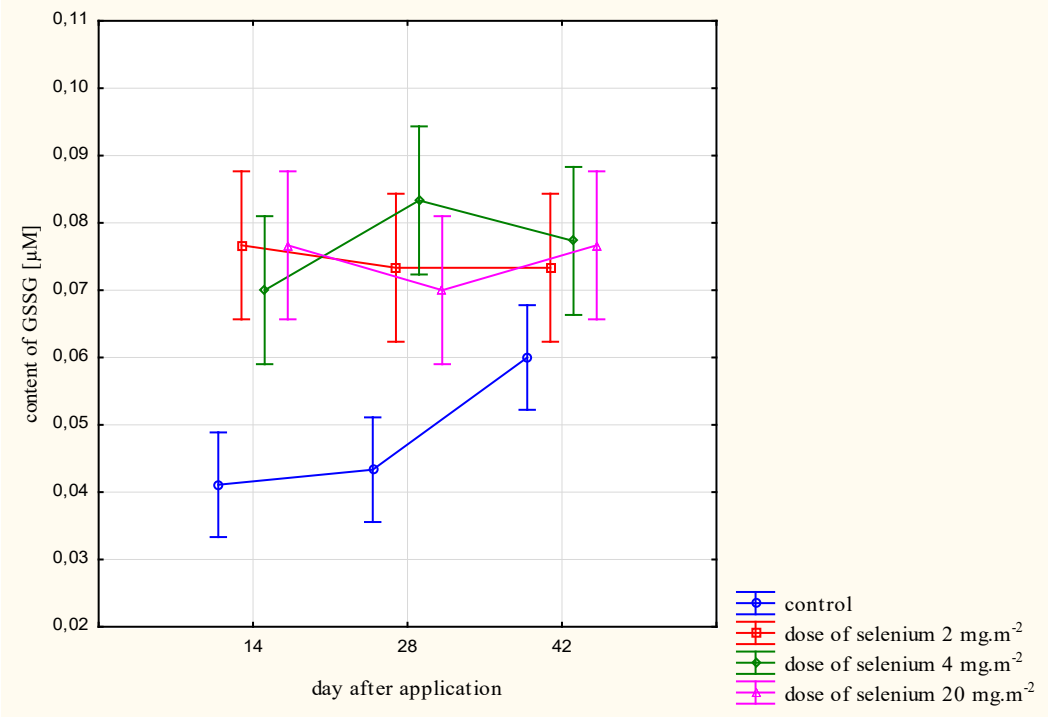


Figure 4 The content of GSSG in forage after foliar application selenite



CONCLUSION

Foliar application of selenate and selenite increased content of glutathione (GSH and GSSG) in the above-ground mass of perennial ryegrass. Increase GSH content after application of selenate was observed for all the doses of selenium to the entire length of the experiment. Between doses showed no differences. Application selenate caused increase GSSG, but it was evident especially in the first 28 days after application. After application selenite increased GSH content of selenium for doses of 4 and 20 mg · m^{-2} Se in all the terms of sampling. Application selenite increased GSSG content for all doses used, and the date of sampling except for 42nd day, when it dropped to the level of the control group. Due to the increase of both forms of glutathione can be assumed that application of selenium to plants acts as a stress factor.

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