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Electrochemical determination of the effect of deoxynivalenol on rats

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Abstract

This study is focused on examining the impact mouldy wheat contaminated with deoxynivalenol (DON) on the health status of rats and their ability to resist oxidative stress. Among the factors determining the defences include the activities of liver detoxification enzymes and content of blood peptides and proteins (glutathione and metallothionein).

Keywords: Deoxynivalenol, rats, metallothionein, *Fusarium*

Introduction

Mycotoxins are secondary metabolites produced by micro-fungi that are capable of causing disease and death in humans and other animals. While aflatoxins are undoubtedly the most important mycotoxins, *Fusarium* toxins have recently been given more attention (Pestka et al. 1990). The biggest risk is associated with the occurrence of a deoxynivalenol (DON). After ingestion of mycotoxin there are activated numerous mechanisms in a body to reduce their toxicity and to induce their subsequent excretion (Jajic et al. 2008). Very important substances providing their detoxifications are thiol compounds contained in metallothionein and glutathione.

Electrochemical methods for metallothionein determination are very suitable methods for protein determination due to the very low limit of detection.

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Materials and methods

This work was aimed at investigation of influence of DON on rats treated with deoxynivalenol for 28 days. The effect of DON was revealed by voltammetric and spectrometric serving for determination of reduced and oxidized glutathione, metallothionein and activities of certain enzymes connected with detoxification of xenobiotics in serum and tissues of rats.

Selected male Wistar albino laboratory rats of 28 days of age were used in our experiments (Fig 2). Experimental animals were kept in a vivarium with controlled air temperature (23 ± 1°C) and photo-period (12 hours day:12 hours night with maximal intensity 10800 LUX). Tempered feed mixtures and drinking water were accessible ad libitum. Animals were weighed once a week and at the same time weight gain, feed intake, conversion and health state were monitored. Feed conversion was calculated according to the following equations: (feed intake)/(weight gain). The experimental animals were divided into eight groups (eight male rats per group). We used feed mixtures of natural barley and barley contaminated by mycotoxins both with different content of vitamins, organic and inorganic zinc (II) form (Table 1). Natural barley contained 40±5μg/kg of DON and naturally contaminated barley 3500±5μg/kg of DON. The rats were administrating these mixtures for 28 days. In the end of the experiment, the animals were put to death and tissues and blood were sampled.

Preparation of Biological Samples

The rats' tissues (liver, kidney, spleen, heart, brain, eye, gonads and femoral muscle) and blood were used for the analysis. The animal tissues were mixed with extraction buffer (100mM potassium

phosphate, pH 6.8) and subsequently homogenized using polo automatic homogenisator (Shutt homogen plus, Germany).

Results and Discussion

We found that administration of deoxynivalenol caused a response in the form of increased synthesis of metallothionein. Another interesting area was the effect of increasing doses of zinc, which was added both to the mouldy and to "control" diet. Synergy effect of mycotoxins is very interesting and very little explored area. The possible link between zinc intake and its influence on the detoxification of mycotoxins via enhanced synthesis of metallothionein, whose transcription is activated by zinc ions presence, seems to be very interesting. Levels MT in different tissues varied. The highest concentration of MT was found in tissues closely connected with the detoxification of xenobiotics, such as kidney (6.69 ± 0.05 µg/ml) and liver (6.06 ± 0.05 µg/ml). Half concentration was detected in heart, brain, testes and muscle (approximately 3.0 µg/ml).

Furthermore, the MT levels differed in individual organs and the difference between groups feeding by natural and mouldy barley was observed ($p<0.05$) except spleen and brain where the difference was not significant. It was observed the similar trend of MT level in single groups. In both groups the highest level had specimens, which feedstuff was enriched with organic chelate zinc (II) form. Significant ($p<0.05$) higher was MT level at feedstuff with organic chelate in liver, kidney and muscle. DON-containing group was slightly increased against the non-DON group. Evident was interaction ($p<0.01$) at gonads. DON-containing group showed after vitamins, organic and inorganic zinc supplementation lower MT level with comparison non-DON group. Inorganic form also increased the MT expression but the results did not reach the values of organic zinc. Feed supplemented by vitamins did not indicate the effect after consumption. Moreover, organic and inorganic zinc (II) and vitamins supplementation have no significant effect on MTs level in brain tissues of rats. To verify the electrochemical detection the Sodium dodecyl sulphate polyacrylamide gel electrophoresis was used. The results obtained from electrochemical detection were similar as the results from gel electrophoresis.

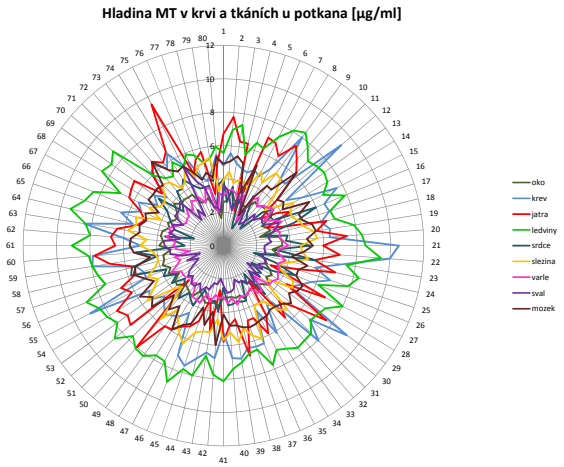

Figure 1: MT levels in single tissues.

Conclusion

Studying the impact of toxic substances in organisms is still very current and provides very interesting results. Toxic substances that are part of our diet, are the more objective of such studies because of the possible benefits. We are able not only to define the level of toxicity, but also gain an idea of the biochemical effect of the studied substance, which can bring new opportunities in its detoxification.

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