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EFFECT OF ZEARALENONE ON HAEMATOLOGICAL PARAMETERS OF PORCINE BLOOD IN VITRO

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ABSTRACT

The aim of this study was to determine the haematological parameters of porcine blood after mycotoxin zearalenone (ZEN) exposure *in vitro*. Samples of porcine blood were incubated with ZEN: 10 $\mu.ml^{-1}$ in E1 group, 100 $\mu g.ml^{-1}$ in E2 group, and 1000 $\mu g.ml^{-1}$ in E3 group for 4 hours at 37°C. The group without any addition served as the control. ZEN caused significant decrease of white blood cells count and lymphocyte count in all experimental groups when compared to the control. Regarding red blood cells (RBC) ZEN significantly decreased the number of RBC in porcine blood followed by increase of haemolysis and decrease of haemoglobin and haematocrit. The count of platelets was significantly decreased in all experimental groups against the control. The results of this study confirm toxicological effect of ZEN on blood cells.

Keywords: zearalenone, porcine blood, haematology, haemolysis

INTRODUCTION

Contamination of food by mycotoxins is serious problem investigated by many researchers **(Biro *et al.*, 2009; Kačániová *et al.*, 2012; Maruniakova *et al.*, 2014**). Mycotoxins are fungal secondary metabolites, commonly found in food crops and considered to be unavoidable contaminants worldwide due to the widespread nature of fungi in the environment **(Kumar *et al.*, 2012)**. They are a permanent challenge in animal nutrition as health and performance of the animals may be compromised as well as the quality of animal derived food **(Döll and Dänicke, 2011).** Toxins produced by *Fusarium* species (e.g. trichothecenes) commonly contaminate cereals **(Larsen *et al.*, 2004)**. Trichothecene toxins such as deoxynivalenol, fumonisins, zearalenone, patulin, and T-2 toxin are the common ones of the most important mycotoxins, which are characteristically stable under changing environmental conditions and have been exhibited a wide array of biological effects **(Smith *et al.*, 1995).** Once trichotecenes cross the plasma membrane barrier, they enter the cell, where they can interact with a number of targets, including ribosomes and mitochondria **(Pace *et* al., 1988).** Zearalenone **(**ZEN) is commonly found in widely-used ingredients for many human and animal foods, such as maize, barley, wheat, oats, sorghum and sesame seeds **(Zinedine *et al.*, 2007)**. It is a resorcyclic acid lactone and is chemically described as 6-(10-hydroxy-6-oxo-trans-1-undecenyl)-β-resorcyclic acid lactone (C18H22O5, MW: 318.36, CAS 17924-92-4) **(EFSA, 2004).** ZEN and its major *in vivo* metabolite α-zearalenol exert estrogenic like action has influence on the reproductive system in animals **(Alm *et al.*, 2006)** and is assigned to the group of endocrine disruptors **(EFSA, 2004)**. In this context, the reproductive system has been regarded as a major target of ZEN toxicity **(Tiemann and Danicke, 2007)** and oxidative stress plays an etiological role in its toxic effects **(Boeira *et al.*, 2014)**. Acute ZEN administration caused deleterious haematological effects in mice, thus blood may also be target for ZEN **(Marin *et al.*, 2013; Boeira *et al.*, 2014)**. In human, ZEA is rapidly absorbed after oral administration. Its derivates are detected in blood about 30 min after oral administration bound to human globulins, as reproductive hormones **(Gajecki, 2002; Minevini and Dell Aquila, 2008).** Among the farm animals, especially pigs react quite sensitively to ZEN contamination **(Döll and Dänicke, 2011).** Our previous study revealed that exposure of porcine blood cells to silver *in vitro* caused changes and imbalance in blood elements. Significant decrease in erythrocytes, haemoglobin content and haematocrit was observed **(Capcarová *et al.*, 2011)**. **Boreira *et al.* (2014)** found changed in blood cells of mice induced by zearalenone**.** In blood ZEN increased the respiratory burst of monocytes and the inflammatory cytokine synthesis **(Marin *et al.*, 2013).** *In vitro* studies are interesting from the view of assessing response of cells in short time **(Sirotkin *et al.*, 2011; Sirotkin 2010a; Sirotkin 2010b; Capcarová *et al.*, 2009**).
The aim of this study was to analyse the effect of ZEN on haematological parameters of porcine blood *in vitro*.

MATERIAL AND METHODS

Animals

Slovakian White gilts (n=24) at the age of 100-120 days were kept under standard conditions at the Experimental Station of the Animal Production Research Centre Nitra. Conditions of their care, manipulations and use corresponded to the instruction of EC no. 178/2002 and related EC documents, and they were approved by local ethics commission. Animals were slaughtered and blood samples were obtained. Blood was collected into EDTA-treated tubes.

ZEN Treatment

Blood was treated in laboratory conditions *in vitro* with zearalenone (ZEN, Romerlabs, Austria). ZEN was dissolved in DMSO to prepare a stock solution of 5 $\mu g.ml^{-1}$ and then diluted in saline to final concentrations. Blood samples were divided to 4 groups (control group and 3 experimental groups). Group (n=5 tubes) without ZEN exposure served as the control. Experimental groups represented by 5 tubes in each group were exposed to ZEN in concentrations: 10, 100, and 1000 $\mu g.ml^{-1}$ (E1, E2, and E3 group).

Haematological analysis

The whole blood was exposed to the ZEN for 4 hours at 37°C. The blood samples were analysed (WBC – total white blood cell count, LYM – lymphocyte count, RBC – red blood cell count, HGB – haemoglobin, HCT – haematocrit, PLT – platelet count) using haematology analyser Abacus junior VET (Diatron MI LtD., Budapest, Hungary). The impedance method counts and sizes cells by detecting and measuring changes in electrical impedance when a particle in a conductive liquid passes through a small aperture. Each cell passing through the aperture – where a constant DC current flows between the external and internal electrodes – causes some change in the impedance of the conductive blood cell suspension. These changes are recorded as increases in the voltage between the electrodes. The number of pulses is proportional to the number of particles. The intensity of each pulse is proportional to the volume of that particle. The volume distribution

diagrams of the particles are WBC, RBC and PLT histograms. Pulse are counted only in channels (in terms of femtoliter, fl) which are between the lower and upper discriminators.

Statistics

The data presented concerning the effects of ZEN are means of values obtained in three separate experiments performed on separate days. Differences between the control (without ZEN administration) and experimental groups (with ZEN administration – E1, E2, E3) were evaluated by one-way ANOVA test using statistical software Sigma Plot 9.0 (Jandel, Corte Madera, USA). Differences were judged for statistical significance at level $P < 0.05$.

RESULTS AND DISCUSSION

Current research was aimed at understanding the role of mycotoxin zearalenone (ZEN) on haematological parameters of porcine *blood in vitro*. Under normal physiological conditions, cells interact with each other to synchronize their metabolic activity, gene expression, and other basic cellular processes **(Capcarova *et al*., 2013).** In this study selected haematological parameters were measured in blood samples after exposure to ZEN *in vitro* for 4 hours at 37°C. The results are shown in Figures 1-7. Our previous studies revealed some changes in haematological and antioxidant parameters in animal cells after an exposure by various environmental contaminants **(Capcarova *et al*., 2009; Petruška *et al*., 2012; Capcarova *et al*., 2013; Zbyňovská *et al*., 2013).** Molybdenum treatments significantly decrease the activity of SOD in hens' granulosa cells **(Capcarova *et al*., 2012)** and caused changes and imbalance in immune cells (**Capcarova *et al*., 2013b)**. In our study ZEN caused significant decrease ($P<0.05$) in WBC in all experimental groups when compared to the control (Fig. 1). Among the experimental groups any significant differences ($P>0.05$) were found. The lowest count of leukocytes was found in E1 group (the lowest amount of ZEN).

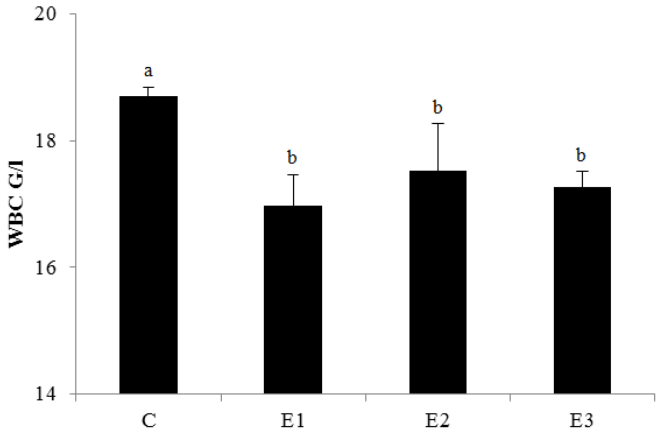

Figure 1 The effect of ZEN on WBC in porcine blood *in vitro*
C- control group, E1-E3 – experimental groups with various doses of ZEN, WBC – white blood cells count, G – Giga ($10^9.l^{-1}$), a-b- means significant differences ($P<0.05$), one-way ANOVA

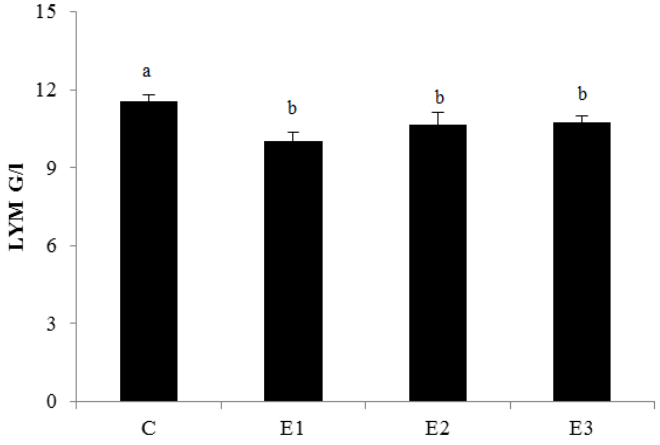

Figure 2 The effect of ZEN on LYM in porcine blood *in vitro*
C- control group, E1-E3 – experimental groups with various doses of ZEN, LYM – lymphocyte count, G – Giga ($10^9.l^{-1}$), a-b- means significant differences ($P<0.05$), one-way ANOVA

Lymphocyte count (Fig. 2) was significantly decreased ($P<0.05$) in all experimental groups against the control. We did not observe any significant differences ($P>0.05$) among the experimental groups. The lowest count of lymphocytes was found, similarly as in the case of leukocytes, in E1 group (the lowest amount of ZEN). It seems that blood white cells, mainly lymphocytes, are sensitive to ZEN exposure. A dose-dependent reduction of the lymphocyte proliferation induced by ZEN was observed in rats **(Atkinson and Miller, 1984)**, monkey **(Bouazis *et al*., 2012)** and humans **(Vlata *et al*., 2006).**
The toxic effect of ZEN may affect the survival of hematopoietic progenitors **(Ficheux *et al*., 2012).** In this study red blood cells count was significantly decreased ($P<0.05$) by ZEN exposure (Fig. 3), followed by significant decrease ($P<0.05$) in haemoglobin (Fig. 4) and haematocrit value (Fig. 5). After ZEN exposure a dose-dependent haemolysis occurred visibly (Fig. 6). The higher dose of ZEN caused more intensive haemolysis. Thus, the effect of ZEN on haemolysis was dose-dependent. In the study with human erythrocytes (**Jilani and Lang, 2013)**, the percentage of haemolysed erythrocytes increased slightly but significantly following exposure of erythrocytes for 48 h to zearalenone. **Lupescu *et al*. (2013)** observed similar effect adding mycotoxin patulin in human erythrocytes. Patulin stimulated Ca(2+) entry into erythrocytes, an effect triggering suicidal erythrocyte death or eryptosis. Suicidal erythrocyte death what can lead to haemolysis of blood was observed after ochratoxin A **(Jilani *et al*., 2012)** and zearalenone exposure **(Jilani and Lang, 2013)**, characterized by cell membrane scrambling and cell shrinkage **(Lang *et al*., 2008).** Circulating eryptotic erythrocytes are cleared from the blood, so after stimulation of eryptosis the percentage of eryptotic erythrocytes remains low *in vivo*. The accelerated loss of eryptotic erythrocytes following *in vivo* stimulation of erythrocytes may lead to anaemia **(Lang *et al*., 2008).**

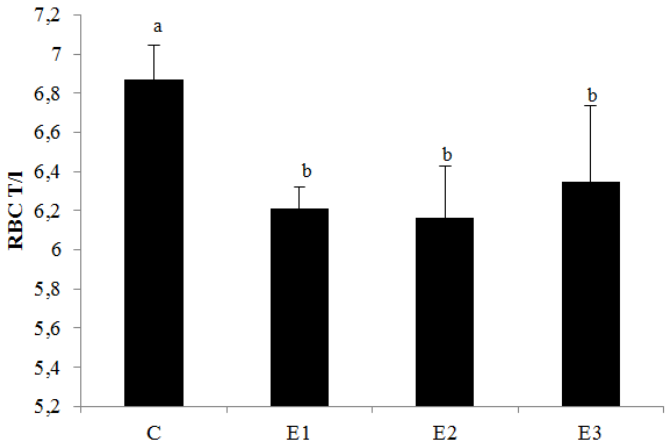

Figure 3 The effect of ZEN on RBC in porcine blood *in vitro*
C- control group, E1-E3 – experimental groups with various doses of ZEN, RBC – red blood cells count, T – Terra ($10^{12}.l^{-1}$), a-b- means significant differences ($P<0.05$), one-way ANOVA

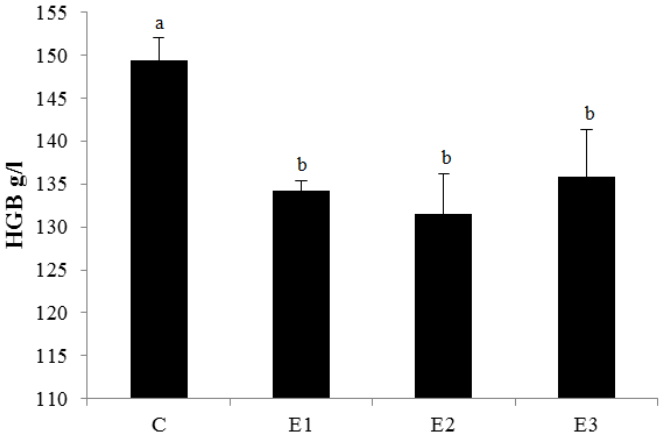

Figure 4 The effect of ZEN on haemoglobin in porcine blood *in vitro*
C- control group, E1-E3 – experimental groups with various doses of ZEN, HGB – haemoglobin, a-b- means significant differences ($P<0.05$), one-way ANOVA

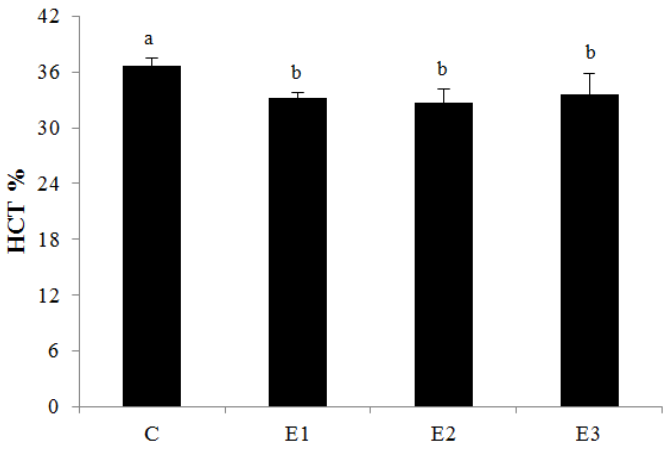

Figure 5 The effect of ZEN on haematocrit value in porcine blood *in vitro*
C- control group, E1-E3 – experimental groups with various doses of ZEN, HCT – haematocrit, a-b- means significant differences (P<0.05), one-way ANOVA

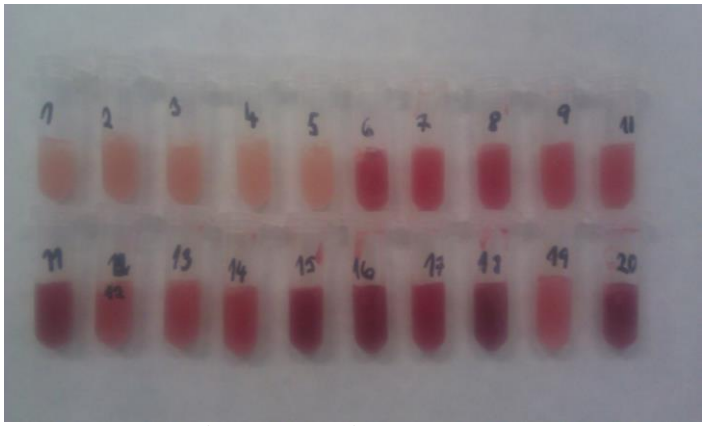

Figure 6 Haemolysis of porcine blood after ZEN exposure
Control group numbers 1-5 (ZEN 0 μg.ml^{-1}), E1 group numbers 6-10 (ZEN 10 μg.ml^{-1}), E2 group numbers 11-15 (ZEN 100 μg.ml^{-1}), E3 group numbers 16-20 (ZEN1000 μg.ml^{-1}) Dark red colour in the tubes represents haemolysis (picture origin: Lea Dufalova)

The mycotoxins may inhibit platelet functions **(Gentry *et al.*, 1988).** After addition of ZEN to the blood samples significant decrease (P<0.05) in platelets count (Fig. 7) was observed in this study. Among the experimental groups no significant differences (P>0.05) were found. According to study of Y**in *et al.* (2014)**, ZEN induced a significant decrease in platelets in blood of the pregnant rats.

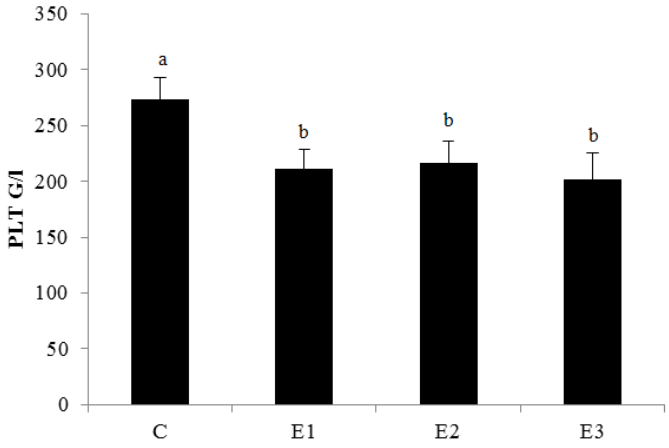

Figure 7 The effect of ZEN on platelets count in porcine blood *in vitro*
C- control group, E1-E3 – experimental groups with various doses of ZEN, PLT – platelets, a-b- means significant differences (P<0.05), one-way ANOVA

Regarding the literature it is obvious that the identification of ZEN effects is not only depending on the toxin doses and ratios, but also on the examined parameters **(Döll and Dänicke, 2011).** Similarly to our study, **Boeira *et al.* (2014)** also observed zearalenone-induced changes in haematological parameters in mice, mainly decrease in number of red blood cells, number of lymphocytes and platelets. The decrease in blood cells could be as a consequence that ZEN can decrease the cell proliferation in blood, what was confirmed in the study of **Marin *et al*. (2013)**. **Abid-Essefi *et al*. (2003)** stated that the decrease in cell proliferation caused by ZEN treatment is probably the result of the cell arrest in G2/M phase and the formation of DNA-adducts.

CONCLUSION

In the present study we showed that ZEN treatment markedly impaired haematological parameters. Results of this study provide a foundation for further analysis and researches on mycotoxins impact on living cells and the system of possible protection against its effects as well as evaluation of various dose dependencies on haematological parameters.

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