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DOSE-DEPENDENT EFFECT OF MOLYBDENUM ON PORCINE BLOOD CELLS: *IN VITRO* ASSESSMENT

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ABSTRACT

The aim of this study was to examine the effect of molybdenum (Mo) on selected haematological parameters of porcine blood *in vitro*. The samples of blood were treated with an ammonium molybdate (NH₄)₆Mo₇O₂₄·4H₂O for 4 hours at 37°C in the concentrations 10, 100, and 1000 µg.ml⁻¹ (E1, E2, and E3 group). Blood without Mo addition served as the control (C). Selected haematological parameters (WBC – total white blood cell count, LYM – lymphocyte count, GRA – granulocyte count, RBC – red blood cell count, HGB – haemoglobin, HCT – haematocrit and PLT – platelet count) were measured using haematological analyser Abacus junior VET. Significant decrease in WBC, LYM, and GRA in the group with the highest dose of Mo (E3) against other groups (C, E1, and E2) was observed. Other results were not influenced by Mo exposure. Our results suggest that Mo can cause the changes and imbalance in immune system.



Keywords: Molybdenum, blood cells, immunology, poultry

INTRODUCTION

Environment plays an important role in affecting the *internal milieu* of animals and humans. The amount of trace elements in the environment is generally low, but these chemicals can interfere with physiological systems (Capcarová and Kolesárová, 2012). The trace element molybdenum (Mo) is an essential component of key physiological systems in animals, plants and microorganisms (Michelis et al., 2011). In eukaryotes, the most prominent Mo enzymes are nitrate reductase, sulfite oxidase, xanthine dehydrogenase, aldehyde oxidase, and the mitochondrial amidoxime reductase (Mendel, 2013) and it is required by enzymes catalysing diverse key reactions in the global carbon, sulphur and nitrogen metabolism (Mendel and Bittner, 2006). Unavailability of Mo is lethal for the organism (Alhendawi et al., 2005). Molybdenum trioxide is used primarily as an additive to steel and corrosion-resistant alloys. It is also used as a chemical intermediate for molybdenum products; an industrial catalyst; a pigment; a crop nutrient; components of glass, ceramics, and enamels; a flame retardant for polyester and polyvinyl chloride resins; and a reagent in chemical analyses (NTP, 1997). Mo toxicity alters normal haematological profile (Kusum et al., 2010). Dietary Mo in high doses caused splenic lesions and lymphocyte apoptosis (Yang et al., 2011), reduced the percentages of peripheral blood T-cell subsets and serum IL-2 contents and caused thymic lesions and could impair immune functions in broilers (Xiao et al., 2011). As it was published previously, the exposure of animals to trace elements caused various alterations in zootechnical parameters (Arpasova et al., 2007; Kalafová et al., 2009) as well as imbalance in internal milieu (Capcarová et al., 2008; Kolesárová et al., 2010). *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). Our previous studies showed some changes in haematological and antioxidant parameters in animal cells after an exposure by various environmental contaminants (Capcarová et al., 2009; Petruška et al., 2012; Capcarova et al., 2013; Zbyňovská et al., 2013). The present study has been designed to investigate the changes in porcine haematological parameters after various concentration of Mo exposure *in vitro*.

MATERIAL AND METHODS

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.

Molybdenum Treatment

Blood was treated in laboratory conditions *in vitro* with an ammonium molybdate (NH₄)₆Mo₇O₂₄·4H₂O, Slavus Bratislava, Slovak Republic). Blood samples were divided to 4 groups (control group and 3 experimental groups). Group (n=6 tubes) without molybdenum exposure served as the control. Experimental groups represented by 6 tubes in each group were exposed to molybdenum in concentrations: 10, 100, and 1000 µg.ml⁻¹ (E1, E2, and E3). The blood was exposed to the molybdenum for 4 hours at 37°C. The blood samples were analysed (WBC – total white blood cell count, LYM – lymphocyte count, GRA – granulocyte count, RBC – red blood cell count, HGB – haemoglobin, HCT – haematocrit, PLT – platelet count) using haematology analyser Abacus junior VET (Diatron MI Ltd., Budapest, Hungary).

Statistics

The data presented concerning the effects of Mo are means of values obtained in three separate experiments performed on separate days. Differences between the control (without molybdenum administration) and experimental groups (with molybdenum 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

Under normal physiological conditions, cells interact with each other to synchronize their metabolic activity, gene expression, and other basic cellular processes (Capcarová et al., 2013). In this study selected haematological parameters were measured in blood samples after exposure to Mo *in vitro* for 4 hours at 37°C. The results are shown in Figures 1-7. The values of WBC count remained similar in the control and the first two experimental groups (E1, and E2). Low doses of Mo caused no significant differences (P>0.05) among the groups. The highest dose of Mo acted vice-versa. In E3 group the significant decrease (P<0.05) in WBC when compared to the control and other experimental groups (E1, and E2) was noted (Fig. 1). Similar situation was in the case of LYM and GRA. Significant decrease (P<0.05) of these parameters between E3 and other groups was found (Fig. 2, 3). Low doses of Mo caused no significant

changes ($P>0.05$) among the groups. To our knowledge there are just a few studies focused on exposure of trace elements or heavy metals on animal blood *in vitro*. Our previous results revealed no significant changes in WBC, LYM and GRA count after mercury exposure in rabbits (Capcarová et al., 2009) and silver treatment in porcine blood (Capcarová et al., 2011) *in vitro*. Yang et al. (2011) found that lymphocytes in chicken blood were histopathologically decreased in high dietary Mo groups. Authors explained this effect as apoptotic influence of Mo on lymphocyte cells as they found higher percentage of cellular apoptosis in high Mo group. It could be also explanation for our results. Mo caused the apoptosis of lymphocytes and other white blood cells and the number of these cells decreased. Also Xiao et al. (2011) found increased frequencies of positive cells containing Bax protein and decreased frequencies of positive cells containing Bcl-2 protein in high molybdenum groups of chickens. Authors concluded that dietary high molybdenum impaired the progression of renal cells from S phase to G(2)M phase obviously and induced renal cell apoptosis. Similar results were obtained by Caicedo et al. (2008). Yiran et al. (2013) found Cd-induced apoptosis and imbalance between pro- and antiapoptotic genes (Bax and Bcl-2) what caused Cd-induced apoptosis in rat hepatic cells.

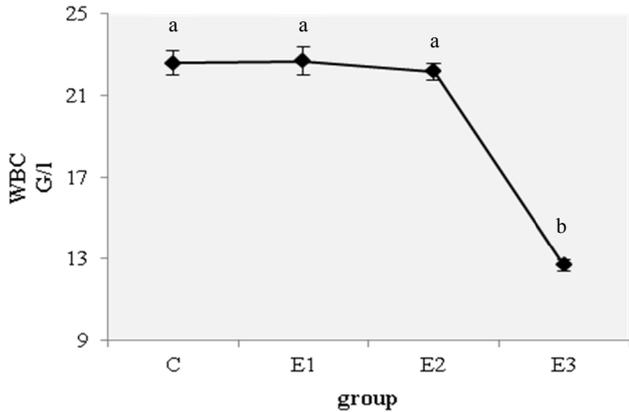


Figure 1 The effect of molybdenum on WBC in porcine blood *in vitro*
 C – control group, E1, E2, E3 – experimental groups with different concentration of Mo (10, 100 and 1000 $\mu\text{g}\cdot\text{ml}^{-1}$), WBC – white blood cell count ($\text{G}\cdot\text{l}^{-1}$), $\text{G}\cdot\text{l}^{-1} = 10^9\cdot\text{l}^{-1}$, data shown as mean \pm SD, a-b = different letters mean statistically significant changes ($P<0.05$), one-way ANOVA

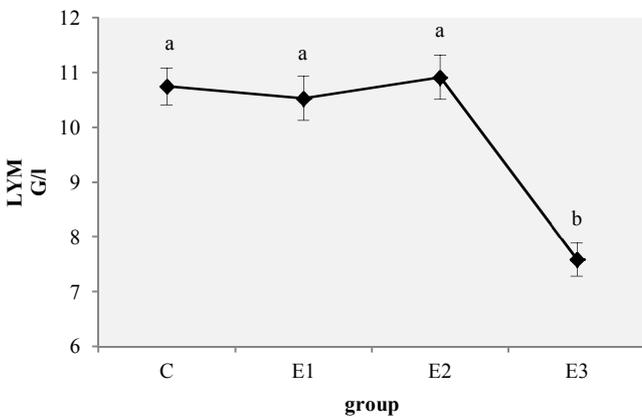


Figure 2 The effect of molybdenum on LYM in porcine blood *in vitro*
 C – control group, E1, E2, E3 – experimental groups with different concentration of Mo (10, 100 and 1000 $\mu\text{g}\cdot\text{ml}^{-1}$), LYM – lymphocyte count ($\text{G}\cdot\text{l}^{-1}$), $\text{G}\cdot\text{l}^{-1} = 10^9\cdot\text{l}^{-1}$, data shown as mean \pm SD, a-b – different letters mean statistically significant changes ($P<0.05$), one-way ANOVA

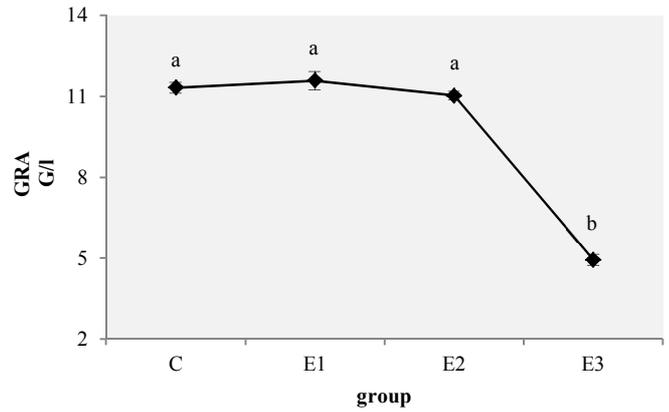


Figure 3 The effect of molybdenum on GRA in porcine blood *in vitro*
 C – control group, E1, E2, E3 – experimental groups with different concentration of Mo₃ (10, 100 and 1000 $\mu\text{g}\cdot\text{ml}^{-1}$), GRA – granulocytes count ($\text{G}\cdot\text{l}^{-1}$), $\text{G}\cdot\text{l}^{-1} = 10^9\cdot\text{l}^{-1}$, data shown as mean \pm SD, a-b – different letters mean statistically significant changes ($P<0.05$), one-way ANOVA

In present study the values of RBC, HGB, HCT and PLT (Fig. 4-7) were balanced during whole exposure time and differences among the groups remained insignificant ($P>0.05$). Different results were found in our previous *in vitro* studies with blood cells of rabbits/porcine after silver and mercury treatment (Capcarová et al., 2009, Capcarová et al., 2011). Tikare et al. (2012) found decrease in RBC, PLT, and HGB after nickel treatment. In our study the doses of Mo were probably too low to cause changes in these parameters and mainly RBC retained good resistance against this element. As other haematological parameters cohere with RBC, their values and differences among the groups analogously remained unchanged after Mo exposure ($P>0.05$).

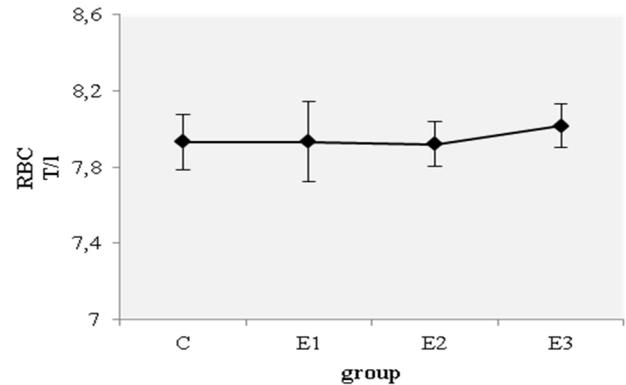


Figure 4 The effect of molybdenum on RBC in porcine blood *in vitro*
 C – control group, E1, E2, E3 – experimental groups with different concentration of Mo (10, 100 and 1000 $\mu\text{g}\cdot\text{ml}^{-1}$), RBC – red blood cell count ($\text{T}\cdot\text{l}^{-1}$), $\text{T}\cdot\text{l}^{-1} = 10^{12}\cdot\text{l}^{-1}$, data shown as mean \pm SD, differences were not significant ($P>0.05$), one-way ANOVA

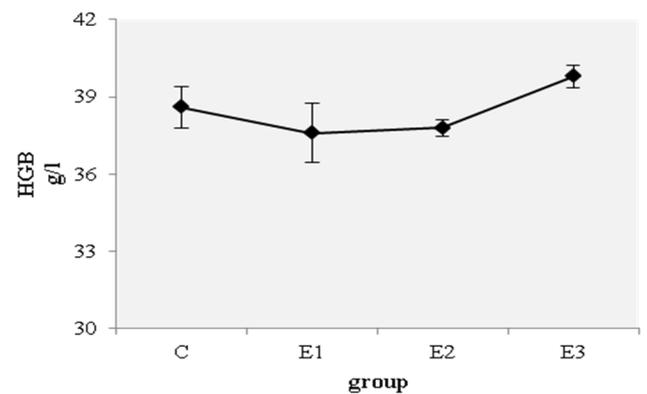


Figure 5 The effect of molybdenum on HGB in porcine blood *in vitro*
 C – control group, E1, E2, E3 – experimental groups with different concentration of Mo (10, 100 and 1000 $\mu\text{g}\cdot\text{ml}^{-1}$), HGB – haemoglobin content ($\text{g}\cdot\text{l}^{-1}$), data shown as mean \pm SD, differences were not significant ($P>0.05$), one-way ANOVA

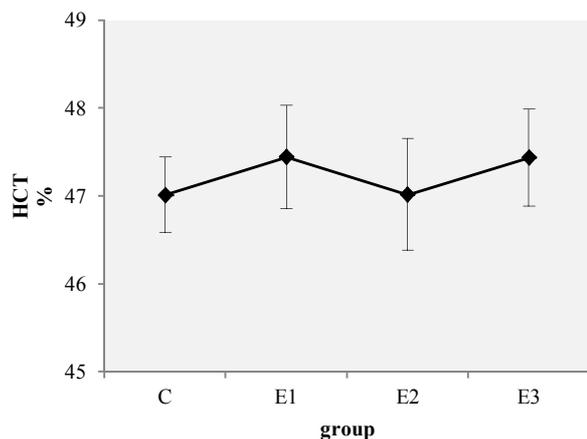


Figure 6 The effect of molybdenum on HCT in porcine blood *in vitro*. C – control group, E1, E2, E3 – experimental groups with different concentration of Mo (10, 100 and 1000 $\mu\text{g}\cdot\text{ml}^{-1}$), HCT – hematocrit (%), data shown as mean \pm SD, differences were not significant ($P>0.05$), one-way ANOVA

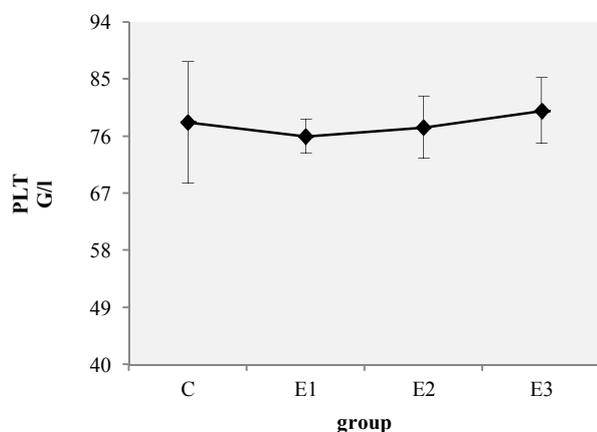


Figure 7 The effect of molybdenum on PLT in porcine blood *in vitro*. C – control group, E1, E2, E3 – experimental groups with different concentration of Mo (10, 100 and 1000 $\mu\text{g}\cdot\text{ml}^{-1}$), PLT - platelets count ($\text{G}\cdot\text{l}^{-1}$); $\text{G}\cdot\text{l}^{-1} = 10^9\cdot\text{l}^{-1}$, data shown as mean \pm SD, differences were not significant ($P>0.05$), one-way ANOVA

CONCLUSION

Results of our study suggest that exposure of blood to molybdenum can cause changes and imbalance in immune cells. Significant decrease in WBC, LYM and GRA in porcine blood was observed what could be interpreted as the result of apoptotic activity of molybdenum on leukocytes.

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