AKUMULÁCIA MINERÁLIÍ V ORGANIZME OVIEC V PRIEBEHU PRIEMYSELNEJ INTOXIKÁCIE MEĎOU PRI SUPLEMENTÁCII ANTIDOT

ACCUMULATION OF MINERALS IN AN ORGANISM OF ANTIDOTE-SUPPLEMENTED SHEEP DURING INDUSTRIAL COPPER INTOXICATION

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Abstract

Diagnostics of experimental copper intoxication by industrial emission from a copper factory on the basis of accumulation and distribution of Cu, Zn, Fe, Mo, Se, As, Cd and Pb in blood serum and organs was evaluated in relation to administration of ammonium molybdate and sodium sulphate. An increase in cupremia in comparison with the starting values was recorded in both groups of sheep on day 17 while maximum concentrations were determined at the end of the experiment. No significant differences in cupremia in relation to administration of ammonium molybdate and sodium sulphate were observed between the A and B groups. With risk elements, the highest concentration of Cu was found in the liver in both groups A and B. Significantly higher ($p<0.05$) Fe content was determined in bones and uterus in group B. The Mo content was increased in the kidneys ($p<0.05$) and bones ($p<0.01$) in group A. Additional differences in the dynamics of Fe, Se, As, Cd, and Pb, observed in the blood serum and organs of sheep from groups A and B during industrial intoxication with copper, were not unambiguously related to the application of ammonium molybdate and sodium sulphate and resulted probably from interactions which occured during the processes of resorption, intermediary metabolism and excretion between the minerals present in the emission.

sheep; copper intoxication; risk elements; distribution

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INTRODUCTION

Heavy metal contamination occurring in the vicinity of ore-processing plants is very dangerous for sheep. In view of other predisposition, sheep as a species seem to be most sensitive to an increased intake of copper, arsenic, cadmium and lead. The toxic effects of risk elements on sheep may become evident in health disturbances and decreased performance and reproductive indices (Riet-Correa et al., 1989; Smith et al., 1991). Many studies have been devoted to the biological effects of sulfur and molybdenum toxicity and their interactions with copper level and liver necrosis are associated with hemolytic crisis of chronic copper poisoning (Kumaratilake et al., 1981). Metals accumulating in organs with high metabolic activity are likely to be more toxic (Baranski, 1987). As most toxic elements accumulate in the liver, all vital functions are disturbed (Freundt and Ibrahim, 1991). Individual tissues differ greatly in their susceptibility to variations in dietary Cu intakes. The liver, kidneys, blood, spleen, lungs, brain, and bones are particularly responsive to such changes, while the endocrine glands, the muscles, and heart are much less so (Henninget et al.,1974).

The aim of our experiment was to determine the copper, zinc, iron, molybdenum, selenium, arsenic, cadmium and lead content in blood serum and organs and muscles of sheep after oral application of the industrial emission alone and the industrial emission plus ammonium molybdate and sodium sulfate

MATERIAL AND METHODS

The experiment was carried out on 10 improved Vallachian non pregnant sheep, age of 5 years, transported from a farm near the copper industry plant, copper intoxication in sheep caused by emissions of an ore – processing plant under both spontaneous and experimental conditions was first described by Vrzgula et al. (1986) and Bíreš et al. (1993). The animals were housed and each was daily fed on 1.5 kg of meadow hay, 0.30 kg of BAK concentrates and water were given *ad libitum*. The sheep were randomly allocated into two groups (n = 5). Before the experiment the animals in group A (GA) had 28.6 ± 5.639 kg average body weight and in group B (GB) 29.2 ± 2.588 kg. GA recieved 2.5 g industrial emission daily by stomach tube diluted by distillate water after the morning feeding. GB in addition to 2.5 g industrial emission, received 400 mg ammonium molybdate [$(NH_4)_6Mo_7O_{24}.4H_2O$ p.a. minimal content MoO_3 80 %] and 800 mg sodium sulphate (Na_2SO_4 p. a.) daily by stomach tube diluted by distillate water after the morning feeding and we stopped the treatment on 24th day. The industrial emission was obtained from the copper factory by dedusting of the electrostatic precipitators from the factory chimney. The industrial emission contains of Cu, Zn, Fe, Mo, Se, As, Cd, and Pb.

Analysis of organs, emission, food and water

Minerals and trace elements in the emission, food, water, blood serum and organs were analysed by atomic absorption photometric method (AAs 306 and Zeeman AAs 4100ZL, Perkin Elmer) – Cu, Zn, Fe, Mo, Se, As, Cd and Pb. Mineralization prepared in the mixture of HNO_3 and H_2O_2 in the micro wave system (Milestone mls 1200)

Blood was collected from the jugular vein at the first time before the starting of the experiment. Then we continued intaking the blood samples during the experiment on days 10, 17, 24, 31, 38 and 45 which was the end of experiment.

RESULTS AND DISCUSSION

An increase in the Cu concentration in blood serum compared with the starting values was obvious in both groups on day 17 and the maximum level was reached on day 45 in both groups A and B. No statistically significant dependence on the administration of ammonium molybdate and sodium sulphate was observed between both groups during the experiment.

The concentration of Zn in blood serum of sheep of group B was insignificantly higher in comparison with the group A during the entire experiment.

A marked increase in Fe level in comparison with the starting values was observed in groups A and B on day 38 of the experiment.

The concentration of Mo in blood serum of sheep after application of antidote in group B hypermolybdenemia was shown from days 10-24. From day 24 administration of ammonium molybdate and sodium sulphate was stopped and we observed decreased level of Mo in group B from day 31 - 45. Significant relationship between the concentration of serum Mo and the administration of ammonium molybdate and sodium sulphate was observed on the days 10, 17 and 24 in sheep of group A $P<0.01$ during the experiment.

The concentration of Se in blood serum of sheep of both groups, determined in our experiment was below the lower physiological limit (dispersion mean values in groups A and B were in the range 0.24 – 0.57 $\mu mol.l^{-1}$ and 0.34 – 0.56 $\mu mol.l^{-1}$, resp. Our investigations of the industrial copper intoxication provided no proof of dependence of serum Se concentrations on the administration of ammonium molybdate and sodium sulphate. There were significant difference in group A recorded on day 45 $P<0.01$ during the experiment.

The maximum As concentration was found in group A at the end of experiment. The results were not statistically significant.

A marked increase in the Cd level in comparison with the starting values was observed in groups A and B on day 38 of the experiment. No statistically significant dependence on the administration of ammonium molybdate and sodium sulphate was observed between both groups during the experiment.

The maximum Pb concetration was found on day 31 in group B and on day 45 in group A. The results were significantly different between untreated sheep and treated sheep in group A on day 24 and 38 ($P<0.05$) while on day 17 and 31 was ($P<0.01$) during the experiment.

The concentration of Cu in the liver of sheep from group B was much higher than in group A. The cumulation level of Cu in other organs; lung, heart, muscles, bones, ovaries and uterus, was higher in group B than in group A. There was not found any statistically significant difference in the concentration of Cu among the analysed organs.

The highest amount of Zn was cumulated in the bones of sheep from group B and group A. It was also found that the level of Zn in kidneys and muscles was much higher in group B than in group A. There was no statistically significant difference in the concentration of Zn among analysed organs.

Although the concentration of Fe was higher in all organs from group B than group A, it was significantly higher only in bones and uterus ($p<0.05$) in group B.

Mo cumulation in spleen, kidneys, bones, liver, heart, uterus, ovaries and muscles was higher in group B than in group A. Statistically significant difference in the Mo level were confirmed in the kidneys ($p<0.05$) and bones ($p<0.01$) in group A. On the other hand the concentration in the lung was higher in group A than in group B.

Distribution of Se in organs analysed was higher in kidneys of sheep from group B than from group A. There was no statistically significant difference in results in organs analysed.

Distribution of As in the analysed organs is different from other investigated element.We found higher amount of As in the liver, bones and uterus of sheep in group B than in group A. However in kidneys, spleen, lung, heart, muscles and ovaries, the cumulation was more in group A than in group B while the difference between organs in statistical results was not found.

High Cd content was observed in all organs from group B comparing to group A. There was no significant difference in statistical results.

Pb was highly accumulated in the liver, bones, ovaries and uterus of sheep in group A more than in group B. There was no statistical significance difference in these analysed organs.

The concentration of Cu in the serum also corresponded to the character of the biological material. At the beginning of the experiment, the Cu level was in the reference range in both groups of sheep which pointed to the cumulative phase of chronic copper intoxication (Soli, 1980; Bíreš et al., 1991a). Hypercupraemia was evident in both groups of sheep starting from day 24 of the experiment. Cu levels characteristic for the toxic phase of poisoning were observed in both groups of sheep, A and B, between days 38 and 45 (Bíreš et al., 1991a; Larry et al., 1991; Edelsten, 1980). The high Cu level in the serum between days 38 and 45 corresponded to the clinical picture of animals, most of them showing clinical signs of intoxication (Elgerwi et al., 1999). The cupraemia, observed in both investigated groups of sheep during our experiment, showed low effectiveness of ammonium molybdate and sodium sulphate in prevention of intoxication because the differences between groups A and B of sheep were statistically insignificant. The low effect of the applied Mo and S in comparison with the group A on the accumulation of Cu in our experiment could be explained by the duration of administration of Mo and S (24 days), the presence of other risk elements in the emission and the character of the biological material (sheep exposed for five years to the industrial emission).

The Zn from the Cu industrial emission (124.34 mg/head/day) did not affect the Zn serum level in both groups. The differences in comparison with the group A were significant ($P<0.05$) at the beginning of the experiment. The probable cause of the low serum Zn was the deficient utilization of Zn from the digestive tract due to interactions with Cu (Bremner et al., 1976; Bíreš, 1989) or the presence of other toxic elements in the industrial substrate (Cd, As, Pb, Fe). The dynamics of Fe in the serum of the treated and untreated sheep showed a stable course in both groups throughout the 45 days of the experiment. The increased Fe intake from the emission (976.60 mg/head/day) did not produce an increase in the Fe serum concentration possibly due to interaction with Cu or other heavy metals in the emission (Kirchgessner et al., 1982; Chowdhury and Chandra, 1987) or damage to the metabolic activity of haemopoietic organs, in young lambs and sheep (spleen, liver, bone marrow) during the industrial intoxication.

During the experiment, the concentration of Mo in the blood serum corresponded to Mo supplementation in the group B of sheep. The hypermolybdenaemia, recorded in the group B of sheep from day 10 of the experiment, persisted up to day 24 and corresponded to the period of supplementation with ammonium molybdate. The Mo levels recorded in this group were characteristic of hypermolybdenasis (Ward, 1978; Pitt et al., 1980). However, no clinical symptoms of molybdenum intoxication were observed in the investigated sheep during this period (Elgerwi et al., 1999). A decrease in serum Mo was recorded in sheep of the group B already on day 31, related to interruption of ammonium molybdate application, and the decreasing trend continued up to the end of the experiment. An increase in the concentration of Mo in the blood serum of sheep of the group A compared to the starting values could be explained by housing both groups of sheep together and by secondary contamination of feed with excrements of the treated sheep.

The Se in the industrial Cu emission (0.031 mg/head/day) did not affect the serum Se level in both groups of sheep. The differences in comparison with the group A were significant ($P<0.05$) at the end of the experiment. The interaction of Cu and other toxic elements in the emission is especially important with respect to the animals exposed to industrial deposits in the area surrounding industrial plants (Bíreš et al., 1991b; Vrzgula et al., 1986).

The concentration of Cu determined in the liver of sheep of groups A and B was in an agreement with the levels considered as characteristic of copper intoxication by Hidiroglou et al. (1984) and Bíreš et al. (1991). However, by comparing our results with those of Bíreš et al. (1995a) and Bíreš et al. (1995b), obtained during industrial intoxication of sheep, we found out that the accumulation of Cu in both groups mentioned was not adequate with regard to the dose of Cu in the emission (429.0 mgCu/sheep/day) and the period of administration (45 days).

Similarly, no effect of ammonium molybdate and sodium sulphate administration on Cu concentration determined in the liver of died off sheep of the group B was observed because the Cu concentration in the group of untreated sheep (group A) was 488.118 $mg.kg^{-1}$ while in the treated animals (group B) it was 561.918 $mg.kg^{-1}$. The interaction of Mo and S with Cu in ruminants is related to the production of thiomolybdates which through binding the present Cu reduce its absorption and metabolic activity (Mason, 1981; Golfman and Boila 1990). The low effect of the applied Mo and S in the experimental sheep of group B in comparison with group A on the accumulation of Cu in our experiment can be explained by the duration of administration of Mo and S (24 days), the presence of other risk elements in the emission tested (As, Cd, Pb, Zn) and the character of the biological material used (all the sheep were reared for 5 years in the area close to the industrial plant which created conditions for accumulation of Cu in the organism of animals at the beginning of the experiment).

The distribution of Zn in the analysed organs corresponded to the biological function of this element (Laurie, 1983). The statistically insignificant differences in the levels of Zn recorded between groups A and B were in the reference range (Underwood, 1977). From the viewpoint of the effectiveness of the applied ammonium molybdate and sodium sulphate it can be stated that similarly to the situation with Cu the antidote had a minimum effect on the concentration of Cu as well in the organs examined.

The highest amount of Fe in the spleen of sheep of both groups was in an agreement with its metabolic activity and corresponded to the observations of Bíreš et al. (1991) who also recorded the highest levels of Fe in the spleen of sheep which died of industrial copper intoxication under experimental conditions. The administration of ammonium molybdate and sodium sulphate was associated in sheep of the group B with higher accumulation of Fe in all the organs analysed. The differences in comparison with the group A were significant ($p<0.05$) in bones and the uterus. The higher accumulation of Fe in sheep of the group B which were supplied with the antidote mentioned was likely related to the effect on the metabolism of Cu during which interactions between Cu and Fe occur on the level of resorption, intermediary metabolism and excretion (Schonewille et al., 1995).

The distribution of Mo in the organs of sheep of the group B reflected the supplementation with ammonium molybdate and sodium sulphate and correlated with the metabolic function of Mo in ruminants (Mason, 1990). Our experiment confirmed the cumulative character of this element because the administration of ammonium molybdate up to day 24 of the experiment was manifested by significantly higher accumulation of Mo in the kidneys and bones of sheep of the group B in comparison with that of the group A also on day 45 of the experiment ($p<0.05$; $p<0.01$, resp.).

With the exception of kidneys, the concentration of Se was lower in all the organs examined from sheep of the group B, however, the difference was insignificant. Selenium can interact with elements present in the industrial emission, particularly with Cu, Zn, Cd, Fe and As (Chowdhury, Chandra, 1987; Bíreš et al., 1991). The values of Se determined in the analysed organs in both groups of sheep were considerably lower than those presented by Bíreš et al. (1991) in sheep that died of intoxication with copper originating from material emitted by copper-producing plant.

The dynamics of As, Cd and Pb in blood serum and distribution of As, Cd and Pb in the organs examined from sheep of groups A and B differed without unambiguous dependence on the administration of ammonium molybdate and sodium sulphate. The results of accumulation of As, Cd and Pb in the biological material from sheep reflected the uptake of the elements mentioned from the industrial emission not only during the 45-days of experimental administration but also during the 5-years exposure to risk elements when reared in the area of industrial deposition. The differences in the concentration of individual elements in the organs examined also point to the complexity of interactions of toxic elements during industrial intoxication as they were described in sheep under experimental conditions and in those reared in the area close to the copper and zinc producing plant by Bíreš et al. (1995b), Elgerwi et al. (1998) and Bíreš et al. (1992). The levels of As, Cd and Pb in the examined biological material taken from died off or slaughtered sheep after 45-days administration of industrial emission from the copper producing plant agree with those determined in the area close to the same industrial plant by Bíreš et al. (1995).

SÚHRN

Diagnostika experimentálnej intoxikácie meďou z priemyselnej emisie zo závodu na výrobu medi bola robená na základe kumulácie a distribúcie Cu, Zn, Fe, Mo, Se, As, Cd a Pb v krvnom sére a orgánoch. Nárast kuprémie oproti počiatočným hladinám sa zaznamenal u obidvoch skupín oviec na 17. deň, pričom maximálne koncentrácie boli na konci experimentu. Nepozorovali sa signifikantné rozdiely v kuprémii v závislosti na aplikácii molybdénanu amónneho a síranu sodného medzi skupinami oviec A a B. Z rizikových prvkov, najvyššia koncentrácia Cu sa v obidvoch skupinách A a B zistila v pečeni. Signifikantne vyšší obsah Fe (p> 0.05) sa zistil v kosti a maternici v skupine B. Celkový obsah Mo vykazoval zvýšenú koncentráciu v obličkách (p> 0.05) a kostiach (p> 0.01) v skupine A. Ostatné rozdiely v dynamike Fe, Se, As, Cd, and Pb, ktoré sa pozorovali v krvnom sére a orgánoch oviec v skupine A a B počas priemyselnej intoxikácie meďou, neboli jednoznačne viazané na aplikáciu molybdénanu amónneho a síranu sodného, ale boli skôr výsledkom interakčných vzťahov minerálnych prvkov obsiahnutých v testovanej emisii v procese resorpcie, intermediárneho metabolizmu a exkrécie.

Kľúčové slová: ovce; intoxikácia meďou; rizikové prvky; distribúcia

Literature in authors.

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