ENVIRONMENTAL INFLUENCE OF CHEMICAL CONTAMINANTS ON FARM ANIMALS AND RODENTS (REVIEW RESEARCH)

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Summary

The presence of chemical environmental pollutants (heavy metals) and their influence on health status of farm animals has been study in long period. We monitored the influence of chemical pollutants on rodents leaving on farms. Heavy metals has special danger for leaving systems, which react with organic molecules to change their structures and function. Heavy metals enter the body through respiratory system, digestive system and skin. The results of our many years of research indicate that there is danger of contamination of animal feed with heavy metals and their position in their body of animals, as well as a negative effect on the reproductive capacity of domestic animals. Heavy metal toxicity general leads to the formation of free radicals, inhibiting the activity of antioxidant defense enzymes as well as glutathione oxidation and the formation malonyl dialdehyde (MDA) as a marker of oxidative stress. Their toxicity stems from the tendency to form covalent bonds with sulfhydryl groups of biomacromolecules or displace certain cofactors, there by inhibiting the activity of certain enzymes. Our recommendation for industrial type farms is to reduce the risk of heavy metals. To introduce multilevel monitoring of the quality of raw materials and final products, as well as to apply adequate protectors against the toxic effect of these agents.

Keywords: farm animals, rodents, chemical contamination

This review article is aimed at presenting the results of our studies carried out in the period (2002-2020). Heavy metals pose a special danger to living systems, which react with organic molecules to change their structure and function. Heavy metals enter the body through the respiratory system, digestive organs and skin. The results of our research indicate that there is danger of contamination of animal feed with heavy metals and their deposition in the body of animals, as well as a negative effect on the reproductive abilities of animals. Heavy metal toxicity generally leads to the formation of free radicals by inhibiting the activity of antioxidant defense enzymes as well as the oxidation of glutathione and formation of maslondialdehyde (MDA) as a marker of oxidative stress from the tendency to form covalent bonds with

sulfhydrylin groups of biomacromolecules or displace certain cofactors thereby inhibiting the activity certain enzymes (3, 4, 7, 46).

Possible monitoring of hepatocytes impairments caused by ROS effects

Heavy metals are highly toxic to all living beings on Earth. They enter organisms of humans and animals via the contaminated food, water, soil and/or air. Toxicity of cadmium (Cd) and lead (Pb) is mostly due to their ability to increase reactive oxygen species (ROS) production. Redox active or redox inactive metals may cause an increase of ROS such as hydroxyl radical (HO), superoxide radical ($O_2^{\cdot -}$) or hydrogen peroxide (H_2O_2). Enhanced generation of ROS can overwhelm cells intrinsic antioxidant defenses then the consequence is “oxidative stress” (14, 35, 47). These heavy metals (Cd and Pb) are not able to induce oxidative stress by means of Haber-Weis and Fenton reactions, but they can generate ROS by indirectly mechanisms which are not completely cleared. From literature data that Cd stimulates creation ROS-a, including O_2, H_2O_2, HO (40, 43, 52).

Increased production of ROS provokes lipid peroxidation (29, 36, 38) of cell membranes, oxidation-related changes in protein molecules (23), as well as changes in DNA molecules (20). Such an action of ROS is responsible for serum albumin (SA) oxidation and fragmentation (22). The consequence of SA oxidation (18, 42) is polymerization of SA molecules and can form dimers reacting with other molecules (28). Lipid peroxidation induced by ROS action cause damages on hepatocyte membranes and destroy receptors for polySA (32, 33, 41, 42, 48, 49). Thus, the concentration of polySA increase in circulation, preventing the transfer of polySA into the hepatocytes for its degradation (53). The occurrence of polySA of increasing concentration cause formation of anti-polySA antibodies, which can be proved by immunodiffusion test against polySA prepared from sheep SA by glutaraldehyde treatment (31). The receptor for polySA on liver cell membrane functions as a binding site for in vivo polymerized albumin conferring the ability of these cells to remove polymerized molecules from the circulation as in normal organism. In this way the hepatocytes would be able to select for catabolism the polymerized albumin from the native one (33). Damage of hepatocytes membranes by lipid peroxidation is also associated by changing protein conformation and packing of phosphatidylcholine (37). Consequence of these effects are enhancement of membrane permeability which is accompanied with lactate dehydrogenase (LDH) leakage from cells increasing LDH activities in surrounding medium (26).

Heavy metals metabolism occurs mostly in the liver where Cd binds to metallothionein (MT), protein rich in cysteine (cys), with high affinity for metals. Cd is in Cd-MT complex which is transferred from the liver to the kidneys over time, then is filtered, and reabsorbed by the renal proximal tubules. Complex Cd-MT is metabolized in lysosomes, where liberate Cd ions which again binds to preexisting or newly made MT. If syntheses of MT cannot keep up with the demand of the content, unbound Cd overwhelm another defense system and Cd toxicity ensues (23, 24, 25).

In liver during heme syntheses Pb increase ROS through inhibition dehydratase of 5-aminolevulinic acid (ALA), causing accumulation of ALA which is potential endogenous free radicals sours (39). ROS is generated and by interaction of ALA and oxyHb increasing the lipid peroxidation. Lipid peroxidation altered composition of cellular membranes, change membrane permeability and enhanced its susceptibility to lipid peroxidation.

At the sheep farm Ile de France, it was noticed that the sheep lost their appetite, weight, and showed no interest in the environment. The sheep were kept free and fell from the farm pasture. During the clinical examination of the animals, the visible clinical symptoms of infectious disease were not diagnosed. The results of the testing of nutrients, grasses and soils from the same site indicated the content of heavy metals, lead, cadmium, and traces of living and arsenic.

The aim of our study was to establish the presence of polySA and SA fragments, as well as the activities of total LDH and LDH5 isoenzyme, as a consequence of ROS action, all indications of damage to the liver evident in the serum of animals previously exposed to Pb and Cd through feed. At the same time, MDA was identified as marker lipid peroxidation.

Materials and methods

Sheep Albumin and Cibacron Blue 3GA-agarose were purchased from Sigma, USA. The molecular weight standard kit was from Pharmacia Biotech, Upsala, Sweden, and other used chemicals were from Merck, Germany.

The experiments were performed on Ile de France sheep (n=20). The control group (n=10) was unexposed to heavy metals from the other ariea. The experimental group (n=10) was exposed to heavy metals (lead and cadmium) through soil and feed (soil Pb = 16.7 ± 0.02 mg/kg DS, Cd = 20 ± 1.0 μg/kg DS and feed Pb = 2.5 ± 0.02 mg/kg DS, Cd = 20 ± 1.0 μg/kg) in the course of three months. After that period, blood and blood serum of these animals was analyzed. The analysis of heavy metals in serum, soil, grass and feed samples was performed by means of atomic absorption spectrometry (Perkin-Elmer Corp., USA) after mineralization in 100A TEKATOR DIGESTOR with nitric acid, and with hydrogen peroxide added. The level of lipid peroxidation (LP) was assayed as thiobarbituric acid reactive substances (TBARS) in the red blood cells (51). The Hb concentration was determined by the cyanmethemoglobin method (13).

Blood was taken from the *jugular vein* with and without anticoagulans. After coagulation the serum was centrifuged at 3000 rpm and kept at -20°C until testing. Before freezing amount of serum was separated, and immediately used to assay the activity total LDH (EC 1.1.1.27) and activity LDH_5. Total LDH activity was carried out the by direction reduction of pyruvate to lactate was measured in Tris pH 7.4, 60.00 mM, containing 1.0 mM pyruvate and 0.18 mM NADH, by UV kinetic method. LDH_5 activity was determined in same buffer mixture with 2.0 M urea as an inhibitor (2, 27).

The PAGE electrophoresis was performed in gel concentration of 8 g/dL in

non-dissociated discontinuous buffer system (19). SDS-PAGE was carried out in gel containing 10 g/dL acrylamide (30). The electrophoretic analyses were performed on a vertical device MINI VE HEFFER.

The presence of polySA in the serum was determined by double immunodiffusion in 1.5 g/dL agarose gel in 16 mmol/L barbital buffer, pH 8.4 against prepared polySA. The preparation of sheep polySA was performed by glutaraldehyde treatment, of sheep albumin (31).

SA fragments were isolated by affinitive chromatography on Cibacron Blue 3GA-agarose mini column (0.9 x 4 cm), using 20 mmol/L sodium phosphate buffer pH 7.2 for equilibration. Elution was performed using three solutions: I 20 mmol/L sodium phosphate buffer pH 7.2; II 1. 5 mol/L NaCl in 20 mmol/L sodium phosphate buffer pH 7.2; III 5 mol/L urea. The absorbance was measured at 280. The results are expressed as arithmetical mean values ($\overline{X}$) ± standard deviation (SD). The data were analyzed by Student's t-test.

Results and discussions

The heavy metals content in the blood serum of the experimental group was analyzed, showing an increased in Pb and Cd concentrations (Pb = 3.61 μmol/L, Cd = 53.40 nmol/L), compared to the control group.

Total enzyme activity LDH in blood serum of the exposed sheep (1176 ± 63 U/L) were significantly higher ($p< 0.001$), than those in the control group (352 ± 59 U/L). LDH_5 isoenzymes from the sheep blood serum, are presented in Fig. 1.

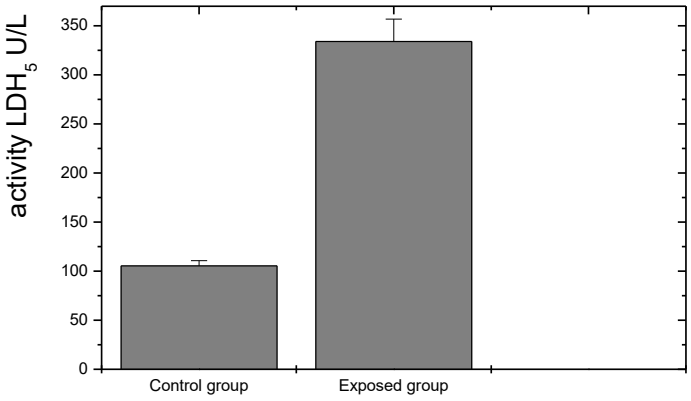

Fig. 1. LDH_5 isoenzymes activities in sheep blood serum

The activities of LDH_5 isoenzymes in serum of the exposed sheep (334 ± 23 U/L), were significantly higher ($p< 0.001$), than those in the control group (115 ± 15 U/L). MDA concentration was significantly increased in the erythrocytes of sheep

(16.95 ± 6.14 nM MDA/g Hb) after three months of taking food from a contaminated site heavy metals ($p<0.0001$) in relation on control group (0.56 ± 0.21 nM MDA/g Hb), (Fig. 2).

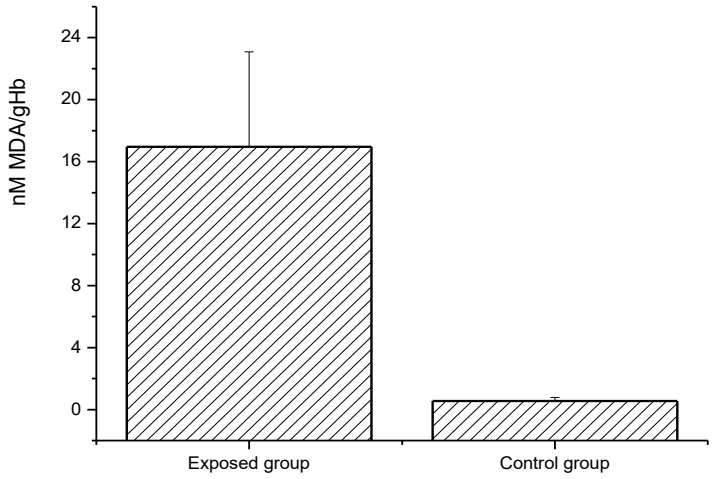

Fig. 2. The content of MDA in erythrocytes in exposed and control group of sheep

All blood samples were examined for polySA presence (Fig. 3).

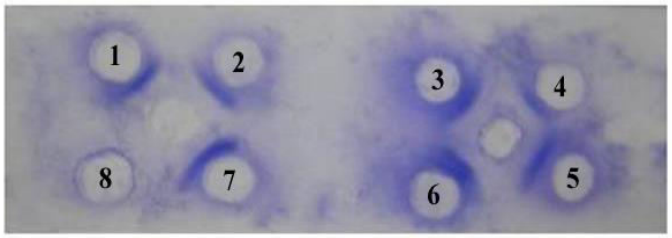

Fig. 3. Immunodiffusion test of polySA

Representative immunodiffusion reaction patterns of serum samples of exposed sheep (wells 1-7) in the control group (well 8) and central wells containing prepared sheep polySA.

The appearance of precipitin lines shown in Fig. 3 indicate the presence of polySA in serum of animals exposed to heavy metals.

Electrophoretic patterns of serum proteins of the exposed and control sheep group, and standard proteins investigated by PAGE are presented in Fig. 4.

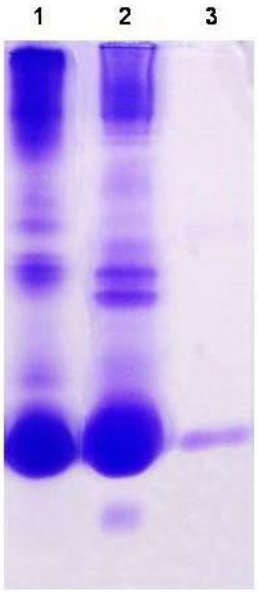

Fig. 4. Representative electrophoretic patterns of serum proteins of sheep

Representative PAGE patterns of the serum proteins in exposed sheep (lane 2), in control (lane 1) and standard proteins (lane 3).

In the PAGE protein patterns of the exposed sheep a protein fraction migrated faster than did the albumin fraction (Fig. 4 lane 2), but in sheep serum in the control group such protein fraction was not revealed (Fig. 4 line 1). The occurrence of this protein fraction was probably due to ROS effects on SA, initiating fragmentation. For confirmation, SA fragment formation in exposed sheep serum affinitive chromatography on Cibacron Blue was performed. Elution profile of exposed sheep serum is presented in Fig. 5.

Elution was carried out with buffers I, II and III and three peaks were eluted. All the obtained peaks were analyzed by SDS-PAGE. SA and SA fragments were eluted in peak 2 which was proved by molecular weight determination, using the molecular weight standard protein kit. These results are presented in Fig. 6.

SDS-PAGE protein patterns on Fig. 6 of peak 2 exhibited the presence of SA and several protein bands with molecular weight from 28 kD to 48.5 kD, indicating that SA was decomposed in fragments.

Heavy metals manifest their toxic effects by provoking oxidative stress, being their oxidation processes consequence. The ROS effect on proteins is mainly the action of hydroxyl radical (23, 24) on aminoacids in polypeptide chain. It has been proven that the hydroxyl radical mostly affects tryptophan, tyrosine and cysteine by

oxidizing them, which cause modification in the polypeptide chain with subsequent formation of fragments (22), which may behave like native molecules (Fig. 4), due to co-precipitation with the entire SA molecule. Therefore, sheep blood serum was analyzed by affinitive chromatography for separation SA with coprecipitated SA fragments from SA Elution profile obtained by affinitive chromatography (Fig. 5) of serum of the animal exposed to Cd and Pb indicate that peak 2 contains SA fragments, as well as molecules SA. This we proved by the SDS-PAGE analysis of the peak 2 (Fig. 6). Namely, results of the SDS-PAGE electrophoretic analysis of the peak 2 shows the presence of SA and SA fragments which are higher mobility proteins than SA with molecular weight 28-48.5 kD (Fig. 6 lane 1). The blood serum in the sheep control group was simultaneously analyzed, and after the affinitive chromatography of serum of these animals in the peak 2 by the SDS-PAGE electrophoretic analyses, only the presence of SA, and not the SA fragments, was proved (Fig. 6 lane 2). Protein modification by ROS-induced fragmentation in vitro, as well as the use of protectors against ROS effects, were examined by Mayo et al. (54) detecting specific cleavage of protein molecules. Oxidatively modified proteins further may spontaneous fragmented and cross linking (34, 45).

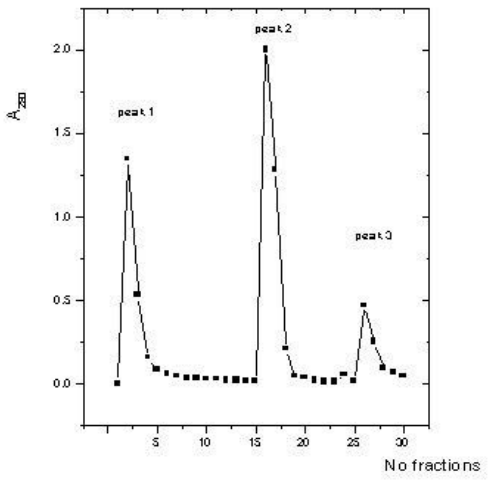

Fig. 5. Elution profile of exposed sheep serum

Representative SDS-PAGE protein patterns isolated from sheep serum by affinitive chromatography peak 2, are presented in Fig. 6 lanes from left to right. Lane 1 peak 2 from sheep serum of exposed animals. Lane 2 peak 2 from sheep serum of control animals. Lane 3 standard molecular weight markers.

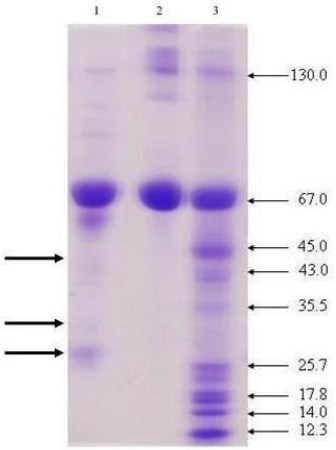

Fig. 6. Representative SDS-PAGE protein patterns of peak 2

Heavy metals thus Cd and Pb metabolism mostly occurs in the liver. The polySA receptors may damage (31, 42, 49), by various agents also by the ROS actions through lipid peroxidation of hepatocyte membranes (44), intravascularly formed polySA enhance in circulation. SA contains a free –SH (thiol group), and is a ROS action target, forming intermediary SA radical can riect with hemoglobin (Hb) forming dimmers Hb-SA and SA-SA types (42). We have tested blood serum for the presence of SA-dimmers and polySA in animals exposed to heavy metals, as well as that of the control group animals (Fig. 3). PolySA was detected by double immunodiffusion (31) in the blood serum as a result of the ROS effect on SA and hepatocyte membranes. The liver damage affects the ability of hepatocytes to remove polySa from circulation and rising its level in serum. It is proved that on liver cell membrane existed specific receptors for polySA (31, 50, 41), which involving transferring polymerized SA in to the cells to metabolism. Consequence of the liver damage breakage the specific receptors conferring polySA to liver cells, affecting the ability of hepatocytes to remove the polySA from the circulation, rising its level. Having new antigenic site induce formation specific anti-polySA antibodies. Using immunodiffusion test antibodies can precipitated by polySA formed by glutaraldehyde treatment standard SA or isolated SA from sheep serum (31). There evidence about ROS effects on hepatocytes membrane, (26)**,** study mechanism of Cd induced cytotoxicity on isolated liver rats' cells. Mechanism of this effects are associated with cellular acidification which stimulate enhancement production of H_2O_2 causing permeabilities changes of plasma membranes link to subsequent extensive breakage (26) found that Cd immediately cause acidification and later alkalinization accompanied with loss of LDH activities in the cell. Our findings of LDH activities in blood serum of exposed animals to Cd and Pb indicate significantly

increasing activities of total LDH as well as LDH_5. In human polymerized SA is detected in sera of patients with liver disease is specially hepatitis cause by HBV (hepatitis B virus). Numerous investigators have reported that polymerized human albumin and HBV have receptors on hepatocytes surface. These receptors can bind HBV and polymerized albumin and are important for the attachment of HBV to hepatocytes and entrance of virus into the cell (1, 2, 21, 50). MDA level may be use as marker of lipid peroxidation and as well as oxidative stress in regard to ROS (11, 17). Our findings indicate significantly increased the level of the MDA in erythrocytes of exposed group of sheep comparison with control one.

Results of our experiments have confirmed the presence of polySA, SA fragments as well as an increased total LDH and LDH_5 activities in serum of those animals exposed to heavy metals. We are of the opinion that these findings may serve as parameters of ROS in hepatocytes impairment monitoring.

Environmental influence on swine

Sound health of pigs is a prerequisite for good reproduction, that is, profitable production. Pig health may be improved in order to achieve the highest possible production and it depends on conditions of their keeping, care, feeding, health control and health care (8, 9, 10).

Lead content in the studied feed samples was 2.0 mg/kg dry substance; cadmium content was 0.22 mg/kg dry substance, while mercury content was 0.0035 mg/kg dry substance. Five boars used for reproduction that consumed the analyzed feed underwent semen analysis in order to determine presence of heavy metals using the method of absorption spectrometry. The obtained results indicated that the metals were transported into the reproductive organs, with the highest level of lead 0.36 mg/kg, cadmium 0.0013 mg/kg and mercury 0.0021 mg/kg. None of the above-mentioned boars underwent cytogenetic analysis. Based on the obtained results it may be concluded that detection of carriers of the chromosomal aberrations is of the great importance in artificial insemination programs. Identification of the carriers of the obscured anomalies is an important task in selection of the breeding animals. The results of the study indicate the risk of feed contamination by heavy metals and their depositing in the boar organisms. We may recommend to the industrial type farms to reduce the risk of heavy metals by introduction of multi-level monitoring of raw material and finished product quality and application of the appropriate protectors against toxic effects of the agents (5, 6).

The results indicate that is danger to contamination of animal feed with heavy metals and their deposition in the body of animals as well as reproductive capacity of boars. In order to reduce the risk of using seeds contaminated with heavy metals, it is necessary to perform an analyses of their presence. Heavy metals cause significant metabolic changes, disrupt biological systems, reduce body weight gain and the mass of individual organs. There are also differences in the accumulation of heavy metals and increased mortality depending on the age of the individuals. Heavy metal toxicity generally leads to formation of free radicals by inhibiting the activity of antioxidant defense enzymes as well as the oxidation of glutation and the formation

of MDA as a marker of oxidative stress. The toxicity is also due to the tendency to form covalent bonds with sulfhidryl groups of biomacromolecules or displace certain cofactors which inhibit the activity of certain enzymes (7, 8, 9, 10).

Environmental influence on rodents

Different storage conditions for agricultural products and the efficacy of environmentally-safe substances were analyzed to formulate a biosecurity standard. Hygienic conditions, the most important such indicator in any facility, are crucial for controlling house mouse (*Mus musculus* L.) populations. It is often impossible to remove all food sources from a facility and its surroundings. A preventive biosecurity programe based on HACCP principles aims to prevent the infection of primary biological vectors in any agricultural storage facility. Our data show the efficacy of sodium selenite and cellulose and their applicability in biosecurity plans considering their application methods at critical control points (15, 16).

Conclusions

Veterinary profession has a very important role in livestock breeding, animal health protects people and the environment. Every day the question how to produce more animal products, but also healthy food intended for human consumption. Therefore, the health care farm animals using more preventive and therapeutic measures less able to fulfill the concept which will be represented at the same time profitable production, and degree control over of toxic agents in the responsibility veterinary professions involved.

Our recommendation for industrial-type farms that need to act to reduce the risk of side effects of heavy metals, for the introduction of multiple-quality monitoring of raw materials and finished products, and implementation of adequate protector of the toxic effects of these agents.

Acknowledgements

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