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Correction of indicators of erythrocytopoesis and microelement blood levels in cows under conditions of technogenic pollution

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This article presents the results of cows examination under condition of technogenic pollution. It was established that erythrocytes blood levels in cows from farms, located in the zone of technogenic pollution were significantly lowel (P<0.001) comparing to control group. Hemoglobin blood concentration in experimental cows was low (72.5±1.74 g/l) in comparison with control group (95.2±1.87 g/l). The hematocrit value ranged from 0.23 to 0.28 l/l and was less than normal (0.35 l/l). The content of "old" erythrocytes, which actively participate in the processes of oxygenation, increases (P<0.01) in the blood of experimental group cows compared with control. The content of "young" forms decreases (P<0.001) is one of the indicators of intensity erythrocytopoiesis. Cobalt blood levels in experimental cows was 1.5 times lowel (0.28 ± 0.008 μmol/l) than in experimetae the control group (0.41±0.016 μmol/l). The maximum content of cobalt in the blood of cows reached the minimum limit (0.50 μmol/l). A negative correlation between the content of cobalt and cadmium was established (r = - 0.545 and - 0.550).
Administration of «Microlact» to animals, wich contains organic compounds (lactate) of microelements increased cobalt (2.3 times) and copper (33.6%) blood levels, hemoglobin content, hematocrit value, MCHC and reduced clinical symptoms of anemia in cows.
Key words: cattle; anemia; erythrocytopoesis; heavy metals; cobalt; copper; lactate.

Introduction

Due to the deterioration of the ecological situation in the world, the possibility of safe farming on the territories under technogenic pollution remains a topical issue.
Anthropogenic pollution of the environment by heavy metals has a negative impact on the health of the population. These biologically active elements have the ability to accumulate gradually in the body, exhibit their toxicity at low concentrations, and adversely affect the health of humans and animals.
Heavy metals include almost 50 elements. In veterinary medicine, the term "heavy metals" is valid only when it comes to hazardous for animals concentration of a particular element in the body. In other cases metals are considered as trace elements. Eight toxic elements (Hg, Cd, Pb, As, Sr, Cu, Zn, Fe) demand strict control in international food trade. The most toxic heavy metals for animals are mercury, cadmium and lead (Kalyn, 2004; Gutyj et al., 2018).
Intensive technogenic influence on the agroecosystem causes environmental pollution and negative impact on the vital activity of the formed biocenoses, exhibits periodic and distant effects on plants with the accumulation of harmful substances in the soil-plant-animal system (Alonso et al., 2004; Slivinska, 2007).
The increasing attention of researchers (Alonso et al., 2004; Jomova et al., 2010) attracts the study of the combined action of heavy metals on the organism of animals, as an ecopatogenic factor of the environment. The source of such action is the heaps of mines located in the Lviv-Volyn coal basin, on the territory of which approximately 101.5 million m^3 of waste are stored, which are more dangerous than coal (Velichko, 2007).

In addition, heavy metals change the metabolism of the essential elements (Cu, Co, Mn, Zn, etc.). Some of them are due to the competitive action of microelements that are involved in the processes of hematopoiesis.
A special place in the development of farming belongs to biologically active substances, used in the prevention and treatment of a number of animal diseases (Martyshuk et al., 2016; Khariv and Gutyj, 2016; Shcherbatyy et al., 2017). Therefore, so-called therapeutic feed and premixes are widely used.
The purpose of the work was to study general blood test parameters, determine heavy metals and trace elements blood levels in cows under conditions of technogenic pollution and to correct established violations.

Materials and methods

The research was carried out at farms of the Volyn region, lpocated in the zone of technogenic influence. The control was the cows of farms of conventionally clean areas. The object of the study were black-and-white cows at the age of 3-7 years with a productivity of 5000-5500 kg of milk. Animals investigated clinically - commonly used techniques (Levchenko et al., 2010) and conducted a laboratory blood test.
General clinical analysis of blood included the calculation of erythrocytes (in the haemocytometer with grid), of hematocrit – by microcentrifuge (by Shkliar), content of hemoglobin – using the hemiglobincyanide method, ESR – according to T. P. Panchenko. Using the results, we calculated the average volume of erythrocytes (MCV) and the content of hemoglobin in a single erythrocyte (MCH) (Levchenko et al., 2010).
The population composition of erythrocytes was studied by in a gradient of sucrose density by (Sizova et al., 1980). The content of trace elements and heavy metals in the blood serum of the cows was defined by the method of atomic absorption spectroscopy using AAS-30. For the correction of the established changes, the drug "Microlact" was used containing organic compounds (%) – lactates of Zn (28.5-29.3), Mn (18.0-19.2), Cu (3.64-3.8), Fe (30.5-30.66), Co (0.33-0.35), Se salt on trilon (2.7-2.8) and J starch (15.0-15.28).
In conducting research, the rules were followed for the implementation of zootechnical experiments on the selection and maintenance of animal-analogues in groups, harvesting technology, use and accounting of consumed feed.
All manipulations with the animals were conducted in accordance with the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Strasburg, 1986). Mathematical analysis was made in Statistica 6.0 (Stat Soft Inc., USA). Differences between the average values were considered statistically significant at $P < 0.05$ (ANOVA).

Results

The conducted research (Slivins'ka, 2007) established that dumps and waste heaps of mines are the main source of technogenic pollution of the environment. Significant flow of heavy metal salts leads to a change in the chemical composition of soils and vegetation. Heavy metals change the metabolism of essential elements (Cu, Co, Mn, Zn, etc.), have a competitive effect on trace elements. The latter participate in the processes of hematopoiesis (Shcherbatyy et al., 2017).
The highest index of heavy metals soil in Ivanychivsky distriet of Volyn regions is on the territory of the mine №6 and near the heap (P1, P2) at the distance of 1 km from the mine №5; 2 km to the mine number 6; 4 km - mine №7 (P4); 13 km - mine number 6; 10 km - mine №7 (P5), where the total content of heavy metals is 2.2-3.7 times higher then the contents background, and in some places excuds maximum permissible concentrations. The content of cadmium in the soil (Fig. 1) decreased with increasy distance from the source of anthropogenic pollution.

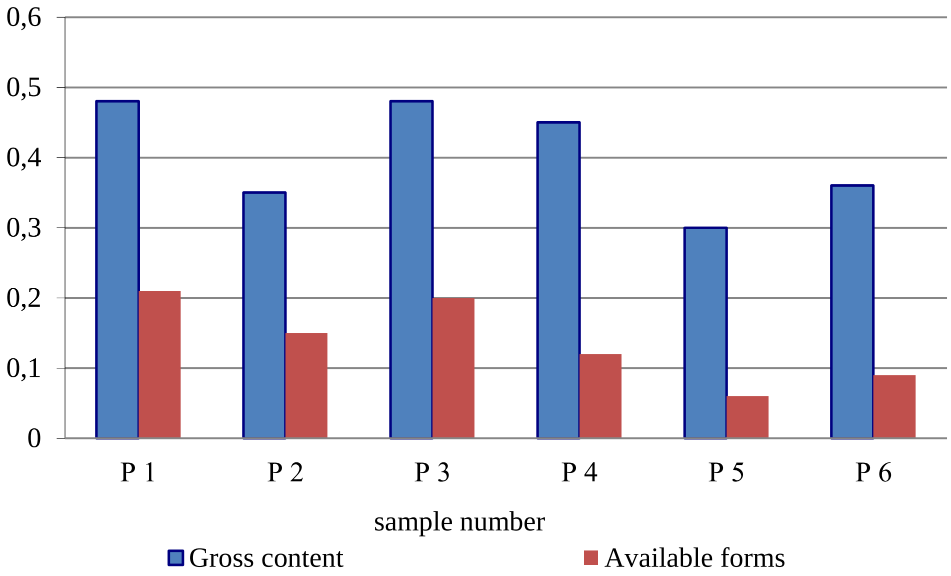
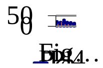


Fig. 1. Cadmium content in soil, mg/kg

At the heaps of mine № 6 (sample P2) cadmium content in soil was 0.15 ± 0.012 mg/kg, at a distance of 2 km - 0.12±0.004, 13 km - 0.06± 0.004 mg/kg. At the same time, the content of gross cadmium almost didn’t change. The content of lead - in the soil near the heaps of mine was 19.0 ± 0.53 mg / kg, at a distance of 2 km - 19.6 ± 0.93, of the available form - 9.6 ± 0.82; 13 km - 17.5 ± 0.38 and 7.6 ± 0.23; 17 km - 15.1 ± 0.26 and 5.1 ± 0.18 mg / kg for the MPC - 6.0 mg / kg (Fig. 2).



Nickel and cuprum concentrations also exceeded the maximum allowable levels.

Soils contamination by heavy metals and their compounds leads of their entry into plants. Therefore, we determined the level of separate trace elements in different feed crops, which are grown and used in the region for cows fuding. We determined the migration of heavy metals in the soil-plant chain in rough feeds (hay meadow) at a distance of 1 km from the mine - P1-P4, juicy (silage, pulp) - P5-P6, root crops (sugar beet) - P7. The results showed than the content of cadmium in hay was 4 times higher than the maximum permissible concentrations in P4 (5 km from the mine) 1.2 times the content of nickel. In samples P1 and P3, the nickel content was 38 % and 34 % higher the maximum permissible concentrations respectively.

The level of lead and nickel in root crops were 4 times higher then the permissible concentrations at a distance of 5 km from the source of contamination (P7). In silage (P5) and bagasse (P6), the content of heavy metals did not exceed the maximum permissible concentrations. Cadmium and lead blood levels were 10-12 times and 1.4-4 times higher than in control groups.

It is known (Zasekin, 1999; Velichko, 2007) that the efficacy of erythrocytopoesis decreases under the influence of by heavy metals. Enzyme systems are inhibit ed and the synthesis erythrocyte precursors in the bone marrow is disturbed. Heavy metals have the ability to destroy erythrocytes, cause significant changes in the hemoglobin system. The sensitive criterion of the organism's response to the action of cadmium is the amount of erythrocytes and hemoglobin content.

It was established that the blood number of erythrocytes in cows from farms, located in the zone of technogenic pollution lower, was then in control group of animals (P <0.001). The concentration of hemoglobin in the blood of experimental cows was low, 72.5 ± 1.74 g/l comparing to control group (95.2 ± 1.87 g/l). The hematocrit value ranged from 0.23 to 0.28 l/l and was less than normal (0.35 l/l). The tendency to develop microcytosis was observel, indicating a lower gas exchange in the tissues.

Reduced erythrocytes number of is accompanied by changes in erythrocytes cell populations in the blood of cows.

The level of "old" erythrocytes, which actively participate in the processes of oxygenation, increases (P <0.01) in the blood of experimental group cows compared to control. The content of "young" forms - decreases (P<0.001). The established changes in the erythrocytes cell population indicate a reduction in the oxygen capacity of blood on cows from experimental group.

Copper blood concentration in cows from experimental group significantly lower (p <0.001) compared with control group. Lack of copper was observed in 100% of cows. A negative correlation relationship was established between the content of cadmium, cuprum and lead in the blood of cows (r = - 0.671 and - 0.646) respectively on hemopoiesis. Probably, there is a competitive influence of cuprum and heavy metals.

In the blood of cows from experimental farms, the content of cobalt was 1.5 times less (0.28 ± 0.008 μmol/l) compariny with control group (0.41±0.016 μmol/l). Maximun cobalt blood concentration in cows from experimental farms reached the minimum limit (0.50 μmol/l). A negative correlation was established between the content of cobalt and cadmium (r = - 0.545 and - 0.550).

The influence of cadmium and lead in combination with cobalt and copper deficiency lead to the changes in the number of erythrocytes, hemoglobin levels, and hematocrit values, indicating the inhibition of hemopoiesis. There was a negative correlation between the number of erythrocytes and cadmium (r = - 0.448), erythrocytes and lead (r = -0.434), hemoglobin and cadmium, lead levels (r = -0.610; r = -0.604 respectively).

The next stage of research was to study the parameters of erythrocytopoiesis, content of microelements (Co, Fe, Cu) in cows during their holding in "Vilna Ukraina"farm in Ivanichiv district of Volyn region using a complex of inorganic and organic compounds (lactates) of microelements “Microlact” for their feeding. 60 cows were selected for experiment and devided into three groups– one control and two experimental (n=20 in each group). Animals from control group received basic ration, animals from the first experimental group - inorganic compounds of microelements (Cu, Zn, Mn, Fe, cobalt chloride, potassium iodide and sodium selenite sulfates) during 45 days in addition to the main ration. Animals from the second experimental group were fed by the complex drug Microlact 4.84-5.0 g per day. The experiment lasted for 45 days.

During the experimental period we observed an improvement in clinical condition and parameters in animals from experimental groups, which indicates the normalization of metabolic processes. Mucous membranes acquired pale pink or pink color, the hair cover was characterized by a peculiar shine, densely adhering to the skin. The folded skin quickly restored its elasticity. The number of rumen reductions in cows from experimental groups was 8-11 times in 5 minutes.

The treatment of cows with a complex of microelements and organic compounds contributed to the activation of hematopoietic function of blood-forming organs (Fig. 3).

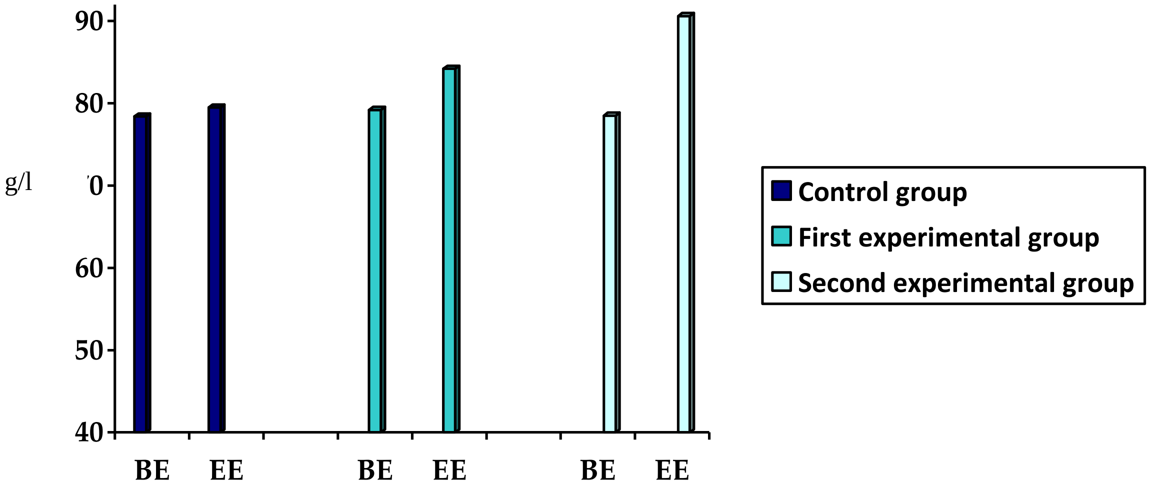
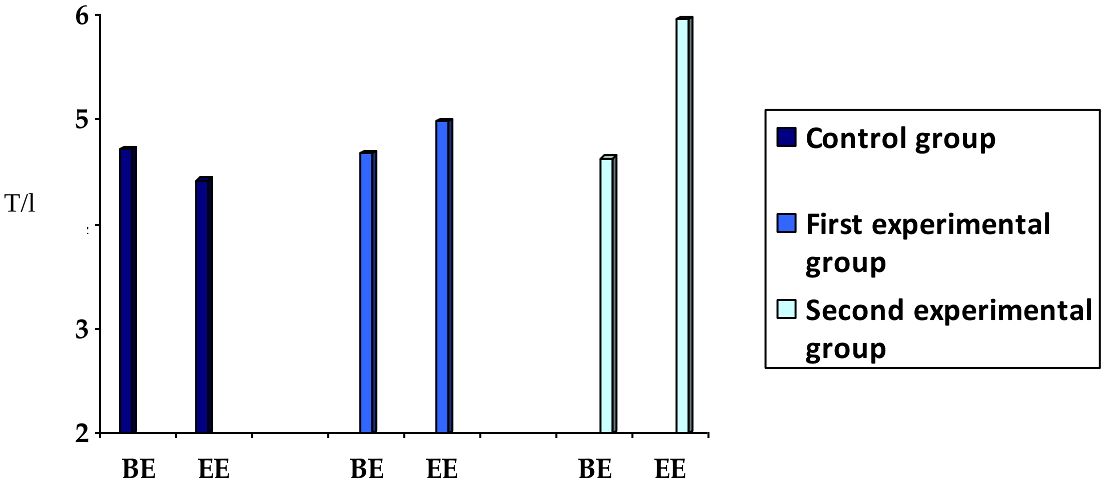


Fig. 3 - Changes in hemoglobin content in the blood of cows
with anemia for the influence of Microlact
Note: BE - beginning of experiment; EE - end of experiment.

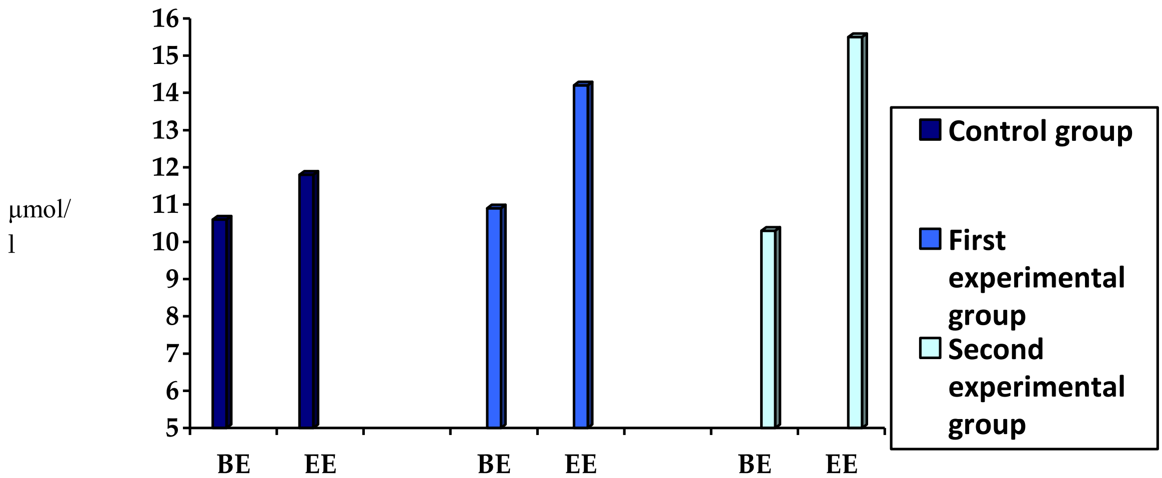
At the beginning of experiment, the hemoglobin blood content in cows from the experimental groups cows was 78.5±2.24-79.2±1.65 g/l, which is actually below the physiological limit (Fig. 3). Blood analysis taken after 45 days of experiment in the same groups of cows, showed an increase in hemoglobin levels on 6.3 % in first experimental group compared with levels at the beginning of experiment, and on 5.9% – comparing to control group. In 100 % of cows from the second experimental group hemoglobin level was reduced at the beginning of experiment. Average hemoglobin content was 78.5±2.24 g/l. At the the end of experiment, it was 15.1 % higher comparing to the level at the beginning of experiment ($p < 0,001$) and 11.1 % higher in comparison with control group ($p < 0.001$). However, the complete restoration of hemoglobin content was established in 40 % of cows.
Erythrocytes blood number in cows from two experimental groups was by 6.5 % lower (4.7±0.42; 4.6±0.24 T/l) comparing to physiological limit (Fig. 4) before feeding inorganic salts of microelements and Microlact.



'Fig. 4 - Changes in number of erythrocytes in the blood of cows,
with anemia for the influence of Microlact
Note: BE - beginning of experiment; EE - end of experiment.

Apparently chronic intoxication by heavy metals decreases the effectiveness of erythrocytopoiesis and inhibits enzyme systems, that provide the synthesis of precursor of heme and formation of erythrocytes in bone marrow. Besides, heavy metals have the ability to destroy erythrocytes and cause significant changes in hemoglobin system (Panas et al., 2003).

Treatment leads to the increase of hemoglobin level and erythrocytes number in the blood of experimental cows (Fig. 4). In cows from the first experimental group erythrocytes blood number was 6.4 % higher ($p<0.1$) comparing with values at the beginning of experiment, 7 (35 %) of animals had optimal amount (more than 5.0 T/l). In the second experimental group, erythrocytes blood number was increased on 28.7 % ($P<0.01$), comparing with values at the beginning of experiment, and on 34.8 % ($P<0.001$), comparing to control. Their average number concluded 6.0±0.28 T/L.
Furthermore, we observed changes of mean corpuscular hemoglobin concentration (MCHC) in the blood of experimental cows as a result of treatment. At the beginning of experiment the average MCHC in cows was 17.2±0.54 pg in the first experimental group; 18.8±1.10 pg– in the second experimental group and 17.4±0.47 pg in control group. After feeding the microelements in form of inorganic salts to pregnant cows of experimental groups, there was only a tendency to decrease this indicator, and after the use of organic compounds (lactates) in complex drug Microlact, the difference was probable ($P<0.001$). Since the number of erythrocytes in cows of this group increased by 28.7 % and the hemoglobin content was only by 15.4 %. It is natural that the saturation of each erythrocyte decreased ($P<0.01$) from 17.4±0.47 to 15.5±0.35 pg, but hypochromia was established only in 25 % cows.
At the beginning of experiment low hematocrit was established in experimental groups comparing to control, which is one of anemia indicators. At the end of experiment, the hematocrit significantly increased ($P<0.001$) in the first and second experimental groups comparing to the beginning of experiment. The tendency to increasing of this parameter was observed in the both experimental groups comparing to control ($p<0.5$; $p<0.001$ respectively), and in the second experimental group comparing to the first experimental group ($P<0.05$). The average hematocrit value was 28.0±0.24 % in the first group, 31.0±0.46 % in the second.
An important parameter of hemopoiesis is mean corpuscular volume (MCV) of erythrocytes. At the beginning of experiment MCV was within the normal range and did not differ in all groups. But in 11.7 % of cows was established microcytosis was established, while in 88.3 % – macrocytosis. At the end of experiment in control group mean corpuscular volume increased ($p<0.05$) comparing to the beginning, and macrocytosis was observed in 100 % of cows. In cows from the first experimental group mean corpuscular volume remained unchanged, the number of cows with microcytosis decreased. The best results are obtained in the second group. Under the influence of microelements lactates, the number of cows with macrocytosis decreased (30 %), and the maximum volume of erythrocytes did not exceed 70 fl.
Microelements have significant effect on the mean corpuscular volume of erythrocytes. Therefore, we determined the content of copper, ferrum and cobalt in the blood of cows in control and experimental groups. At the beginning of experiment, ferrum blood level in all groups of cows was within the normal range, and in individual cows exceeded the maximum normal level (26.8 μmol/l). At the beginning of experiment copper blood level was decreased in 100 % cows of the control, both experimental groups. The average content was 10.64±0.43; 10.9±0.35 and 10.3±0.26 μmol/l respectively (Fig. 5).



'Fig. 5 - Changes in copper content in the blood of cows
with anemia for the influence of Microlact
Note: BE - beginning of experiment; EE - end of experiment.

At the end of experiment85 % of cows in control group had reduced level of copper. Copper blood content In increased in cows from the first experimental group on 23.2 % comparing to the beginning of experiment, on 17.0 % comparing to control group (P<0.001). In the second experimental group on 33.6 % and 23.7 % (p<0.001) respectively. Bedises in cows from the second group, the copper content was 8.4% higher in comparison with the first group (Fig. 5).

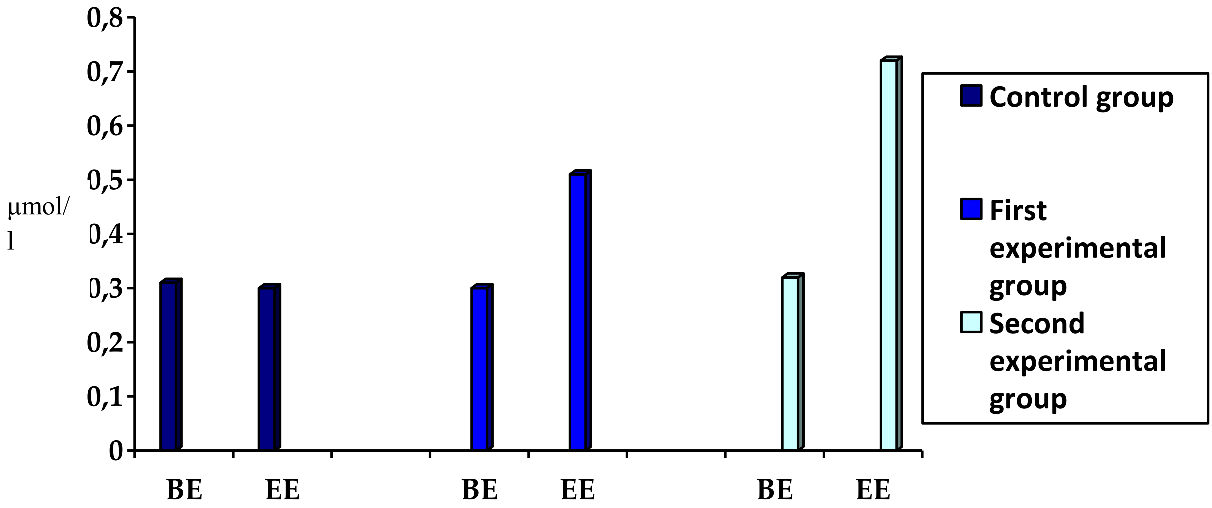


Fig. 6 - Changes in cobalt content in the blood of cows
with anemia for the influence of Microlact
Note: BE - beginning of experiment; EE - end of experiment.

The main changes were found in cobalt content (Fig. 6). At the beginning of experiment the maximum content of cobalt did not reach even minimum physiological limit (0.50 µmol/l) in all groups. At the end of experiment low cobalt level was determined in 100 % of cows from control group (Fig. 6), and the maximum level did not even reach 0.36 µmol/l. In the first experimental group cobalt blood concentration became 1.7 times higher (P<0.001) comparing to the beginning of experiment, average ranged 0.411 - 0.669 µmol/l (0.51±0.016) and in 60 % of cows was higher than 0.50 µmol/l. It was significantly higher (70 % ; p<0.001) comparing to control group. The average cobalt blood content was 0.72±0.013 µmol/l in the second experimental group. This was 2.3 times higher comparing to the beginning of experiment; 2.4 times - control group and 1.4 times - first experimental group (P<0.001).
Adding microelements to the ration of cows increases cobalt and copper blood levels to physiological and eliminates the symptoms of anemia.

Discussion

The distribution of heavy metals has become so large that they are already decisive environmental factors in many ecosystems (Uetani et al., 2005; Gutyj et al., 2017). It is known that many of the chemicals that are used systematically and continuously on organisms, 28 are of the most dangerous chronic long-acting toxicants, and among them, along with known organic solvents, pesticides and nitrogen and sulfur oxides, the heavy metal salts occupy a significant place (Liu et al., 2008; Khariv et al., 2016).
Increase in the arrival of heavy metals in the natural environment from anthropogenic sources and as a result of violations of the cycle of mineral compounds of lithosphere and hydrosphere led to an increase in their content in the biosphere and organisms, which leads to a decrease in the productivity of ecosystems and poses a potential danger to humans and animals (Eichenberger, 1982; El -Shahat et al., 2009). By the scale of pollution and action on biological objects, heavy metals occupy a special place among the pollutants. Under certain conditions, many of them are required for living organisms as microelements, but as a result of intensive atmospheric dispersal in the biosphere and significant concentration in the soil, they become toxic to the biota (Salvatori et al., 2004).
The importance of research related to heavy metals is due, on the one hand, to the increasing volumes of these metals entering the environment as a result of human activity, and on the other hand – the harm they inflict on living organisms.

The research determined increased content of leat and cadmium in soil compariny to the maximum permissible concentrations and the proportion of available elements increases due to the acidic reaction of soil. The available cadmium content in soil decreased with increasing distance from the source of technogenic pollution more intensively (13 km - 2.5 times) than lead (only content 1.3 times). In addition to cadmium and lead nickel and copper concentration also excuded the maximum permissible contents. This contributes to the excessive accumulation of cadmium and nickel in hay, lead and nickel in root crops (4 times more than the maximum permissible concentrations). The coefficient of cumulation of cadmium in hay is 0.73-1.1, nickel – 0.95-1.0, lead – only 0.083.
Fodder is the main source of heavy metals incorne in the body of animals. The blood levels on cows located near mine (Grybovytsia and Bilichi villages) was the cadmium 12 and 10 times higher than in the control group, at distances 12-14 km – 4.3-6.3 times (2.14±0.110-0.72±0.36 µmol/l, in control zone cows – 0.17±0.010); - 1.43-3.96 times excudet the parameters in control zone.
Intoxication of cows by Plumbum and Cadmium causes a complex negative influence on various parameters of their organism. Many studies are devoted to the study of influence of heavy metals on the blood composition, protein, carbohydrate, lipids metabolism, energy status, tissue respiration and enzymatic activity of the main ways of energy supply, etc. (Liu et al., 2008; Gutyj et al., 2016; Vishchur et al. , 2016; Gutyj et al., 2017).
The negative effect of heavy metal ions on the organism is appered both on molecular and metabolic levels (Gutyj et al., 2017; Grynevych et al., 2018). Bioaccumulation determines the rate of metal flow to the body and its removal outside. However, it depends on the binding ability of tissues and cellular structures. As a result of the interactions complex of metals accumulation and distribution is specific process, depending on time and nature of metal. Features of the dependence of metal accumulation varies from their concentration. The degree of permeability of metal ions depends on their overall concentration and forms in the environment and organism. The composition of the blood plasma, the content of formed elements, and the structural and functional integrity of hemoglobin system, as is known, determine the functional and adaptive capacity of animal organism (Gutyj et al., 2016; Martyshuk et al., 2016; Khariv et al., 2017). Therefore, these indicators can detect the degree of toxicants danger. Toxicants increase the sensitivity of hemoglobin to denaturing agents, and also reduce its affinity to oxygen.
Cumulation of cadmium and lead in the body causesnadverse negatively affects on hemopoiesis: in 38 (95 %) of 40 cows, oligochromia was established, and in 21 cows (52.5%) it was combined with oligocyteemia. There is negative correlation of medium degree between the number of erythrocytes and and cadmium blood level, between the lead content and hemoglobin. In 73.7 % of cows normochromic anemia was established, in 23.7 % – hypochromic and only in 2 % – hyperchromic, mostly normocytic (86.8 %). According to the etiology the anemia in cows under the condition of technogenic pollution by lead and cadmium is hypoplastic due to effect myelotoxic, bone marrow (Sukmans'kiy, 2000).
The population composition and acid resistance of erythrocytes also change under the influence of heavy metals. In particular, the relative proportion and absolute number of "young" forms of erythrocytes, as well as the absolute number of "mature" cells decreases, while the number of "old" increases. Reducing the number of "young" erythrocytes is the result of disturbed hematopoiesis in the bone marrow. An increase in the proportion of "old" forms can be explained by a more intense course of their oxygenation processes. The lipid peroxidation in such cells increases, they reduce the ability to synthesize macroergic compounds and transport amino acids (Paranyak et al., 2007).
Erythrogram of cows in the area of technogenic pollution is characterized by earlier growth of main peak and complete completion of hemolysis on 5.5-6.0 minute (in the control group of cows – 7.5 minute), indicating a decrease in the resistance of erythrocytes to the influence of acid hemolytics, and therefore – to changed biochemical structure of their membranes (Slivinska et al., 2018).
Copper and cobalt levels in blood of cows from area of technogenic pollution is decreased. There is a negative correlation between the contents of copper and cadmium (r=-0.53), copper and lead (r=-0.60). Low copper blood level in combination with excessive concentration in the soil can be explained by a violation of its absorption by plants.
For the purpose of treatment and prevention of anemia, we tested the developed scheme, incending the use of drug “Microlact” - contains lactates of cobalt, copper, zinc, manganese, ferrum, sodium selenite on trilon. The experiment lasted 45 days.
The results of treatment show a tendency of restory hemopoiesis in experimental cows. Blood copper level increased on 23.2 %, and cobalt concentration was 1.7 times higher comparing to the initial level.

The best therapeutic effect was observed in animals of the second experimental group (receives Microlact) which maximally contributed to growth of cobalt and copper content in the blood of cows and positively influenced morphological parameters, hemoglobin content and hematocrit.

Conclusion

In 95% of cows from area of technogenic pollution with cadmium and plumbum develops normocytic anemia. A negative correlation between the number of erythrocytes and cadmium (r=-0.448), plumbum (r=-0.434) content, between hemoglobin and cadmium level (r=-0.64), hemoglobin plumbum lead content (r=-0.604) was established. The number of "old" erythrocytes in the blood of cows the increases population of “young” decreases. Adding “Microlact” to the diet of cows increases cobalt and copper blood levels to the physiological parameters and climnanates symptoms of anaemia.

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