ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

# Reviewing The Influence Of Copper, Lead And Zinc Accumulation On The Morphofunctional Liver And Kidney State In Broiler Chickens Under Experimental Toxicosis.

**Irina M Donnik[1]*, Olga G Loretts[1], Nikolai V Sadovnikov[2], Anatoly D Shusharin[2], Alexander V Elesin[2], Natalia N Semenova[3], and Mikhail I Barashkin[1].**

[1]Grand PhD in Biological sciences, Academician of Russian Academy of Sciences; Federal State Budget Educational Institution of Higher Education "Ural State Agrarian University", 42, Karla Libknehta St., Yekaterinburg, Sverdlovsk region, 620075, Russian Federation.
[2]Grand PhD in Veterinary sciences, Federal State Budget Educational Institution of Higher Education "Ural State Agrarian University", 42, Karla Libknehta St., Yekaterinburg, Sverdlovsk region, 620075, Russian Federation.
[3]Academic secretary, Federal State Budget Educational Institution of Higher Education "Ural State Agrarian University", 42, Karla Libknehta St., Yekaterinburg, Sverdlovsk region, 620075, Russian Federation.

**ABSTRACT**

Modeling of liver and kidneys toxic injury in broiler chickens in the early period of post-natal development can contribute to the studying of Cu, Pb and Zn salt mixture influence on the structure-functional formation of organs. Morphologically toxic hepatitis is manifested by hepatocyte dystrophic damage and necrosis, necrosis of kidney glomerular apparatus and tubules dystrophy. The purpose of the work was to study dynamics of structure-functional disturbances in the liver and kidneys using the model of the experimental toxicosis caused by addition of heavy metals salts mixture to a diet.
**Keywords:** broilers, heavy metals salts in feeding, morphofunctional disturbances.

***Corresponding author***

ISSN: 0975-8585

## INTRODUCTION

At the initial stages of toxic hepatitis, leukocyte infiltration of intralobular stroma is observed, but at the same time, there is activation of sinusoidal cells followed by alteration and inflammation processes in the liver, and macrophage activity increase influence the intensity of cell regeneration. Thus, counteraction of chickens' organism to the actions of fodder toxic components results in the structure-functional change in the formation of internals, and delay of growth and development of broilers are the external manifestation of this disorder.

## MATERIALS AND METHODS

The experiment was performed in broiler chickens of "Smena" cross at different age periods (7, 14, 28, 38, 40, 42 days). The chickens were kept in specially allocated cells in the conditions of a poultry farm, fed and bred according to the standards provided by the period of chickens' life. The first control group of chickens received a fodder diet adopted in the poultry farm, and the second, test group in addition to the diet received metal salt mixture consisting of trihydrate lead acetate in amount of 3.9 g for a group of 60 chickens; zinc sulfate – 12.8 g and copper sulfate – 2.05 g for a group of 60 chickens. The salt mixture was added to the combined chickens' feed beginning from the 7 day of age by preliminary mixing and main final mixing with the combined feed. The period of chicken feeding, with addition of heavy metal salts, was 33 days from the beginning of the first feeding. Blood and tissue samples of the internals were taken from 5 chickens in each age period on 14, 28, 38 and 42 days of chicken lives. Internals tissues were taken from 5 chickens from the control group at the same age periods of their life, as from the test group chickens. They were anesthetized and decapitated. The organs under study were fixed in formalin solution, then the tissue sections were embedded in paraffin, prepared in a microtome MPS-2 and stained by hematoxylin and eosin.

Pre-treatment of chicken organ samples was carried out in the poultry farm laboratories and then definition of Pb, Cu, Zn concentration was made on an atomic-absorbing spectrometer AAS Vario 6 Analytik Jena AJ (Germany). Errors in concentration of elements in organ tissues, their mean values were determined using the one-way analysis of variance, differences between the groups were considered to be reliable at $p \leq 0.05$.

## RESULTS

The liver of the test group chickens, whose main diet was added with the salt mixture, consisting of heavy metal salts, showed copper level increase up to 11.9±0.1mcg/g in organ tissues 14 days after the salt mixture addition to the broilers' diet compared to the copper level in the liver tissue of the control group chickens (10.4±0.1 mcg/g). Copper concentration in the liver significantly increased with chicken age from 7.2±1.0 mcg/g to 11.9±0.4 mkg/g, $p \leq 0.05$, and on day 38 the content of this metal in the liver tissue reached 14.8±0.5 mkg/g comparing with the control group chickens of the same age (12.0±0.6 mcg/g, $p \leq 0.05$).

On day 41 the copper content in the liver tissue of the test group chickens slightly decreased to 11.9±0.7 mkg/g, but was significantly higher than in the control group chickens 9.2±0.5 mcg/g, $p \leq 0.05$.

Thus, copper maximum accumulation in the liver tissue of the test group chickens was registered on day 38 (14.8±0.5 mcg/g) and difference in dynamics of copper accumulation in intact chickens was insignificant (copper entered the chicken organism from the main diet which contained the same amount of copper as in the test group, 10 mg/kg) 12.0±0.6 mcg/g, and by 41 day of life of chicken from the control group its contents decreased to 9.2±0.5 mcg/g, $p \leq 0.05$. In addition, in chickens from the test group the amount of metal was at the level of-11.93±0.72 mcg/g.

Such copper distribution in the liver tissues of the test and control group chickens is probably connected with their growth and development, as well as the maximum development of organs (structure-functional organization) was apparently followed by a high need in nutrients, oxygen, provided by the increased volume of blood flow and active microcirculation in liver tissues at this age. By the end of the chickens' liver structure-functional organization, 41 day of life, the spontaneous accumulation of copper in the liver tissues of both the control and test group chickens significantly decreased to 9.2±0.5 mcg/g and 11.9±0.7 mcg/g, $p \leq 0.05$, respectively. The chickens had also quite close dynamics of copper accumulation in renal

tissue, both in the test and control groups. Thus, in the test group chickens' kidneys copper content was assessed at the age of 28 days – 11.9mcg/g; in the control group chickens of the same age the copper content in their kidneys made 7.2±1.0 mcg/g. On the 40 day of life in the test group chickens, the copper level in their kidneys was within 11.2±0.2 mcg/g, while in the control group chickens, this figure raised to 9.6±0.3 mcg/g, p≤0.05, in comparison with the copper level in the kidneys of the control group chickens, that is 7.19±1.02 mcg/g).

On 41 day of supervision over the test group chickens, copper concentration decreased in the renal tissue 7.41±2.12 mcg/g, p≤0.05, in comparison with the copper content in the renal tissue of the test group chickens at the age of 38 days and in the control group chickens at the age of 41 days, the copper content in their kidneys decreased to 6.3±0.3 mcg/g. At the same time, the general tendency to a gradual decrease in copper content in the control and test group chickens' kidneys was registered with age (Table 1).

**Table 1: Copper content in chickens' parenchymal organs (mcg/g dry weight)**

| The organ studied | Control group | Test group | P |
|---|---|---|---|
| 28-day-old chickens | | | |
| Liver<br>kidneys | 10.23±0.14<br>7.19±1.02 | 11.41±0.67<br>11.96±0.42 | p>0.05<br>p<0.05 |
| 38-day-old chickens | | | |
| Liver<br>kidney | 12.00±0.64<br>9.57±0.30 | 14.80±0.51<br>11,20±0,20 | p<0.05<br>p<0.05 |
| 41-day-old chickens | | | |
| Liver<br>kidneys | 9.18±0.53<br>6.32±0.27 | 11.93±0.72<br>7.41±2.12 | p<0.05<br>p<0.05 |

Thus, the liver lead level was significantly high in the test group chickens at the age of 28 days (6.2±0.5 mcg/g) comparing with its content in the control group chickens (4.5±0.7 mcg/g, p≤0.05). In 24 days of feeding chickens with additives, containing heavy metal salt mixture, its content in the liver of the test and control group chickens showed nether considerable changes, nor a decrease (Table 2). In the test group chickens, its amount was at the level of 5.82±1.22 mcg/g, and in intact chickens, lead concentration in the liver even tended to decrease up to 2.9±0.7 mcg/g, p≤0.05. By day 41 the liver lead content in the control group chickens decreased even more up to 1.14±0.30 mcg/g, while in 41-day-old chickens from the test group it remained at the level of 5.7±0.7 mcg/g, i.e. just like at the previous age periods of chickens' life. Lead accumulation in the renal tissue shows the most contrast picture of its distribution in chickens of various age groups, in comparison with its accumulation in the liver tissue. Thus, in 14 days of feeding the chickens with heavy metal salts mixture the renal lead concentration significantly increased to 15.3±2.1 mcg/g, p≤0.05, in comparison with its concentration in the renal tissue of the control group (4.8±1.3 mcg/g). In 24 days of feeding the chickens with the addition of heavy metal salts mixture to their diet the quantitative index of this element was maximally increased up to 22.4±0.7 mcg/g, p≤0.01, whereas in the control group chickens this parameter remained at the level of 5.1±0.8 mkg/g. The same level was observed in the intact chickens' kidneys following 14 days after the introduction of heavy salt metals, together with the main diet into their organisms. At the age of 41 days during 27 days of observation beginning from the introduction of heavy metal salts to their diet, the amount of lead in the chickens' kidneys remained rather high - 14.4±0.5 mcg/g, in comparison with its content in the control group chickens' kidneys – 1.9±0.3 mcg/g, p≤0.01. In general, in this age group its concentration decreased in the control group chickens, which, apparently, spontaneously received this element from the combined feed. This element was absent, though, in the fodder due to the data of the laboratory analyses.

**Table 2: Content of lead in chickens' parenchymal tissues (mcg/g dry weight)**

| The organ studied | Control group | Test group | P |
|---|---|---|---|
| 28-day-old chickens | | | |
| Liver<br>kidney | 4.50±0.72<br>4.87±1.33 | 15.33±2.10<br>6.21±0.47 | p<0.01<br>p<0.05 |
| 38-day-old chickens | | | |
| Liver | 2.90±0.65 | 5.82±1.22 | p<0.05 |

| | | | |
|---|---|---|---|
| kidney | 5.10±0.85 | 22.38±0.74 | p<0.001 |
| 41-day-old chickens | | | |
| Liver<br>kidney | 1.14±0.30<br>1.91±0.32 | 5.70±0.68<br>14.44±0.45 | p<0.05<br>p<0.01 |

In 28-day-old chickens, high zinc concentrations were found in the liver – 166.17±16.76 mcg/g, in comparison with the content of other metals, copper and lead, in the liver tissue. With age, zinc concentration in the liver tissue decreased in the control group chickens and was at the level of -149.67±16.69 mkg/g in 38-day-old chickens and at the level of 130.85±15.77 mcg/g, (P <0.02) in 41-day-old chickens.

Significantly less zinc concentrations - 110.43±7.21mcg/g, (P <0.02) were found in the renal tissue of the intact 28-day-old chickens, comparing with that in the liver of chickens of the same age. By 38 day of life of the control group chickens the zinc content in their kidneys was significantly higher (126.29±11.04 mkg/g), than its contents in the kidneys of 28-day-old chickens. By 41 day of life the zinc content (Table 3) significantly decreased (114.17±11.12 mkg/g), (P <0.05), in comparison with its content in the kidneys of the previous age group chickens. Comparing the obtained data of the zinc content in the kidneys and liver of the control group chickens from three age groups, we can make a conclusion that in the liver and kidney tissue of the same age groups of chickens, but receiving a high concentration of heavy metal salts mixture together with the basic diet, zinc concentration in the liver did not increase in the 28-day-old test group chickens (152.92±18.21 mcg/g). In 38-day-old chickens from the test group, the zinc content increased to (164.75±12.43 mcg/g), (P <0.05), and in 41-day-old chickens the liver zinc content remained at the same level (157.54±7.14 mcg/g), and slightly increased in comparison with its content in the liver of the control group chickens.

**Table 3: Content of zinc in chickens' parenchymal organs (mcg/g dry weight)**

| The organ studied | Control group | Test group | P |
|---|---|---|---|
| 28-day-old chickens | | | |
| Liver<br>kidney | 166.17±16.76<br>110.43±7.21 | 152.92±18.21<br>104.34±11.60 | p>0.05<br>p>0.05 |
| 38-day-old chickens | | | |
| Liver<br>kidney | 149.67±16.69<br>126.29±11.04 | 164.75±12.43<br>134.52±7.42 | p<0.05<br>p>0.05 |
| 41-day-old chickens | | | |
| Liver<br>kidney | 130.85±15.77<br>114.17±11.12 | 157.54±7.14<br>122.13±6.71 | p>0.05<br>p>0.05 |

**Morphological changes in the liver of 28-day-old test group chickens** showed a significant focal interstitial swelling; the portal path vein plethora with vascular thrombosis. Besides preportal lymph node infiltration and macrovesicular liver steatosis were found.

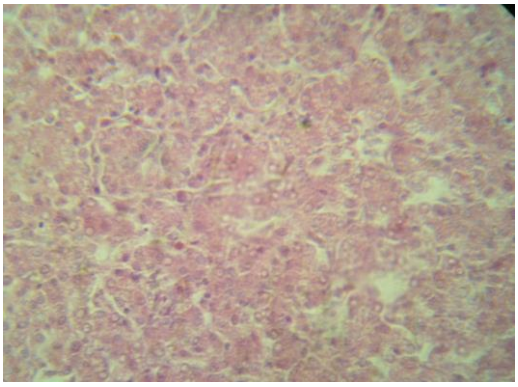

**Fig 1: Liver 28-day-old test group chickens. Liver sinusoidal dilatation and macrovesicular liver steatosis. Staining by hematoxylin and eosin, x400**

ISSN: 0975-8585

The studied material taken from the control group chickens at the age of 28 days provided us with the data about the formation of lymphoid follicles, plethora of the central veins in the liver tissue, perivascular swelling, an active proliferation of endothelial vessels and granular-fatty dystrophy of hepatocytes.

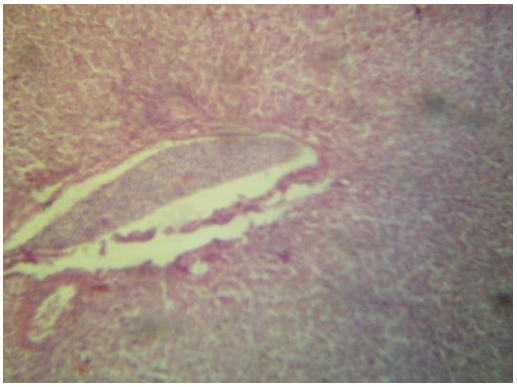

**Fig 2: Liver 28-day-old chickens, control group. Perivascular swelling, endothelial vessel proliferation. Staining by hematoxylin and eosin. x100**

**Morphological changes in the kidneys of 28-day-old test group chickens** showed that the cortical and marrow substance was differentiated. There is a considerable number of glomeruli of embryonic type in the cortical substance. There is a focal plethora of sinusoidal vessels. Focal hemorrhages and widespread sites of wavy tubule epithelium necrosis can be observed. The kidney histological analysis showed that in 28-day-old control group chickens cortical and marrow substances were differentiated; and glomeruli of embryonic type were found under the capsule. There was also a focal desquamation and epithelial necrosis of wavy tubule epithelium.

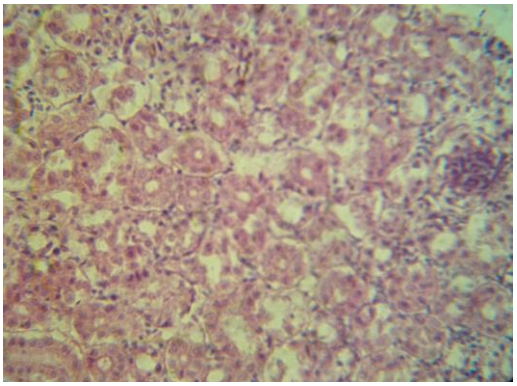

**Fig 3: Kidney 28-day-old control group chickens. Focal desquamation and necrosis of wavy tubule epithelium. Staining by hematoxylin and eosin. X200**

**Morphological changes in the liver of 38-day-old test group chickens**. The conducted histological examination revealed widespread hemorrhages in the liver parenchyma, congestive hyperemia of vascular microvasculature, pigmentation disturbance, granular-fatty dystrophy, hepatocyte focal necrosis, as well as thromboses of multifarious vessels.

ISSN: 0975-8585

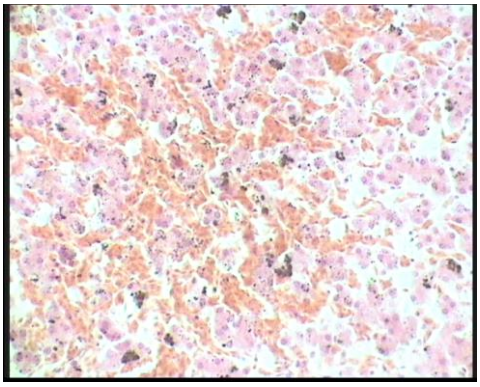

**Fig 4: Liver 38-day-old test group chickens. Widespread hemorrhages in the liver. Hemosiderosis Staining by hematoxylin and eosin. X400**

**Chickens from the control group** of the same age showed insignificant hemorrhages and venous hyperemia of liver vessels. Some chickens revealed perivascular swellings, with no other serious disorders found.

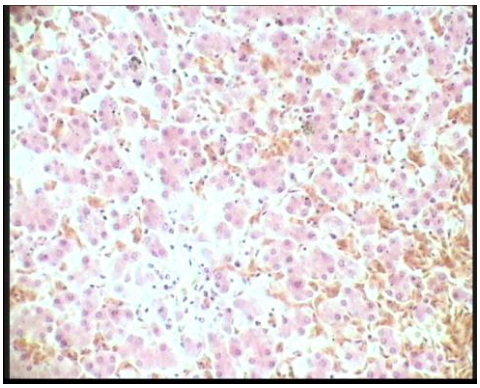

**Fig 5: Liver 38-day-old control group chickens. Granular-fatty dystrophy, congestive hyperemia of the liver vessels. Staining by hematoxylin and eosin. X200**

**In 38-day-old chickens' kidneys** an interstitial swelling was found with the formation of cystic cavities. Hyperemia of the kidney vessels and blood clot formation were observed. In the majority of the liver tissue samples from the test group chickens, focal formation of epithelial cylinders, hemorrhage, Bowman-Shumensky capsule deformation up to their break were registered. Histological examination of all five chickens' kidneys revealed focal neurosis of wavy tubules.

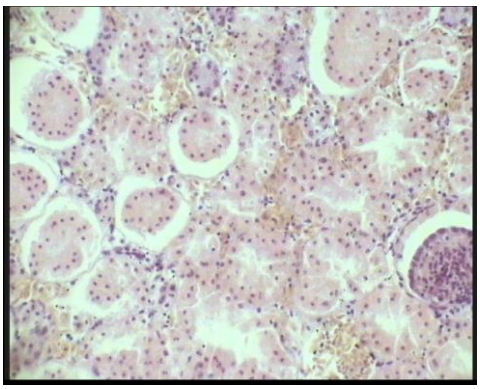

**Fig 6: Kidney. 38-day-old test group chickens. Congestive kidney vessel hyperemia and formation of epithelial cylinders. Staining by hematoxylin and eosin. X h400**

ISSN: 0975-8585

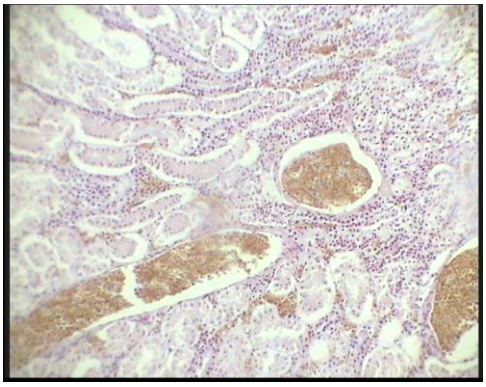

**Fig 7: Kidney. 38-day-old control group chickens. Stagnant hyperemia of kidney vessels. Staining by hematoxylin and eosin. X200**

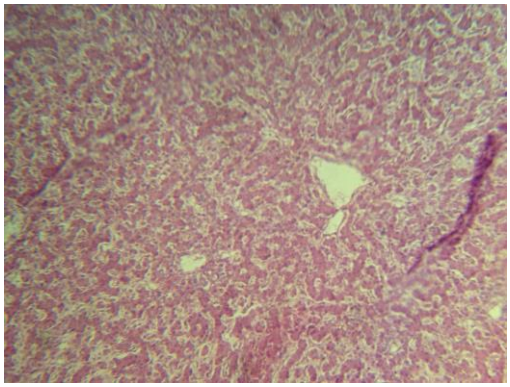

**Fig 8: Liver 41-day-old control group chickens. Congestive hyperemia of the microvasculature is found. X100**

**Morphological changes in the liver of 41-day-old control group chickens.** A moderately expressed interstitial swelling with the sinusoidal space dilation, as well as focal plethora of the central veins and portal ways, can be seen. Congestive hyperemia of the microvasculature is found. Hepatocytes remain unchanged. Periportal infiltrates are noted in two cases.

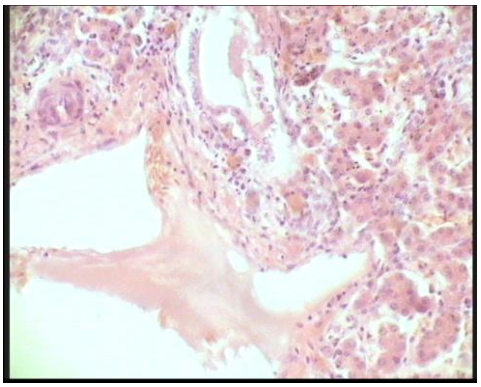

**Fig 9: Liver 41-day-old test group chickens. Thrombus formation, desquamation of the bile duct epithelium. Staining by hematoxylin and eosin. X400**

**Morphological changes in the liver of 41-day-old test group chickens** revealed the interstitial swelling being more expressed in comparison with that in the control group. Focal fatty dystrophy of hepatocytes, hemosiderosis, swelling around the vessels and bile ducts are noted. Bile duct epithelial desquamation and blood vessel obliteration are seen. Central and portal veins are full-blooded. Focal growth of connecting tissue and periportal necrosis of hepatocytes with cellular reaction are found, and in some chickens of this group progressive necrosis of hepatocytes can be observed.

ISSN: 0975-8585

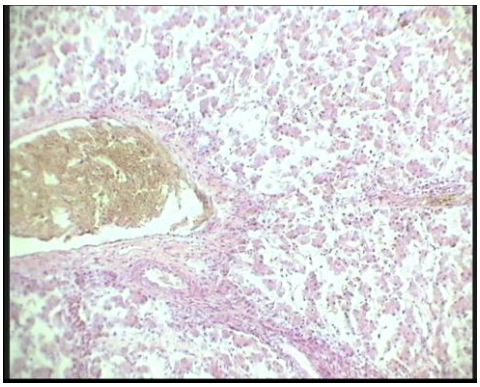

**Fig 10: Liver 41-day-old test group chickens. Thrombus formation, hepatocyte necrosis, growth of the connective tissue. Staining by hematoxylin and eosin. X100.**

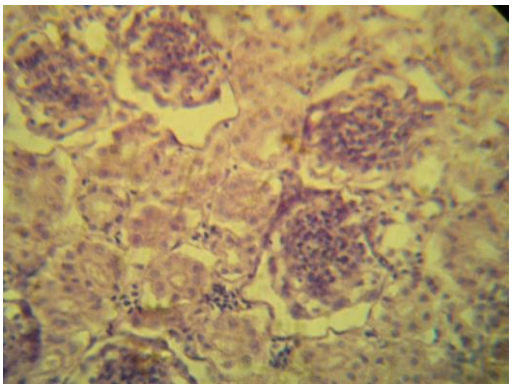

**Fig 11: Kidney 41-day-old test group chickens. Deformation of the glomeruli capsule, glomeruli swelling. Staining by hematoxylin and eosin. X400.**

**Morphological changes in kidneys of 41-day-old control group chickens.** Glomeruli of the embryonic type are found in the kidneys under the capsule. The tubular epithelium is not changed; proteinosis is registered only in several cases.

**Morphological changes in the liver of 41-day-old test group chickens.** There are focal deformations and necrosis of tubular capsules with cellular reaction observed, along with glomeruli swelling. Capillary sinuses are full-blooded. A moderately expressed interstitial swelling of the parenchyma, focal desquamation and epithelial necrosis of the kidney wavy tubules are noted. Formation of cystic cavities is noted. Around large vessels, there is a fat tissue deposition. Inflammation foci are noticeable.

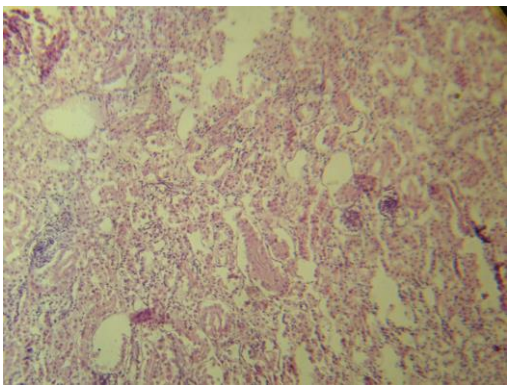

**Fig 12: Kidney 41-day-old test group chickens. Focal necrosis of the wavy tubule epithelium and formation of cystic cavities. Staining by hematoxylin and eosin. X100.**

ISSN: 0975-8585

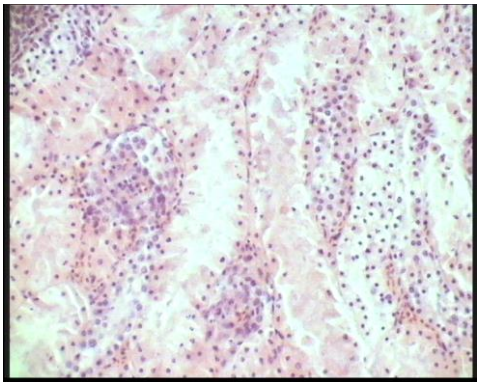

**Fig 13: Kidney. 41-day-old test group chickens. Focal necrosis of the wavy tubule epithelium. Staining by hematoxylin and eosin. X400.**

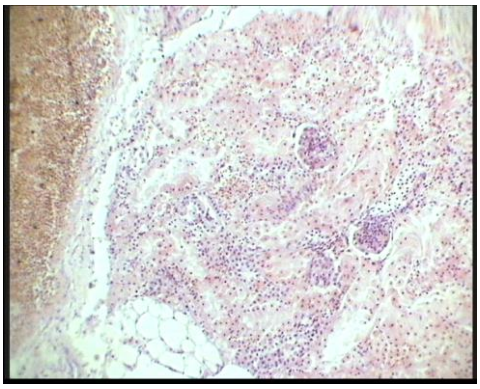

**Fig 14: Kidney 41-day-old test group chickens. Perivascular fatty infiltration. Staining by hematoxylin and eosin. X200.**

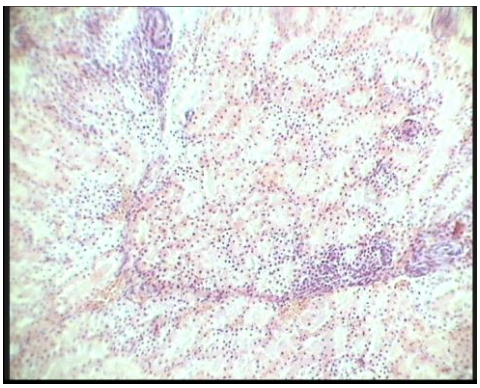

**Fig 15: Kidney 41-day-old test group chickens. Inflammation foci. Staining by hematoxylin and eosin. X200.**

## DISCUSSION

### Chicken biochemical blood analysis

Direct and indirect entering of technogenic pollutants of various nature into the human body provokes a large group of diseases and causes development of the accompanying disturbances of the body functions (14). Heavy metals enter the human body in various ways including alimentary one with food stuffs (Vyaizenen G.N. 2004, Madan V.J. and Stafigur R. 2007). Production of poultry products, free of substances toxic to the human body, is impossible without its complex analysis at all the stages of technological process. Complex evaluation of chemical pollutant structure and concentration in poultry products should, in our

opinion, consist at least of three stages: intravital examination of birds with carrying out a laboratory analysis of biological fluids, postmortem examination of meat, by-products, an analysis of the finished goods in compliance to sanitary standards. It is necessary to note that poultry is quite tolerant to high doses of zinc and copper. During feeding there were no clinical signs of intoxication in chickens receiving high doses of Zn, Cu, Pb salts, though a decrease in average daily weight gain and growth retardation in the chickens was observed by 28-32 days. The fact was also mentioned by other authors (Madan V.J., Stafigur R. 2007). At the age of 28 and 38 days, the samples of blood serum were taken in the chickens for a biochemical analysis. Some indexes of protein, carbohydrate, mineral exchanges and enzyme activity, both in the intact and test group chickens, were determined in blood serum.

The total protein level in 28-day-old chickens' blood serum was 34.7 g/l, globulins of 29.3 g/l thus exceeding similar figures in the control group (31.1 and 27.2 respectively). This tendency was also observed in 38-day-old chickens, when total protein in the blood serum of the test group chickens (49.3) increased significantly in comparison with the content of total protein in the blood serum in the control group chickens (36.9 g/l). In addition, globulin fraction in the blood serum of the test group chickens was high-increasing (41.6 g/l) in comparison to the content of globulin in the serum of the control group chickens (34.5 g/l) (Table 8). This increase in globulin at primering with heavy metals is possibly connected with the fact that during a dystrophic processes development autoimmune processes are triggered – autoantibodies synthesis against the body own autoantibodies, but changed membranes of hepatocytes. Besides, the injured hepatocytes begin to synthesize paraglobulins, which are discharged into the blood, thus providing their increase in the blood of the test group chickens. The increase in total protein level is probably connected with the increase of globulin concentration.

Unlike in other animals, uric acid is a product of protein metabolism in birds, which considerably exceeds the urea level. Its amount considerably increases in chickens from the test group, both at the age of 28 days and 38 days – 601.8 mcmol/l and 612 mcmol/l, thus showing the increased purine exchange at disintegration of nucleic acids in tissue cells, and also it can be connected with the reduced protein metabolism in cells and hepatocytes injury, caused mainly by accumulation of lead and its damaging action which led to the synthesis of paraglobulins in hepatocytes. Besides, the injured hepatocytes could activate T-dependent cellular reactions, antigenic properties increases in them and autoimmune liver injuries developed. The latter resulted in substantial increase of globulin fraction content in the blood serum in the test group chickens at the age of 38 days (Table 5), which is quite characteristic for the chickens from the test group.

Significant differences are noted in the enzyme exchange parameters. Thus, at the age of 28 days the test group chickens had in their blood serum ALT 6.6, MU/l, AST 193.6 MU/l, amylase 836.6 MU/l, glutamyl transferase 15.5 MU/l, lactate dehydrogenase 2395 MU/l, creatinine kinase 2531.6 MU/l and lipase 70.8 MU/l, whereas in the blood serum of the control group chickens these figures were ALT 8.6 MU/l, AST 174.6 MU/l, amylase 492 MU/l, glutamyl transferase 13 MU/l, lactate dehydrogenase 1638 MU/l, creatinine kinase 1630 MU/l and lipase 57 MU/l.

**Table 4: Biochemical indicators of blood serum chicken broilers 28**

| | | | |
|---|---|---|---|
| Total protein (g/l) | 30.8±2.15 | 34.6±1.96 | P<0.05 |
| Albumin (g/l) | 5.2 ± 0.2 | 6.2 ± 0.37 | P<0.05 |
| Globulins (g/l) | 25.6 ± 1.96 | 29.0 ± 1.73 | P<0.05 |
| Glucose (mmol/l) | 9.92 ± 0.33 | 12.76 ± 0.87 | P<0.05 |
| Cholesterol (mmol/l) | 2.49 ± 0.2 | 2.56 ± 0.16 | P>0.05 |
| Triglycerides (mmol/l) | 0.79 ± 0.05 | 0.91 ± 0.08 | P<0.05 |
| Creatinine (mcmol/l) | 15.75 ± 0.25 | 19.00 ± 0.63 | P<0.05 |
| Urea nitrogen (mmol/l) | 0.82 ± 0.05 | 0.76 ± 0.04000 | p>0.05 |
| Uric acid (mcmol/l) | 546.6 ± 51.30 | 602.0 ± 44.65 | p>0.05 |
| ALT (MU/l) | 7.2 ± 1.2 | 6.0 ± 0 | p>0.05 |

| | | | |
|---|---|---|---|
| AST (MU/l) | 177.0 ± 11.98 | 193.4 ± 8.71 | p>0.05 |
| Amylase (MU/l) | 489.6 ± 21.48 | 836.84 ± 98.60 | P<0.05 |
| Glutamyl transferase (MU/l) | 13.4±0.75 | 15.4±0.92 | P<0.05 |
| Lactate dehydrogenase (MU/l) | 1785±146.17 | 2494.4±109.37 | P<0.05 |
| Creatinine kinase (MU/l) | 1354.2±288.98 | 2531.8±205.75 | P<0.02 |
| Lipase (MU/l) | 55.4±2.29 | 70.6±9.61 | p<0.02 |

Studying the fine mechanisms of lead adverse influence on organs and systems, many authors established the lead enzymopathic effect (Dlin V.V. 1997; Ershov Yu. A.1989). Binding with sulfhydryl, carboxyl and amine groups of the active centers of a number of enzymes and thus inhibiting them, the specified polytropic poison damages cellular, mitochondrial, lysosomal membranes, causing deterioration in energy metabolism and oxidation-reduction processes in different organs and tissues. Lead causes damage of the respiratory chain and disturbance of the tricarboxylic acid cycle in mitochondria and other disturbances that cause tissue hypoxia, which results in inability of cells to utilize oxygen. Most often, it is the ability of lead to inhibit enzymes that underlies pathologies of hematopoietic, cardiovascular and other systems.

A characteristic symptom of poisoning with heavy metals in our experiment was the increase in amylase activity in the test group chickens. Changes of the enzyme content in the chickens' blood serum show the lead cytotoxic effect on cells of the heart, liver and kidneys.

**Table 5: Biochemical blood serum parameters in broiler chickens at the age of 38 days**

| Parameter | Control group | Test group | Student's test |
|---|---|---|---|
| Total protein (g/l) | 35.75±1.38 | 49.40±1.63 | P<0.05 |
| Albumin (g/l) | 5.60 ± 0.24 | 7.60 ± 0.24 | P<0.05 |
| Globulins (g/l) | 29.75 ± 1.18 | 41.80 ± 1.56 | P<0.05 |
| Glucose (mmol/l) | 1.41± 0.41 | 11.69± 0.69 | P<0.05 |
| Cholesterol (mmol/l) | 2.87± 0.14 | 3.25± 0.12 | P>0.05 |
| Triglycerides (mmol/l) | 0.96±0.05 | 1.08±0.24 | P<0.05 |
| Creatinine (mcmol/l) | 20.00±1.41 | 21.20± 1.11 | P<0.05 |
| Urea nitrogen (mmol/l) | 0.64±0.05 | 0.80±0.05 | p>0.05 |
| Uric acid (mcmol/l) | 353.75±6.62 | 666.80±68.72 | p>0.05 |
| ALT (MU/l) | 6.0±0 | 6.0 ± 0 | p>0.05 |
| AST (MU/l) | 215.40± 26.09 | 193.4 ± 8.71 | p>0.05 |
| Amylase (MU/l) | 421.80±79.35 | 603.60±49.89 | P<0.05 |
| Glutamyl transferase (MU/l) | 18.2±1.46 | 18.6±2,46 | P<0.05 |
| Lactate dehydrogenase (MU/l) | 2402.4±462.41 | 2059±613.53 | P<0.05 |
| Creatinine kinase (MU/l) | 3198±401.79 | 4221,.4± | P<0.02 |
| Lipase (MU/l) | 40.2±4.8 | 80.8±18.15 | p<0.02 |

Chronic intoxication of chickens' organism is also characterized by an increase of nonheme iron in the blood serum. The studies performed also testify inefficiency of erythropoiesis caused by lead. All results indicate the mediated hematotoxic influence of lead; it, most likely, inhibits the erythrocyte active systems of protection against the effects of peroxidates on membranes with subsequent hemolysis of a cell (S. Ribarov et al. 1980). The blood shows a hemoglobin decrease that, perhaps, is connected with the mediated effect of the lead suppressing exchange of cobalt and copper - stimulators of B12 vitamin synthesis and, respectively, hemoglobinpoiesis.

Calcium, magnesium and phosphorus levels in the blood serum of the test group broilers were practically the same as in the control group ones throughout the experiment.

**Table 6: Content of mineral substances in blood serum of broiler chickens 28 days**

| Parameters | Control group | Test group | Student's test, p |
|---|---|---|---|
| Calcium, mmol/l | 1.288±0,03 | 1.528±0,03 | p >0.05 |
| Magnesium,mmol/l | 1.022±0,12 | 1.1±0,05 | P>0.05 |
| Phosphorus, mmol/l | 2.24±0,13 | 2.404±0,10 | P>0.05 |
| Nonheme iron, mcmol/l | 16.92±1,50 | 19.48±2,85 | p <0.05 |

**Table 7: Minerals content in blood serum of chicken-broilers at the age of 38 days**

| Parameters | Control group | Test group | Student's test, p |
|---|---|---|---|
| Calcium, mmol/l | 1.518±0.08 | 1.642±0.06 | p >0.05 |
| Magnesium mmol/l | 1.168±0.22 | 1.446±0.13 | P>0.05 |
| Phosphorus, mmol/l | 2.622±0.20 | 3.156±0.09 | P>0.05 |
| Nonheme iron mcmol/l | 19.06±2.89 | 27.32±3.28 | p <0.05 |

Such an influence on calcium, magnesium, phosphorus content dynamics is possibly due to the combined effect of the introduced toxicants and antagonistic interaction of zinc and copper with lead. Some authors (T. I. Gerasimenko, S. G. Domnin, et al. 2000) note that the combined effect of lead with zinc and copper calculated on the basis of the experimental data was significantly lower, than at an isolated action. That indicates a decrease of the subcellular acute toxic lead effect caused by zinc and copper. There is an antagonism especially marked at the influence of binary mixture lead-copper. In relation, developing between lead and zinc, and especially between lead and copper, in the process of their simultaneous introduction into the organism, it seems possible to consider changes of metal kinetics in the organism.

Sverdlovsk region is characterized with a high concentration of industrial enterprises: emission of contaminants from them into the atmosphere is 1 million 320 thousand tons per year. The soil and plants in these territories are polluted by radionuclides, heavy metal salts (N. V. Sadovnikov 1996, 1998, I.A. Shkuratova 1998, 2001). Such steps as evaluation of foodstuffs, especially those of cattle breeding and poultry breeding at all the stages of their production include: intravital analysis of biosamples of productive animals and birds, postmortem analysis of corpses, analysis of the end products should be included into the complex measures for human body protection from alimentary penetration of ecotoxicants with food and water. Evaluation of quality and structure of fodder, which is fed to animals and birds, is not less important component in protective measures.

Heavy metal salts in living tissues are known to be deposited selectively. As a rule, lead in large amounts accumulates in liver, kidneys, and bones, zinc maximally accumulates in muscles and liver, copper - also in liver and cardiac muscle (A. A. Malygina 2001, V. Korenikova et.al. 2002). The obtained results analysis confirms this fact as well. However, in the organs of birds the following changes were observed.

Lead accumulation in broilers' organ tissues remained unchanged with age during their cultivation. Background doses of lead in combined feed were not found. However, distribution of this metal in chicken tissues is of interest. Thus, for example, in 28-day-old chickens, the greatest amount of lead in light meat was 2.85±0.28 mcg/g and in red meat – 1.42±0.14 mcg/g. In the cardiac muscle, lead concentration was high in comparison with other tissues, and was at one level during the whole period of chicken fattening (5.15±1.60 – 5.45±1.96 mcg/g). It is possible to assume that liver detoxicating function is reduced resulting in lead accumulation in the cardiac muscle.

The lead content in the control group chickens' kidneys and liver was also high at the age of 28 and 38 days of life (4.87±1.33 – 4.50±0.72 mcg/g, respectively and 5.10±0.82 – 2.90±0.65 mcg/g). At the same time, it should be noted that in the control group chickens at the age of 41 days of life lead was not found in light and red meat, small intestine. In the kidneys and liver its concentration decreased almost 2.5 and 3.9 times, respectively (Table 9). Possibly, reduction of this level is connected with competitive influence of several elements.

Change of the copper content in the tissues of the control and test group chickens at the age of 28, 38 and 41 days of their life showed that copper accumulation in tissues considerably increases in the cardiac muscle – 8.10±2.20 mcg/g, small intestine – 3.14±1.01 mcg/g and kidneys – 11.96±0.42 mcg/g of dry matter in test group chickens. A similar tendency for copper accumulation in these tissues is also observed at the age of 38 and 41 days.

At the same time, it should be noted that the zinc content in chickens' tissues at the age of 28 days, that is 21 day after introducing a high concentration of heavy metal salts mixture with fodder, was significantly lower in light meat (sternalis muscles) – 36.3±12.3 mcg/g, red meat (skeletal muscles) – 55.23±0.40 mcg/g, cardiac muscle – 42.12±1.37 mcg/g, small intestine – 28.17±4.85 mcg/g, than in chickens from the control group, that may be connected with inhibition of zinc accumulation in these tissues due to the increased concentrations of copper and lead in them. The zinc level in the liver and kidneys was identical both in control and test groups. The same tendency of zinc distribution in organs of the test group chickens was observed at the age of 38 and 41 days of life. At the age of 38 days, both groups of chickens showed a significant decrease of zinc concentration gradient in the organs. Zinc concentration in tissues of chickens continued to decrease with age, but at the same time zinc level in the liver and kidneys increased in chickens at the age of 38 and 41 days in the test group, in comparison with this metal content in the control group chickens, 122.13±6.71 mcg/g and 114.17±11.12 mcg/g in kidneys, respectively, and in the liver – 157.54±7.14 mcg/g and 130.85±15.77 mcg/g in chickens at the age of 41.

The received data confirm that selective accumulation of heavy metal salts in a bird organism is connected with biological role, participation in enzymatic reactions, physiological and morphological features of the organ. It coincides with V. V. Kovalsky's research outcomes of 1983.

A high content of heavy metal salts in kidneys demonstrates that they enter kidneys in two ways from the liver and directly from the blood.

The results of the studies performed testify to the ability of heavy metals accumulation in the organism that leads to the development of pathological changes in a bird's organism.

At the combined effect of heavy metal salts, the whole complex of morphological changes was revealed, at first they become apparent as acute processes, which pass into chronic with age, the fact confirmed by other researchers - L. N. Aristarkhova, A. A. Malygin (2000), O. V. Vinogradova (2003). Common pathological processes revealed at an early age are dystrophic discioculary, disturbance of hemodynamics organs. Such changes were observed in the liver, kidneys and heart. The liver is the indicator and a barrier to toxicants effects, therefore at the combined toxicosis in the early period of chickens' development, granular dystrophy was found. Further on congestion in liver vessels occurred and signs characteristic of fatty dystrophy appeared. Later vascular thromboses, mass hemorrhages, hemosiderosis, hepatocyte necrosis of focal character were registered, thus showing progressive intoxication, passing into chronic. Morphological changes are also confirmed by biochemical blood analyses of chickens (the level of enzymes and uric acid increases). All this stands in accordance with the results of L. I. Drozdova, I. A. Shkuratova and E. P. Ermoshkayeva (2004).

In the conditions of a technogenic pressure, the maximum load is on the excretory system and therefore its state characterizes the condition of the external environment.

At the beginning of the experiment the chicken kidney analysis at combined poisoning showed organ hemodynamic disorder, granular dystrophy of the kidney wavy tubules. By the end of fattening - fatty dystrophy, focal necrosis of epithelium tubules, in some epithelial cylinders in tubule lumen, swellings, deformations of glomeruli, hemorrhages and urate deposits were observed. (That is confirmed by biochemical studies) and confirms a renal failure. Similar changes were also described by other authors both in humans and animals (M. V. Nezhdanova 1998, E. P. Ermoshkayeva 2004). At the combined poisoning liver and kidneys are critical organs, accumulating the maximum amount of heavy metal salts and assume the main loading on their utilization. Liver in such situation plays the role of an adaptive body, and kidneys - compensatory. Therefore, a complex of changes characterizing the development of chronic intoxication is found in these organs (L. I. Drozdova, I. A. Shkuratova, O. V. Vinogradov 2003 and E. P. Ermoshkayeva 2004).

ISSN: 0975-8585

## CONCLUSIONS

1. The content of metals - copper, zinc, lead in liver and kidneys of the chickens bred for the meat cross "Smena" on 28, 38 and 41 days of their life was determined.
2. Copper and zinc cumulation in the liver and kidneys of those chickens receiving high doses of heavy metal salt vixture of copper, zinc and lead as an additive to the basic diet was determined. At the same time the insignificant difference of their increase in the organs in relation to copper and zinc cumulation was established.
3. A higher concentration of lead in the liver and kidneys of those chickens receiving heavy metal salt mixture with a fodder in comparison with the content of lead in the intact chickens' liver and kidneys were registered.
4. Chronic intoxication in chicken broilers caused by the 33rd day of introduction into the organism of heavy metal salts causes a delay of growth and decrease in live weight gain in the chickens at the end of fattening.
5. Due to our observations the greatest cumulative ability and the damaging action on the liver and kidney tissues is mostly expressed in lead.
6. A chronic toxic effect of copper, zinc and lead on the chickens' organisms up to 28 days caused insignificant damages in their organs in the form of microcirculation disturbance, change in permeability of the vascular wall, desquamation of the organ epithelium.
7. The prolonged cumulation of heavy metals in the liver and kidneys in the test group chickens, during the period from 28 days to 38 days of broiler fattening, caused more significant damages to the liver and kidneys. The damage nature was in a form of hemosiderosis, necrosis, hemorrhages in organs. Also compensatory abilities developed, which manifested in the form of chronic intoxication.
8. The most significant damages are caused by high doses of heavy metal salts entering the organism, from 38 to 41 day of chicken fattening. Thus, in the liver and kidney changes were in the form of fatty hepatosis development, tissue necrosis and formation of cystic cavities in kidneys with deformation of structural blocks.

## REFERENCES

[1] Bakulin V. A. Diseases of birds / V. A. Bakulin. - SPb., Ed. V. A. Bakulin, publishing code under OKVED 22.11.1, 2006. - 688 p.
[2] Drozdova L. I., Shkuratova I. A. Clinical and morphological diagnostics of noncontagious diseases of animals in the conditions of ecological trouble/ - Yekaterinburg, UrGSHA, 2002. - 115 p.
[3] Ermoshkayeva E. P. Morphological changes in the organism of laboratory rats and their posterity at poisoning with lead acetate and zinc oxide: Author's abstract of candidate dissertation/E.P. Ermoshkayeva; "IRA UTK". - Yekaterinburg, - 2004. - 22 pp.
[4] Gerasimenko T. I., Domnin S. G., Rosly O. F., Fedoruk A. A. Assessment of the combined effect of binary mixes lead-copper and lead-zinc (Experimental study) //Medicine of work and industrial ecology-2000. No. 8. – P. 36-38.
[5] Gerasimenko T. I., Domnin S. G., Rosly O. F., Fedoruk A. A. Features of the combined effect of lead, copper and zinc// Medicine of work and industrial ecology-2000. No. 10. – P. 28-30.
[6] Gerasimenko T. I., Domnin S. G., Rosly O. F., Fedoruk A. A. Experimental study of spermatogenesis at the combined lead and zinc influence //Medicine of work and human ecology in the mining and metallurgical industry: Collection of research papers. - Medical scientific center of prevention and health protection of workers of industrial enterprises of the Ministry of Health of the Russian Federation, - Yekaterinburg, 1998. – P. 104-109.
[7] Hygienic criteria of the environment state. Lead. - Geneva, 1980. - 192 p.
[8] Hygienic criteria of the environment condition. 3. Lead / WHO. - M.: Geneva, 1980. - 193 p.
[9] Malygina A. A. Level of heavy metal accumulation in the organism of cattle and methods of decreasing of their abnormal contents in the conditions of Central Ural Mountains: Author's abstract of candidate dissertation / Malygina A. A.- Sverdlovsk
[10] Nezhdanova M. V. Influence of lead environmental pollution on children kidneys state / M. V. Nezhdanova//Problems of the environment and natural resources. - 1998. - No. 11. – P. 157 - 167.
[11] Kovalsky V. V. Geochemical environment, minerals, reaction of the organism/ Problems of geochemical ecology: Works of biogeochemical laboratory. - M.: Science, 1991. - V.22. – P. 5-22.

[12] Ribarov S., Benov L., Benchev I. Hemolitic effect of lead in vitro experiments// Pharmacology and toxicology. -1980 No. 5 – P. 620-621. Benchev//Pharmacology and toxicology. -1980. №5. – P. 620-621.

[13] Rosly O. F., Gerasimenko T. I., Fedoruk A. A. Experimental and hygienic assessment of two binary mixes lead-copper and lead-zinc // Hygiene and sanitation. - 2001. - No. 3. - Page 65 - 67.

[14] Sharoyko V.V., Nureeva G. V., Zhuravskaya A. N., Kershengolts B. M. Influence of lead cations and some complexes of biologically active agents of plant origin on activity and stability of plant genome //Siberian ecological journal -2002 - 2 - 127-135.

[15] Tatarchuk A. T., Shkuratova A., Aristarkhova L. N., et al. Interrelation of ecological features of the environment with the development of pathology of farm animals// Concept of scientific providing veterinary medicine of the Northeast region of the Nonchernozem zone of the Russian Federation: Theses of the conference report. 24 - June 25, 1999. - Nizhny Novgorod, 1999. - Page 18 - 20.

[16] Tatarchuk A. T., Shkuratova A., Malygina A. A. Interrelation of ecological features of the territory with accumulation of heavy metals in the organism of animal, their immunological status and incidence// Food security - the 21st century: ecologic and economic aspects: Collection of research papers. - Yekaterinburg, 2000. - V.1. – P. 186-197.

[17] The state report on sanitary and epidemiological situation in the Russian Federation in 1999//Ecological bulletin of Russia. - 2001. - No. 2. – P. 3 - 12.

[18] The state report on the surrounding environment condition and influence of habitat factors on health of the population of Sverdlovsk region in 1997//Yekaterinburg: Editorial publishing center URTs "Agrokosmoekologiya" at Goskomecology of Sverdlovsk region, 1997. - 269 p.

[19] Vasilyeva S. V. Biochemical aspects of age dynamics of microelements in laying hens in the ecosystem of poultry farm: Author's abstract of candidate dissertation / S. V. Vasilyeva; SPb., 2007. - 22 p.

[20] Vorobeychik E. L. Ecological rationing of toxic loads on land ecosystems: Author's abstract of candidate dissertation / E. L. Vorobeychik; Institute of plant and animal ecology UrORAN. - Yekaterinburg, 2004. - 48 p.

[21] Yershov Yu. A., Pletneva T. V. Mechanisms of toxic effect of inorganic compounds / - M.: Medicine. 1989. – P. 138.