THE EFFECTIVENESS OF L-CYSTEINE IN THE INTOXICATION WITH THE COMBINATION OF CADMIUM AND LEAD

Natalia Sergeevna Pavlova[1,*], *Galina Ivanovna* Pavlenko[1], *Natalia Anatolevna* Brichko[1], *Dmitry Aleksandrovich* Drozdov[1] and *Vasily Ivanovich* Dorozhkin[1]

[1] All-Russian Research Institute of Veterinary Sanitation, Hygiene and Ecology – Branch of Federal State Budget Scientific Institution «Federal Scientific Center – K.I. Skryabin, Ya.R. Kovalenko All-Russian Research Institute of Experimental Veterinary Medicine, Russian Academy of Sciences» Moscow 123022, Russian Federation

Abstract. The anthropogenic zones formation with an abnormal heavy metals degree negatively affects the safety and quality of livestock products, the health status and animals productivity. Detoxification by complexation with metal-binding proteins and peptides with a high content of cysteine (up to 33%) is one of the intracellular mechanisms of tolerance to heavy metals (HMs). L-cysteine is a natural feed component, practically it does not have any side effects and does not reduce the quality of products, the duration of its use is not limited, while complexing agents cannot be feeded for a long time, as well as the liver and kidneys deseases. The aim of this research was to study the biological effect of the L-cysteine in case of combined chronic cadmium and lead poisoning of white rats in laboratory conditions. Two-month experiment on white rats feeded with a combination of lead and cadmium at the level of 50 and 5 mg/kg respectively were held. It was found that L-cysteine led to the body weight and the function of the central nervous system normalization, the restoration of protein metabolism, and alkaline phosphatase activity, and hematopoietic functions. The feed additive significantly reduced the specific thiolotropic effect of lead and cadmium.

Keywords: toxicity, l-cysteine, feed additive, cadmium, lead, heavy metals

1. Introduction

Increasing number of studies in various branches of science point to constantly occurring changes in the composition of the natural environment as a result of the chemicals ingress into it

[*]Corresponding author: ns2008p@mail.ru

[1, 2]. The main pollutants are chemical plants, fuel and energy complex, agriculture and transport [3]. It leads to the cadmium, copper, mercury, lead appearance in the composition of air, soil, feed and products therefore [1, 4, 5]. Transport emissions are also significant - hydrocarbon, methane and lead, which, enter the soil, enter the human body with food [6, 7]. In regions with the increased toxigenic load, farm animals are exposed to prolonged systematic exposure to ecotoxicants, including heavy metals (HMs), which affects their health, productivity, product quality and safety [2, 8-10]. One of the ways to obtain safe products under these conditions is the protective pharmacological agents and feed additives that reduce the toxicants accumulation and their negative effects both [5, 7].

Being absorbed, Cd and Pb in a protein-bound state (metallothionein) are carried by the blood throughout the body. The half-life of cadmium in the blood is from 75 to 128 days, lead from soft tissues is about 20 days, however, it is not excretion but accumulation of metals in organs. Approximately 30% of cadmium is deposited in the liver and kidneys, 90% of lead in the bones, the rest is distributed throughout the body with a half-life of 20-25 years [8, 9]. Cd and Pb levels in blood, wool and urine only reflect recent heavy metal intake and not actual accumulation.

One of the main negative effect lead and cadmium mechanisms is oxidative stress [9]. The results of their intake are changes in gene expression and DNA damage, disruption of the transport and enzymes functioning, heme synthesis inhibition and impaired mitochondrial function, glutathione depletion. The negative effect of metals is enhanced by their joint intake [10].

Lead and cadmium bind to a variety of biomolecules: enzymes, regulatory proteins, signaling proteins. The particular interest is the interaction of these elements with thiolate-rich sites found in metal regulatory proteins and enzymes [11]. Thiol (-SH) functional group causes a combination of useful properties in some medicinal compounds. Thiol-containing agents reduce the radicals degree, form stable complexes with heavy metals such as lead and cadmium. Therefore, they are used in HM poisoning as radical scavengers or metal chelators. Many of these compounds have been used for a long time, however, a detailed study of the action mechanisms allows optimizing their use and developing new drugs and methods of treatment [12].

Amino acids are important in animal nutrition, L-cysteine is one of them. L-cysteine (L-1-amino-2-mercaptopropionic acid) is a chemically and biologically active isomer of an aliphatic sulfur-containing amino acid. Cysteine is a source of organic sulfur for body cells. It plays an important role in immunity, central nervous system (CNS) functions, is an essential component of bile acids, and plays the role of an intracellular antioxidant [11-14]. Cysteine is important in neutralizing some toxic substances and protecting the body from the damaging effects of radiation [11, 14-16]. This is due to the high affinity thiol group to heavy metals, so that proteins containing cysteine, such as metallothionein, are able to bind metals such as mercury, lead, and cadmium [9, 17-20]. L-cysteine and its derivatives are a large group of exogenous antioxidant drugs that protect against oxidative tissue damage. This effect can be associated, for example, with the inactivation of hydroxyl radicals or with the secondary induction of glutathione production [19, 20].

So, it seems promising to develop complex feed additives to reduce the negative impact of HMs, especially with their long-term combined intake, using L-cysteine [12, 13, 19. 21].

The purpose of this study is to study the effectiveness of the feed additive L-cysteine in combined cadmium and lead poisoning for white rats in the laboratory terms.

2. Materials and Methods

The object of the study was a feed additive sample L-cysteine (L-Cystein), manufactured by Wuhan Grand Hoyo Co., Ltd. (China). White outbred male rats (LLC "Krolinfo") were used in the experiment. The choice of doses and duration of the experiment were carried out in accordance with the literature data and the "Instructions for feed additive L-cysteine". According to the instruction L-cysteine is given in doses of 0.1-5.0 g/kg of feed.

Experimental studies were carried out in the vivarium of All-Russian Research Institute of Veterinary Sanitation, Hygiene and Ecology – Branch of Federal State Budget Scientific Institution «Federal Scientific Center – K.I. Skryabin, Ya.R. Kovalenko All-Russian Research Institute of Experimental Veterinary Medicine, Russian Academy of Sciences», on 30 white male rats. The initial weight of rats 197-210 g. Clinically healthy rats were taken in the experiment, after a 15-day quarantine. Animal studies were performed in accordance with the rules adopted by the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes.

White rats were divided into three groups. The first group - control, the animals received normal food. The rats of the second experimental group were added cadmium sulfate and lead acetate to the feed in doses (in terms of pure metal) 5 and 50 mg/kg of feed, respectively. It is 10 MPC for feed. The rats of the third group were fed with cadmium and lead salts in the same doses with the feed additive L-cysteine at a therapeutic dose of 5.0 g per 1 kg of feed. The duration of the experiment was two months.

Animals were examined in one and two months after the start of the experiment respectively. Integral and specific indicators were used.

Some dynamic and static performance indicators of animals were used to assess the state of the nervous system. Dynamic motor activity was determined using the "vertical" motor component (the number of animals getting up on their hind legs in 3 minutes); static muscle performance - a method of keeping animals on a horizontal rod.

To determine the state of the CNS, the summation threshold index (SPP) was used. The SPP-01-M device was used. The neuromuscular excitability of the animals was determined by the contraction of the interdigital muscles using electrodes with an increase in the current supply.

The state of blood was assessed using conventional methods: determination of the amount of hemoglobin, leukocytes, and erythrocytes with a hematological analyzer Medonic CA-620 BALDER (Sweden), Uni-Gem reagents for hematological analyzers (LLC “Reamed”).

The effects of L-cysteine on the liver were determined by the content of total protein in the blood serum refractometrically (IRF-22 refractometer, Russia). At the end of the experiment, the amount of SH-groups in the blood serum was determined; the determination method is based on the equivalent interaction of molecular iodine with free SH-groups of proteins and low molecular weight compounds (540 nm light filter, KFK-2-UHL4.2, Russia). The functional state of the kidneys - according to the results of measuring diuresis, the specific gravity of urine, the content of protein and chlorides in the urine. The immune status of the body was determined by the level of immunoglobulins using a turbometric method based on the precipitation reaction with zinc sulfate. After the end of the experiment, the mass coefficients of the internal organs of the animals were determined.

Statistical processing of the results was carried out using Microsoft Excel 2013 for Windows 10, using Student's t-test for independent samples. Differences were considered statistically significant at $p<0.05$. The results were presented as the arithmetic mean and standard deviation ($M \pm m$).

3. Results

At the beginning of the experiment, and then after one and two months, the body weight of the animals and the value of the excitability threshold of the nervous system according to the SPP were determined. The results of the examination of animals after one and two months in Table 1 are shown.

Table 1. Body weight dynamics and SPP of white rats in a 2-month experiment (M±m).

Term, month	Group	Body weight, g	SPP, c.u.
Background value	Control Pb+ Cd Pb+ Cd+ L-cysteine	202±5 204±6 203±6	3,7±0,3 3,8±0,2 3,5±0,22
1	Control Pb+ Cd Pb+ Cd+ L-cysteine	298± 10 277±6* 289± 6	3,9±0,3 4,6±0,1* 3,1±0,4
2	Control Pb+ Cd Pb+Cd+ L-cysteine	373±7 348±4* 364±6	3,5±0,3 5,5±0,2* 4,0±0,5

*) *P< 0,05*

A month later, in the experimental groups, there was a clear trend towards weight loss in the group receiving the combination of lead and cadmium. By the end of the second month, the decrease in body weight in animals of this group continued and reached 9% compared with the control. In animals of the second group, body weight does not significantly differ from control, although there is a tendency to decrease it. The addition of L-cysteine to the feed practically normalized body weight.

SPP significantly increased in the group of animals treated with lead and cadmium both after one and two months, which indicates a decrease in the excitability of the nervous system. The addition of L-cysteine to the feed led to the normalization of the SPP at all periods of animal examination.

The results of blood analysis are presented in table 2.

Table 2. The results of blood analysis

Group	I. Control		II. Pb+ Cd		III.Pb+Cd+ L-cysteine	
Terms, month	1	2	1	2	1	2
Erythrocytes, 10^{12}/l (RBC)	9,02 ±1,12	8,4 ±0,5	8,4±0,72	8,52±0,75	8,38±0,92	8,57±0,72
Mean Erythrocyte Volume, (MCV)	46,2±0,43	49,3±0,9	45,9±1,23	48,2±1,3	45,9±2,1	49,7±2,5
Distribution width erythr. by volume,% RDW)	17,1±0,8	13,8±0,3	16,2 ±0,2	12,6±0,3	16,7±0,3	14,5±1,1
Hemoglobin, g/l (HGB)	167,7±9,36	158,8±5,8	152,3±5,8	156,0±6,1	150,8±7,4	155±3,42
Avg. conc. Hb	403,2 ±8,6	382,2±2,1	395,0±3,7	376,2*±2,2	392,5±4,4	365,2±1,9*

in erythr., g/l (MCHC)						
Hematocrit, % (HCT)	41,6±4,96	41,6±2,4	38,6±3,01	41,08±3,51	38,4±3,9	42,5±1,8
Platelets, 10^9/l (PLT)	313,0±19,4	468,0±25,8	310,8±23,2	495,7±20,1	307,4±18,6	451,1±28,0
Leukocytes, 10^9/l (WBC)	6,9±0,5	7,1±0,9	8,5±2,1	8,9±1,8	7,9±1,9	6,5±1,5

*) *P< 0,05*

In rats fed with a combination of lead + cadmium, after a month there was a tendency to decrease in the level of hemoglobin, and after two months a significant decrease in the average concentration of hemoglobin in erythrocytes (MCHC - reflects the degree of saturation of the erythrocyte with hemoglobin; characterizes the ratio of the amount of hemoglobin to the volume of the cell.) compared with control (at $p<0.05$). There were no significant changes in other indicators. The use of L-cysteine did not lead to the normalization of this indicator.
To assess the state of protein metabolism, as well as the function of the liver and kidneys, the content of total protein and alkaline phosphatase was determined in blood serum. The data are presented in table 3.

Table 3. Integral and specific indicators of the state of animals in a two-month experiment (M±m).

Indicators	Terms, month	Control	Cd + Pb	Cd + Pb +L-cysteine
Urine				
Daily diuresis, ml	1 2	3,9±1,1 4,6±1,5	4,5±1,3 2,1±1,1	4,0±1,3 4,4±1,5
Specific gravity of urine, g/ml	1 2	1,013±0,005 1,011±0,004	1,013±0,001 1,012±0,003	1,013±0,001 1,012±0,003
Protein in the urine, mg/ml	1 2	3,6±1,3 4,9±1,3	2,9±0,9 5,8±1,1*	5,9±1,1 4,5±1,3
Chlorides in urine, mg/ml	1 2	5,9±0,35 6,3±0,6	6,3±0,45 5,8±1,1	6,6±0,55 5,4±1,5
Serum				
Total protein in blood serum, g/l	2	65,5±3,1	53,5±2,3*	58,9±2,1
Alkaline phosphatase in blood serum, U/l	2	252,5±12,5	297,5±13,5*	289,5±11,4*
Common SH-groups in blood serum, µmol/l	2	14,8 ± 1,5	11,0 ± 1,3*	13,9± 1,5
Non-protein SH-groups in blood serum, µmol/l	2	8,9 ± 1,1	5,3 ± 0,3*	8,5 ± 0,6
Protein SH-groups in blood serum, µmol/l	2	4,9 ± 1,3	4,8 ± 0,8	5,2 ± 1,3

Immunoglobulin, units/l	2	7,9 ± 0,6	5,5 ± 0,5*	7,3 ± 0,6
Mass coefficients of internal organs of animals				
Liver	2	3,53 ± 0,22	4,67± 0,33*	3,56 ± 0,16
kidneys	2	0,37 ± 0,01	0,45±0,02*	0,36 ± 0,01
Spleen	2	0,42 ± 0,03	0,44 ± 0,05	0,43 ± 0,05
Heart	2	0,31 ± 0,05	0,30 ± 0,02	0,29 ± 0,01

*) P< 0,05

In the study of the functional state of the kidneys, a set of indicators was determined: diuresis, specific gravity of urine, protein and chloride content in the urine (Table 3). In animals fed with metals, there was a significant increase in the protein content in the urine (at P<0.05) and a trend towards a decrease in diuresis compared to the control, which indicates a pathology of the kidneys, that is, the development of renal failure. The introduction of L-cysteine into the feed restored these indicators to normal.

As follows from Table 3, the combination of metals caused a significant decrease in the content of total protein and an increase in the level of alkaline phosphatase in the blood serum compared with the control (at P<0.05) at the end of the two-month experiment. The introduction of L-cysteine into the diet led to the normalization of protein levels, and there was also a tendency to normalize the activity of alkaline phosphatase.

The results of determining the content of SH-groups, presented in table 3, are typical for the action of heavy metals. So, at the end of the experiment, the combined action of metals caused a significant decrease in the content of SH-groups due to the low content of non-protein groups compared to the control (at P<0.05). Animals treated with L-cysteine along with HM had a complete recovery of this index.

The results of the study of immunoglobulins (Table 3) showed that in the group of animals fed food with lead and cadmium, there was a significant decrease in the content of immunoglobulins at the end of the experiment compared to the control (at P<0.05). With the introduction of L-cysteine into the feed, this indicator normalized. Thus, the feed additive is important as a tool that plays a key role in strengthening immunity in this poisoning.

Animals treated with a combination of lead and cadmium showed a significant increase in the weight coefficients of the liver and kidneys compared with the control (at P<0.05), while the heart and spleen did not differ significantly from the control (Table 3). In the group of animals that were fed with L-cysteine simultaneously with heavy metals, the weight coefficients of all organs did not differ from the control values.

4. Discussion

In case of chronic intoxication of lab rats with a combination of lead and cadmium, after two months, a significant decrease in body weight, compared with the control (at P<0.05) is observed [22]. In the group of animals that received L-cysteine against the background of metal poisoning, there was no significant change in body weight compared to the control, that is, normalization occurred.

Starting from the first month, receiving HM with food, there was a statistically significant increase in SPP, that characterizes a decrease in the excitability of the nervous system. With the introduction of L-cysteine in feed, this indicator normalized. This is explained by the fact that cysteine is used in the body for the production of taurine, that plays an important role in central nervous system functioning, as well as in reducing oxidative stress [11, 14, 15].

In the analysis of blood in rats treated with a combination of lead + cadmium, there was a

tendency to a decrease in the level of hemoglobin in the blood, and a significant decrease its average concentration in erythrocytes (MCHC), which may indicate the onset of anemia or impaired liver and kidney function. A pathological hemoglobin decrease in the blood is the result of an accelerated destruction of red blood cells in the peripheral bloodstream (hemolytic anemia) and a violation of the formation of red blood cells. Anemia is a characteristic symptom of lead and cadmium poisoning associated with a decrease in the lifespan of erythrocytes and inhibition of heme synthesis [22]. The content of erythrocytes in the blood of animals in the experimental groups was at the level of control values. The use of L-cysteine did not lead to the normalization of this indicator. Therefore, it is not enough to use only L-cysteine to normalize this indicator.

In the rats blood serum that received only heavy metals, there was a decrease in total protein and an increase in the activity of alkaline phosphatase, which indicates a violation of the function of the liver and kidneys. A change in these indicators is observed in many liver diseases and is typical for acute and chronic cadmium and lead poisoning. L-cysteine, introduced into the feed, restored these indicators to normal meanings.

A significant increase of the protein content in the urine and a tendency to a decrease in diuresis also indicate a violation of kidney function under the influence of heavy metals. This syndrome is observed in heavy metal poisoning, the cause is a violation of renal circulation, which causes a sharp decrease in glomerular filtration, the development of tubular anuric nephropathy and necrosis of epithelial cells of the proximal and distal tubules [23]. These indicators were restored while L-cysteine was added to the feed.

It is especially important that L-cysteine significantly reduced the specific negative thiolotropic effect of lead and cadmium. Lead and cadmium act as thiol poisons at the molecular level. The leading role belongs to the processes of interaction with sulfhydryl groups, which determine the activity of many enzymes involved in various stages of protein, carbohydrate, and mineral metabolism [14, 22]. This is due to the presence of a thiol group in cysteine, which has a high affinity for heavy metals and allows binding such metals as mercury, lead and cadmium. Thus, due to the ability of SH-groups, which are part of the cysteine molecule, to enter into redox reactions, this feed additive has antioxidant properties. Quantitative and functional changes in immunoglobulins may be due to a violation of their synthesis as a result of chemical intoxication, leading to a decrease in serum levels.

There was an increase in liver weight by 32% and kidney weight by 22% under the influence of elevated concentrations of HM ($P<0.05$). The animals mass of the internal organs changes often consequence of lead and cadmium intoxication [22]. The morphofunctional hepatic-renal complex integrity violation is one of the integral pathogenetic mechanisms in the thiol poisons toxic effect. This is determined by their ability to bind sulfhydryl groups of proteins, replace Ca^{2+} and Zn^{2+} in proteins, lipids, and other biologically active compounds [9, 17-19]. Cadmium and lead cause a number of enzyme systems inhibition in liver and kidneys, that leads to organs fatty degeneration and their enlarging. L-cysteine forms compounds with heavy metals and reduce the number of free radicals. Being the main component of proteins that bind heavy metals in the body, L-cysteine reduced the amount of damaging elements, reducing the load on the liver and kidneys thereby. Liver and kidneys mass coefficients decreased to control values in animals received L-cysteine compared with the experimental group rats fed with lead and cadmium.

The presented results are consistent with our data on the accumulation of cadmium and lead in the organs and tissues of white rats when L-cysteine introduced into the feed at a dose of 5.0 g per 1 kg feed against the background of cadmium and lead poisoning at a dose of 5 and 50 mg/kg, respectively in terms of pure metal. The maximum accumulation was found in the kidneys and liver of lead (8.15 and 7.44 mg/kg) and cadmium (2.51 and 1.8 mg/kg) in animals

fed with metals. The intake of L-cysteine significantly reduced the accumulation of lead in all the studied organs by 42-58%, cadmium - by 2-4 times [24].

5. Conclusions

A sufficiently full-fledged protective effect of L-cysteine was shown in chronic poisoning with heavy metals. The obtained data correspond to the administered doses and are consistent with the data of other researchers. The effectiveness of L-cysteine due to the high content of thiol groups involved in the specific binding of HMs, that reduces their entry into the body at the stage of absorption sharply, accelerated the process of excretion and neutralization, reduced the toxic load on the liver and kidneys, the nervous and immune systems, as well as reduce the oxidative stress.

The use of L-cysteine did not normalize the hemoglobin content in erythrocytes. So it was necessary to supply its action with other means to minimize the negative effects of heavy metals.

L-cysteine could be recommended as a means capable to reduce cadmium and lead intoxication during chronic intake with food, separately and in combination with sorbents and detoxifying substances both.

References

1. Hana R., Zhoua B., Huanga Y., Lu X., Li S., Li N., Journal of Cleaner Production,**276**: 123249 (2020) doi: 10.1016/j.jclepro.2020.123249
2. Li C., Zhou K., Qin W., Tian C., Qi M, Yan X., Yan X., Han W., Soil & sediment contamination, **28, 4**: 380-394 (2019) doi: 10.1080/15320383.2019.1592108
3. Kinuthia G.K., Ngure V., Beti D., Lugalia R., Wangila A., Kamau L., Sci Rep., **10**: 8434 (2020) doi: 10.1038/s41598-020-65359-5
4. Li S., Zhao B., Jin M., Hu L., Zhong, H., He, Z. Journal of Hazardous Materials,**400, 5**:123255 (2020) doi: 10.1016/j.jhazmat.2020.123255
5. Dahmardeh Behrooz R., Kaskaoutis D.G., Grivas G., Mihalopoulos N. Chemosphere,**262**, 127835 (2021)doi: 10.1016/j.chemosphere.2020.127835
6. Zakharova L.L., Zhorov G.A., Obryvin V.N., Brichko N.A. Russian journal "Problems of veterinary sanitation, hygiene and ecology", 1(33): 92-99. (2020) doi: 10.36871/vet.san.hyg.ecol.202001014
7. Zhorov G.A., Rubchenkov P.N., Zakharova L.L.,. Obryvin V.N. Russian journal Problems of veterinary sanitation, hygiene and ecology, **4**(20): 113-121 (2016)
8. Noori N., Noudoost B., Gandomi H., Akhondzadeh Basti, A. Toxin Reviews, **35 (3-4)**: 196-200 (2016) doi: 10.1080/15569543.2016.1225768
9. Vázquez M., Calatayud M., Jadán Piedra C., Chiocchetti G.M., Vélez D., Devesa V., Food and Chemical Toxicology, **86**: 163-175(2015) doi: 10.1016/j.fct.2015.10.006
10. Robin A. Bernhoft, The Scientific World Journal, **2013**: 394652 (2013) doi.org/10.1155/2013/394652.
11. Sisombath N.S., Jalilehvand F., Chemical Research in Toxicology,**28 (12):** 2313-2324 (2015) doi: 10.1021/acs.chemrestox.5b00323
12. Pfaff AR, Beltz J, King E, Ercal N., Mini Rev Med Chem., **20(6):**513-529 (2020) doi: 10.2174/1389557519666191119144100.
13. Pisarenko O.I., Serebryakova L.I., Tskitishvili O.V., Studneva I.M., Vanin A.F., Chazov E.I. Izvestiya Rossiiskoi Akademii Nauk. Series Biological, **1**: 110-114 (2008)

14. Luczak M.W., Zhitkovich A., Free Radical Biology and Medicine,**65**: 262-269 (2013) doi: 10.1016/j.freeradbiomed.2013.06.028 Status and Ne
15. Pfaff A.R., Beltz J., King E., Ercal N. Mini-Reviews in Medicinal Chemistry, **20(6):** 513-529 (2020) doi: 10.2174/1389557519666191119144100).
16. Krzywoszyńska K., Swiątek-Kozłowska J., Potocki S.,Ostrowska M., Kozłowski H. Journal of Inorganic Biochemistry, **204**: 110957 (2020) doi: 10.1016/j.jinorgbio.2019.110957
17. Jalilehvand F., Sisombath N.S., Schell A.C., Facey G.A. *Inorg. Chem.,***54, 5**: 2160–2170 (2015) doi: 10.1021/ic5025668 10.1021/acs.chemrestox.5b00323
18. Cardiano P., Foti C., Giuffrè O., Journal of Molecular Liquids, **223**: 360-367 (2016) doi: 10.1016/j.molliq.2016.08.050
19. Vandenbossche M., Jimenez M.,. Casetta M, Traisnel M., Crit. Rev. Environ. Sci. Technol., **45**: 1644-1704 (2015) doi: 10.1080/10643389.2014.966425
20. Shen X.,Liu W., Chen Y.,Guo Y., Gao M., Chen W., Liu Y., Liu S., Chemosphere, **229**: 32-40 (2019) doi: 10.1016/j.chemosphere.2019.04.192)
21. Pedroso T.F., Oliveira C.S., Fonseca M.M., Oliveira V.A., Pereir M.E. Biological Trace Element Research,**180 (2):** 275-284 (2017) doi: 10.1007/s12011-017-1009-z
22. Paithankar J.G., Saini S., Dwivedi S., Sharma A., Chowdhuri D.K., ChemosphereIn press, journal pre-proof **16**: 128350 (2020) doi: 10.1016/j.chemosphere.2020.128350
23. Cabral M, Garçon G, Touré A, Bah F, Dewaele D, Bouhsina S, Cazier F, Faye A, Fall M, Courcot D, Verdin A. Toxicol Rep., **8**:386-394 (2021) doi: 10.1016/j.toxrep.2021.02.009
24. Dorozhkin V.I., Pavlenko G.I., Pavlova N.S., Brichko N.A., Drozdov D.A. Russian journal Problems of Veterinary Sanitation, Hygiene and Ecology, **3**(35):378-383 (2020) doi: 10.36871/vet.san.hyg.ecol.202003014