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ORIGINAL ARTICLE

The effect of cadmium loading on protein synthesis function and functional state of laying hens' liver

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In the article are considered issues related to the study of the effect of cadmium on the body of birds, in particular laying hens. The effect of cadmium sulfate at doses of 2.0 and 4.0 mg/kg body weight on protein synthesis function and functional state of the liver were investigated of cross-laying Hysex white hens, of 78 weeks old. Three groups of chickens were formed: two experimental and one control groups. The laying hens of the experimental groups were subjected to a cadmium load. Laying chickens of the first experimental group were given cadmium sulfate at a dose of 2.0 mg/kg body weight. Laying chickens of the second experimental group were given cadmium sulfate at a dose of 4.0 mg/kg body weight. Laying chickens in the control group were in the normal diet without cadmium. Increased activity of aminotransferases, in particular alanine and aspartate aminotransferases in their serum of hens, was observed during cadmium binge drinking in doses of 2.0 and 4.0 mg/kg body weight, respectively. These changes indicate a violation of the functional state of the liver of laying hens under conditions of cadmium load. In the study of protein synthesis of the liver of laying hens under conditions of cadmium loading, it was found that cadmium sulfate in the above doses contributed to a significant decrease in the level of total protein by 14, 21 and 30 days of the experiment. The reduction of total protein in the blood of laying hens was due to a decrease in the level of albumin, which in the second experimental group, respectively, decreased to 28.16 ± 0.75%, whereas in the first – 29.62 ± 0.98%. In the study of the level of globulins, it is possible to increase this indicator in both experimental groups of poultry for 21 days. Drinking with water cadmium sulfate at a dose of 4.0 mg/kg body weight was associated with a more likely decrease in total protein and albumin than drinking cadmium sulfate at a lower dose.

Keywords: Toxicology; Cadmium; Hens; Blood; Immune system

Introduction

Uncontrolled excessive emissions of industrial enterprises and motor vehicles, violations of the fertilizer application system, the treatment of plants with pesticides, technological accidents and other anthropogenic factors lead to the accumulation of heavy metals in soil and plants, adversely affecting the health of agricultural animals and humans. (Uetani et al., 2005; Nazaruk et al., 2015; Sachko et al., 2016; Gutyj et al., 2016; Khariv et al., 2016). Over the past decades, an increase in the content of this metal in the soils of Ukraine and other countries has been accompanied by the accumulation of Cd^{2+} in agricultural products and feed, an increase in the threat to human and animal health. (Hutyi, 2013; Gutyj et al., 2015; Hradovych et al., 2016; Grushanska, 2017; Gutyj et al., 2017; Grynevych et al., 2018).

Cadmium and its compounds belong to the immunotoxicant, which cause disruptions in the functioning of the body's immune system, reduce resistance to infections, contribute to the formation of allergic, autoimmune and oncological pathologies (Ali et al., 1986; Salvatori et al., 2004; El-Refaiy and Eissa, 2012; Peng et al., 2015; Gutyj, 2015). A feature of the biological action of cadmium is its ability to adversely affect the health of animals with prolonged exposure to low levels of pollution due to the high coefficient of biological cumulation (up to 40 years). It is known that cadmium can significantly change the metabolism and functions of such essential elements as zinc, iron, copper, manganese, calcium, selenium (Ostapyuk & Gutyj, 2018; Gutyj et al., 2019).

Cadmium adversely affects the vital systems of humans and animals, causing pathological changes in tissues and organs (kidneys, lungs, bone tissue, organs of the reproductive and endocrine systems), inhibiting the process of erythropoiesis and immune system function (Fregoneze et al., 1997; Rodríguez et al., 2001; Lu et al., 2005; Liu et al., 2008; Al-Azemi et al., 2010). Disorders caused by the long-term intake of cadmium in the body are determined by the level of accumulation of Cd^{2+} in cells and the manifestation of cumulative toxicity. The vulnerability of cells to cadmium largely depends on the level of expression of the genes of metal-binding proteins, metalothioneone, in them. (Antonio et al., 1998; Pavan Kumar and Prasad, 2004; El-Shahat et al., 2009).

Cadmium poisoning occurs when it enters the stomach or by inhalation. Absorbed cadmium accumulates in the liver and kidneys as a complex with metalthionein. That is why our researches were aimed at studying the effect of cadmium on the protein synthesis function and the functional state of the liver of laying hens.

Materials and Methods

For the experiment we selected 24 Hisex white class hens at the age of 78 weeks, from which three groups were formed: the control group and two research groups. Groups were formed on the principle of analogs (age and live weight). Chickens from different groups were labeled with persistent organic dyes. The chickens of the control group were on a normal diet, they were fed

with mixed feed and watered without making cadmium sulfate. Within 30 days, cadmium sulfate was added to the drinking water of chickens from the experimental groups in doses: the first group – 2 mg/kg, the second group – 4 mg/kg body weight.
Conditions and parameters of the indoor microclimate for all groups of birds were similar. During the experiment we took into account the amount of food consumed and water.
All experimental interventions and slaughter of animals were carried out in compliance with the requirements of the European Convention for the Protection of Vertebrate Animals used for Experimental and Scientific Purposes (Strasbourg, 1985) and the decisions of the First National Congress on Bioethics (Kiev, 2001).
Blood from laying hens was taken from the axillary veins in the periods: before the start of the cadmium sulfate task and the first, seventh, fourteenth, twenty-first and thirieth days of the experiment. Investigated the concentration of protein, its fractions, the activity of aminotransferases by the method (Vlizlo, 2012).
Analysis of the research results was performed using the software package Statistica 6.0. The probability of differences was assessed by Student's t-test. The results of the mean values were considered statistically significant at * – P<0.05, ** – P<0.01, ***– P<0.001 (ANOVA).

Results and Discussion

Enzymes are biological catalysts that accelerate metabolic processes in cell cytoplasm. They are continuously synthesized in cells and penetrate the bloodstream. For enzymes inherent the localization in the corresponding cells and their structural elements (cytoplasm, mitochondria, nuclei). When cell membranes are permeable, enzymes enter the bloodstream and create increased activity in the blood. The study of the activity of enzymes in serum is great diagnostic and prognostic value in various pathological conditions of the structure of parenchymal organs. In the presence of a pathological process in the blood of mammals and birds changes in the qualitative and quantitative composition of enzymes that reflect the intensity and direction of the development of pathology.
The results of studies of the activity of aminotransferases in the serum of laying hens under cadmium loading are shown in table 1. It was found that the activity of alanine aminotransferase in the serum of chickens of the first experimental group has probably increased since the 7th day of the experiment by 13.3%. For the 14th day of the experiment, the activity of the enzyme continued to increase. And on the 14th day of the experiment fluctuated within 0.38 ± 0.007 mmol/g/l, whereas in the control group of laying hens, this indicator was much lower. On the 21st day of the experiment, the activity of alanine aminotransferase in the serum of the birds of the first experimental group was the highest, where compared to the control it increased by 29%.
During the feeding for chickens of the second experimental group of cadmium sulfate at a dose of 4.0 mg/kg body weight, similar increases in the activity of this enzyme were found throughout the experiment. Thus, for the 7th day of the experiment the enzyme activity increased by 20%, whereas on the 14th day – by 21.2%, respectively. The highest activity of alanine aminotransferase was on the 21st day of the experiment, where, respectively, it was 0.45 ± 0.009 mmol/g/l. This increase in enzyme activity is due to an increase in the permeability of hepatocyte cell membranes and mitochondrial membranes and the intracellular enzymes flow into the bloodstream.

Table 1. The activity of aminotransferase serum of laying hens for cadmium loading (M ± m, n=8).

Experimental groups	Before drinking	Days of research			
		7	14	21	30
		ALT, mmol / g / l			
C	0.31 ± 0.01	0.30 ± 0.01	0.33 ± 0.01	0.31 ± 0.01	0.30 ± 0.01
E1	0.32 ± 0.01	0.34 ± 0.01*	0.38 ± 0.01***	0.40 ± 0.01***	0.38 ± 0.01***
E2	0.30 ± 0.01	0.36 ± 0.01**	0.40 ± 0.01***	0.45 ± 0.01***	0.43 ± 0.01***
		AST, mmol/g/l			
C	4.27 ± 0.18	4.30 ± 0.18	4.29 ± 0.17	4.35 ± 0.15	4.33 ± 0.13
E1	4.31 ± 0.13	4.49 ± 0.15	4.82 ± 0.20	5.14 ± 0.19**	4.93 ± 0.24*
E2	4.28 ± 0.17	4.60 ± 0.19	5.16 ± 0.23**	5.70 ± 0.21***	5.54 ± 0.25***

In the study of aspartate aminotransferase activity it was found that under cadmium loading the activity of this enzyme increased significantly in the first experimental group at 21 and 30 days of the experiment, whereas in the second experimental group respectively – at 14, 21 and 30 days of the experiment. Thus, the activity of aspartate aminotransferase in the serum of the first and second experimental group of laying hens increased by 12.4 and 20.3%, respectively, relative to the indicators of the control group. During the 21 days of the experiment, enzyme activity increased slightly compared to the previous day. It was the highest in the serum of the second experimental group of laying hens, where it increased by 31% relative to control, while in the first experimental group – by 18.2%. At 30 days of the experiment, the activity of aspartate aminotransferase in the serum of both study groups remained significantly higher than the control.
We think that aminotransferase hyperenzymemia in the serum of diseased animals results from the action of cadmium on the liver, which has a destructive effect on cell membrane phospholipids. This causes an increase in their permeability and release of aminotransferases from hepatocytes into the bloodstream.
Protein is considered the main element, due to which the internal process of "building" in the body. It maintains the fluidity of blood, its viscosity. The protein determines the volume of blood needed in the bloodstream. At the expense of proteins, the shaped elements are kept in a suspended state, and also the transportation of the most important exogenous and endogenous substances is carried out. Thanks to proteins, the pH of the blood is regulated. Protein takes an active part in immune reactions (Hariv & Gutyj, 2016).
It was found that the cadmium load in the blood of laying hens is inhibited by the protein synthesis function of the liver, as indicated by the low level of total protein in their blood. Thus, in the blood of chickens of the first experimental group on the 14th day of the experiment revealed a decrease in the level of total protein by 6.7% relative to control. On the 21st day of the experiment, the test indicator decreased and was 42.11 ± 1.25 g/l, respectively, whereas in the control group it fluctuated within 4673 ± 095 g/l (Table 2).

During bird feeding of cadmium sulphate at a dose of 4,0 mg/kg body weight, a significant decrease in total protein was observed from the 7th day of the experiment, where it decreased by 42% compared to the control group of chickens. At 14 and 21 days of the experiment revealed a decrease in the level of total protein by 9 and 165%. At the 30th day of the experiment, the level of total protein fluctuated within 3965 ± 111 g/l, which was 15% higher than the values of the control group of chickens in the specified period of studies.

The decrease in total protein in the blood of cadmium-loaded chickens was due to a decrease in the albumin fraction. It was found that in the 14th day of the experiment the level of albumin in the blood of the first experimental group decreased by 2.6%, and in the second experimental group – by 4.1% relative to the control group of laying hens. On the 21st day of the experiment, the level of albumin in the blood of the experimental groups was the lowest, where it was 29.62 ± 0.98% in the blood of the first experimental group of chickens and 28.16 ± 0.75% in the blood of the second experimental group. At the 30th day of the experiment, the level of albumin in the blood of chickens of the first and second experimental group remained low and compared to the control group of chickens it decreased by 2.22 and 4.51% respectively.

Table 2. Total content of protein and its fractions in blood of laying hens forcadmium loading (M ± m, n = 8).

Experimental groups	Before drinking	Days of research			
		7	14	21	30
		Total protein, g/l			
C	46.43 ± 0.95	46.60 ± 0.87	46.52 ± 1.05	46.73 ± 0.95	46.60 ± 0.90
E1	46.71 ± 0.80	45.84 ± 1.20	43.38 ± 1.22*	42.11 ± 1.25**	42.41 ± 1.19*
E2	46.58 ± 1.06	44.62 ± 0.96	42.31 ± 1.15*	39.04 ± 1.23***	39.65 ± 1.11**
		Albumins,%			
C	33.59 ± 0.85	33.92 ± 0.90	33.74 ± 0.70	34.10 ± 0.79	33.85 ± 0.94
E1	34.12 ± 0.90	32.38 ± 0.70	31.14 ± 0.85*	29.62 ± 0.98**	31.63 ± 0.92*
E2	33.78 ± 0.93	31.84 ± 0.89	29.62 ± 0.98**	28.16 ± 0.75***	29.34 ± 0.97**
		Globulins,%			
C	66.41 ± 1.94	66.08 ± 1.57	66.26 ± 1.70	65.90 ± 1.64	66.15 ± 1.80
E1	64.88 ± 1.65	67.62 ± 2.10	68.86 ± 1.95	70.38 ± 2.05*	68.37 ± 2.11
E2	66.22 ± 1.87	68.16 ± 2.35	70.38 ± 1.85	71.84 ± 2.10*	70.66 ± 1.95
		Coefficient A/G			
C	0.51	0.51	0.51	0.52	0.51
E1	0.53	0.48	0.45	0.42	0.46
E2	0.51	0.47	0.42	0.39	0.41

When examining the globulin fraction in the blood of laying hens, it was found that the globulins in the blood of the second experimental group probably increased on the 7th day of the experiment. On the 14th day of the experiment, the level of albumin probably increased in all study groups. Thus, in the blood of the first experimental group the level of globulins increased to 68.86 ± 1.95%, and in the second – 70.38 ± 1.85%, which in comparison with the control group it increased by 2.6 and 4.1%, respectively. On the 21st day of the experiment, the level of globulins in the blood of the first and second experimental groups of chickens ranged from 70.38 ± 2.05 to 71.84 ± 2.10%, whereas in the control this indicator was 65.90 ± 1.64%. At 30 days of the experiment, this indicator remained high in the blood of both experimental groups of chickens.

In laying hens during the feeding of cadmium sulfate at doses of 2.0 and 4.0 mg/kg body weight, there is an albumin-globulin disproportion. As a result, the value of A/G coefficient at day 14 of the experiment was 0.45 and 0.42 against 0.51 in clinically healthy birds. Such a magnitude of the coefficient undoubtedly indicates the inhibition of the protein synthesizing function of the liver of laying hens. Increased levels of serum globulin in chickens reflect the intensity of inflammatory processes in their body under cadmium load.

Conclusion

In terms of cadmium loading, laying hens suppress protein-synthesizing liver function, which is manifested by a decrease in total blood protein, a decrease in albumin level and an increase in globulin level. Feeding cadmium sulfate with water at a dose of 4 mg/kg body weight was more likely to be accompanied by a decrease in the level of total protein and albumin than that of cadmium sulfate at a dose of 2.0 mg/kg body weight. Drinking cadmium sulphate laying hens at doses of 2.0 and 4.0 mg/kg body weight promoted aminotransferase activity in their blood. The highest activity of alanine and aspartate aminotransferases was on the 21st day of the experiment. These changes indicate a violation of the functional state of the liver of laying hens under cadmium load.

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