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ORIGINAL ARTICLE

Cadmium burden impact on morphological and biochemical blood indicators of poultry

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The article reveals the experimental results of the impact of various doses of cadmium sulfate on morphological and biochemical blood parameters of poultry. The purpose of the research was to figure out how cadmium sulfate at a dose of 2 and 4 mg/kg of body weight effects the morphological parameters of the blood and liver function of the laying chickens. According to the assessment of the morphological analysis of the quantitative and qualitative composition of the blood, it is possible to come to fairly objective conclusions about the functional state of the hematopoietic system of the hen's body under cadmium burden. Three groups of chickens were formed for the experimant: a control group and two research ones. Chickens of the control group were on a regular diet, they were supplied with nutrition and water without adding cadmium sulfate. Cadmium sulfate was added to drinking water for hens of experimental groups during 30 days. at such doses: the first group – 2 mg/kg, the second group – 4 mg/kg of body weight. Living conditions and microclimate parameters in the room for all groups of birds were the same. The chicken blood was drawn from the axillary veins before drug administering and on the first, seventh, fourteenth, twenty-first and thirtieth days of the experiment. Hematological studies provide an opportunity to study the effect of cadmium on the chicken body in detail, which will lead to more accurate development of a specific prevention and treatment regimen for cadmium toxicosis in poultry.
Feeding the laying chickens with cadmium sulfate at doses of 2 and 4 mg/kg of body weight contributed to a decrease in the number of red blood cells, hemoglobin level and an increase in the number of white blood cells. Changes in morphological parameters are observed on the 21st day of the experiment in chickens of the second experimental group which were given cadmium sulfate at a dose of 4 mg/kg of body weight.
Imposing the body of chickens with cadmium burden contributed to the liver functional state disorder which is demonstrated by the increased activity of aminotransferases in their blood serum. The activity of alanine and aspartate aminotransferases was higher in the serum of chickens of the second experimental group on the 21st and 30th day of the experiment.
Keywords: Toxicology; cadmium sulfate; poultry; morphological parameters; blood

Introduction

The problem of the environment pollution by cadmium, which stems from the intensification of industrial and agricultural production, has now become the issue of a particular concern (Uetani et al., 2005; Gutyj et al., 2017; Sachko et al., 2016). The increase in the content of this metal in the soils of Ukraine and other countries, which has been observed for the last decades, is accompanied with the accumulation of Cd2+ in agricultural goods and animal nutrition products causing the threat to human and animal health (Hutyi, 2016; Gutyj et al., 2016; Hradovych et al., 2016; Grushanska, 2017).
Cadmium toxic exposure appears in a number of tissues and organs (kidneys, liver, lungs, reproductive glands) as well as systems (excretory, cardiovascular, hematopoietic) (Fregoneze et al., 1997; Rodríguez et al., 2001; Lu et al., 2005; Liu et al., 2008; Al-Azemi et al., 2010). Cadmium is known by its long half-life in human and animal bodies, by interaction with bivalent metals, both at the level of absorption and the one of metabolism in tissues and organs (Antonio et al., 1998; Pavan Kumar and Prasad, 2004; El-Shahat et al., 2009).

Cadmium and its compounds belong to the immunotoxicants which cause the functioning disorder of the body immune system; reduce resistance to infections, lead to appearance 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; Vishchur et al., 2016).
In this regard, the impact of cadmium on the human and animal body has been the subject of a detailed investigation, especially for the last decade.
The aim of the research was to study the effect of cadmium sulfate at doses of 2 and 4 mg/kg of body weight on morphological and biochemical parameters of laying chicken blood.

Materials and methods

24 Hisex White laying chickens ages 78 weeks were selected for the experiment. Three groups, one control and two experimental, were formed out of that number. The groups were formed on the principle of analogy (age and live weight). Chickens from different groups were labeled with persistent organic dyes. The chickens of the control group were on their regular nutrition, they were provided with the combined feed and water without adding of cadmium sulfate. The drinking water for the chickens from the experimental groups was supplied with cadmium sulphate for 30 days at different doses: the first group - 2 mg/kg of body weight, the second group - 4 mg/kg of body weight.
The living conditions and microclimate parameters in the room were similar for all groups of poultry. The amount of consumed combined feed and water was recorded during the experiment.
All experimental intrusion, interventions and slaughtering of animals were carried out in compliance with the requirements of the *European Convention for the Protection of Vertebrate Animals Used for Experimental and other Scientific Purposes* (Strasbourg, 1986) and the decisions of the *First National Congress on Bioethics* (Kyiv, 2001).
The blood of the laying chickens was drawn from the axillary vein at different time: before adding cadmium sulfate to nutrition and on the first, seventh, fourteenth, twenty-first and thirtieth days of the experiment.
The amount of erythrocytes and leucocytes was counted on the Goryaev camera according to generally recognizable method. The level of blood hemoglobin was determined by the cyanide hemoglobin method with the usage of FEK-M according to the method of H. V. Derviz and A. H. Vorobiov. The amount of hematocrit was determined by blood centrifugation in micropipettes 3000 rpm. The serum was determined by the activity of alanine aminotransferase (AlAT) and aspartate aminotransferase (AsAT) – by the method of Reitman and Frenkel modified by K. G. Kapetanaki (1962) (Vlizlo, 2012).
The analysis of the research results was performed by using the software package Statistica 6.0. The probability of difference was assessed by applying the Student's t-test. The results of the mean values were considered statistically significant when * – $P<0.05$, ** – $P<0.01$, *** – $P<0.001$ (ANOVA).

Results and discussion

Blood composition is a relatively stable indicator which is one of the labile systems of the body of laying chickens as well. The physiological processes occurring in the body largely affect the qualitative composition of the blood (Gutyj et al., 2016; Gutyj et al., 2017; Grynevych et al., 2018). Hematological studies grant the opportunities to investigate thoroughly the effect of cadmium on the body of poultry, which enables more efficient and specific development of a treatment regimen for cadmium toxicosis in poultry.
According to Table 1, the number of red blood cells in the blood of chickens of the experimental and control groups before adding the experiment preparation were within the range of normal physiological values, namely within 3.15 ± 0.10 - 3.22 ± 0.10 T/l. After administration of water with cadmium sulfate at a dose of 2 mg/kg of body weight, the number of erythrocytes increased to 3.29 ± 0.16 T/l on the 7th day of the experiment, whereas this count in the control group was 3.21 ± 0.13 T/l. The chickens of the second experimental group demonstrated the lowest number of erythrocytes in the specified period of time.
Further decrease in number of erythrocytes in the blood of chickens from the experimental groups was observed. On the 14th day of the experiment the erythrocyte level went down by 6.2% in the blood of poultry in the first experimental group and by 11.7% in the blood of the experimental group E_2. On the 21st and 30th days of the experiment the number of red blood cells continued to decline, which resulted in the decrease of up to 2.69 ± 0.13 T/l in the first experimental group and and 2.36 ± 0.14 T/l in the second group, while the figure ranged within 3.23 ± 0.13 T/L for the chickens of the control group. On the 30th day of the experiment, the number of erythrocytes in the blood of chickens of the experimental groups increased slightly in comparison with the previous day, however, compared to the chickens from the control group the count remained low level.
Similar changes were observed while exploring hemoglobin levels. Hemoglobin is known to be a respiratory blood pigment that mainly serves as a carrier for molecular oxygen from respiratory organs to tissue (Khariv et al., 2016). The data in Table 1 demonstrate that the hemoglobin levels in the blood of chickens from the control group fluctuated within 97.67 ± 0.76 g/l throughout all the experiment.

Table 1. Morphological parameters of the blood of laying chickens under cadmium burden (M ± m, n=8).

Experimantal groups	Before supplying water with cadmium sulfate	Days of experiment 7	14	21	30
Erythrocytes, T/l					
C	3.19 ± 0.12	3.21 ± 0.13	3.25 ± 0.12	3.23 ± 0.13	3.20 ± 0.12
E1	3.22 ± 0.10	3.29 ± 0.16	3.05 ± 0.11	2.69 ± 0.13**	2.85 ± 0.15*
E2	3.15 ± 0.10	3.07 ± 0.15	2.87 ± 0.14	2.36 ± 0.14***	2.49 ± 0.16**

Hemoglobin, g/l					
C	97.67 ± 0.76	98.46 ± 0.63	99.11 ± 0.70	98.55 ± 0.77	97.67 ± 0.76
E1	99.15 ± 0.66	102.43 ± 1.12*	93.23 ± 1.32**	86.61 ± 1.11***	90.72 ± 1.21**
E2	98.58 ± 0.73	87.32 ± 1.10**	81.22 ± 1.20***	72.17 ± 0.73***	83.56 ± 0.93**
Hematocrit, %					
C	39.18 ± 0.70	39.63 ± 0.73	39.44 ± 0.68	39.71 ± 0.75	39.60 ± 0.73
E1	39.54 ± 0.67	38.23 ± 0.67	37.84 ± 0.90	36.10 ± 0.74*	37.44 ± 0.85*
E2	39.43 ± 0.75	37.56 ± 0.81	36.71 ± 0.80*	34.11 ± 0.63**	35.23 ± 0.90*
Leukocytes, G/l					
C	31.7 ± 0.75	31.5 ± 0.58	31.9 ± 0.55	31.8 ± 0.55	31.7 ± 0.65
E1	31.9 ± 0.70	33.9 ± 0.55*	34.2 ± 0.70*	34.9 ± 0.60*	33.8 ± 0.70*
E2	31.8 ± 0.70	34.1 ± 0.70*	34.7 ± 0.65*	35.8 ± 0.66**	34.1 ± 0.70*

When cadmium sulfate was administered with water at a dose of 2 mg/kg of body weight, a slight increase in the hemoglobin level in the blood of this experimental group was established, which could happen due to the body's protective and adaptive response to the toxicant intake. On the 14th and 21st days of the experiment hemoglobin levels in the chicken blood of the first experimental group gradually rose up to 86.61 ± 1.11 g/l, whereas in the control group this indicator was 98.55 ± 0.77 g/l, which means that experimental indicators were by 12% lower than the control ones.

When giving poultry cadmium sulfate water at a dose of 4 mg/kg of body weight, gradual decrease in hemoglobin levels was established beginning on the 7th days of the experiment. The subsequent periods of experiment were marked with the continuing decline in hemoglobin levels. On the 14th day it went down by 18%, on the 21st day – by 26.8% compared with indicators of the control group of chickens.

The hematocrit value reflects the ratio between the clusters of blood cells and blood plasma. After consuming cadmium sulfate at doses 2–4 mg/kg of body weight the laying chicken demonstrated a gradual deacreae in the hematocrit value (Table 1). The lowest level of this indicator was observed in the second experimental group on the 21st day of the experiment, when it was 34.11 ± 0.63%.

The outcome of the research on laying chickens (Table 1) showed that after feeding them the with cadmium sulphate water at a dose of 2 mg/kg of body weight the number of leukocytes in their blood gradually increased during the whole experiment: on the 7th day it rose by 7.6%, on the 14th day – by 7.2%, on the 21st day – by 9.7%, on the 30th day – by 6.6%. Supplying chickens with cadmium sulfate water at a higher dose (4 mg/kg of body weight) led to an increase in number of leukocytes in the blood of the second experimental group as well. The number of leukocytes in the blood of this experimental group underwent the greatest boost on the 14th and 21st days of the experiment, when, compared with the control group of chickens, it increased by 8.8 and 12.6% respectively. Apparently, leukocytosis was caused by the development of inflammatory processes in the digestive tract under the influence of cadmium toxic exposure, which typically results in the increase in quantity of leukocytes in the chicken blood.

Since cadmium negatively affects the liver functioning, it is therefore necessary to investigate the functional state of the liver. The activity of aminotransferases was indicated in the blood of chickens as these enzymes reflect the functional state of the liver. Aminotransferases are known to be involved in transamination processes. They carry amino groups from amino acids to keto acids. One of the enzymes that belongs to this group of aminotransferases is the alanine aminotransferase. The activity of this enzyme under cadmium burden is shown in Table 2. Alanine-aminotransferase is a special enzyme that is included in the classification of transferases, the main function of which is to catalyze the turnover of the amino group NH_2 directly from the amino acid alanine for alpha ketoglutarate, which leads to the creation of pyruvic acid and glutamate. Alanine-aminotransferase is one of the main indicators of liver cell damage. According to the the data in Table 2, the activity of alanine-aminotransferase in the blood serum of the poultry before administering cadmium sulfate water was within the range of values from 0.30 ± 0.009 to 0.32 ± 0.005 mmol/g/l After adding the toxicant mentioned above to the chicken nutrition, the activity of the enzyme increased in the poultry experimental groups. It was established that, under cadmium burden, the activity of the enzyme started frowing from the 14th day of the experiment, when it reached the level by 15% and 21% highter than the initial one in the first and second experimental groups respectively. On the 21st day of the experiment, the activity of alanine aminotransferase ranged from 0.40 ± 0.008 – 0.45 ± 0.009 mmol/g/l, whereas in the control group of chickens this indicator was 0.31 ± 0.007 mmol/g/l. On the 30th day of the experiment, AlAT activity in the serum of the first experimental group was by 26.7% higher, and in the second experimental group – by 43% higher than in the control group of chickens.

In the course of exploring the activity of aspartate aminotransferase, it was found out that its amount in the serum of the control and two experimental groups ranged from 4.27 ± 0.18 to 4.31 ± 0.13 mmol/g/l at the beginning of the experiment. After feeding poultry with cadmium sulfate, an increased activity of this enzyme was established starting from the 7th day of the experiment. On the 14th day of the experiment, an increase in the activity of AsAT in the blood serum of chickens which were given cadmium sulfate at a dose of 2 mg/kg of body weight was found to be 12.4%, respectively. The enzyme activity was slightly lower in the blood serum of chickens from the second experimental group, where it rose up to 5.16 ± 0.23 mmol/g/l, whereas in the control group this indicator ranged within 4.29 ± 0.17 mmol/g/l. On the 21st day of the experiment, the activity of AsAT in the serum of chickens from both experimental groups continued to grow and increased relatively to the indicators of the control group of chickens by 18.2 and 31%, respectively.

Table 2. Aminotransferase activity of laying chicken blood serum under cadmium burden (M ± m, n=8).

Experimantal groups	Before supplying water with cadmium sulfate	Days of experiment			
		7	14	21	30
AlAT, mmol/g/l					
C	0.31 ± 0.008	0.30 ± 0.006	0.33 ± 0.005	0.31± 0.007	0.30 ± 0.005
E1	0.32 ± 0.005	0.34 ± 0.009*	0.38 ± 0.007***	0.40 ± 0.008***	0.38 ± 0.008***
E2	0.30 ± 0.009	0.36 ± 0.007*	0.40 ± 0.009***	0.45 ± 0.009***	0.43 ± 0.008***
AsAT, 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***

On the 30th day of the experiment, the activity of the enzyme AsAT, in comparison with the previous days, began to diminish to 4.93 ± 0.24 and 5.54 ± 0.25 mmol/g/l.

High aminotransferase activity in the serum of chickens under cadmium burden indicates destructive processes in the liver, which cause an increase in the emission of aminotransaminases from cellular organelles in the blood of experimental poultry. Thus, the results obtained indicate an increase in destructive processes in the body of chickens under cadmium burden.

It is worth mentioning that the activity of alanine aminotransferase increased to a greater extent than the activity of aspartate aminotransferase. This is due to the fact that AlAT, even with minor destructive lesions of the hepatocyte membranes under cadmium burden, is easily released from them and enters the blood, while AsAT is contained in the hepatocyte mitochondria and, consequently, the penetration of this enzyme into the blood is complicated by the fact this enzyme must also penetrate the mitochondrial membrane alongside with the surface cell membrane, which occurs at high doses of cadmium exposure.

Conclusion

Feeding the laying chickens with cadmium sulfate at doses of 2 and 4 mg/kg of body weight contributed to the decrease in the number of red blood cells, hemoglobin level as well as the increase in leukocytes. Noticeable morphological changes are observed on the 21st day of the experiment with the chickens from the second experimental group which were provided with cadmium sulfate at a dose of 4 mg/kg body weight.

The cadmium burden on the body of chickens affected harmfully the functional state of the liver, which is supported by the fact of increased activity of aminotransferases in their blood serum. The activity of alanine and aspartate aminotransferase was higher in the serum of chickens of the second experimental group on the 21st and 30th day of the experiment.

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