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Effect of Copper On Biological and Productive Parameters of Laying Hens.

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ABSTRACT

Copper is an essential trace element performing important biological functions in many organisms. It usually affects organisms in a strictly dosage dependent manner being essential at low and toxic at higher concentrations. In the present study the influence of higher doses of copper on biological and productive parameters, the patterns of copper accumulation in various organs and tissues and the pathological changes of internal organs have been investigated in laying hens. It was revealed a differentiation of organs and tissues according to the degree of copper accumulation. The most copper was accumulated in the feather, liver and kidney of laying hens. It was found that doses of copper that are excessing the maximum permissible level (MPL) in 20 and 30 times lead to a sharp decrease in viability,body weight and egg productivity of laying hens as well as to severe pathological changes in organs and tissues. The toxic effect of higher doses of copper was also manifested in the decrease of the serum total protein and bilirubin concentration as well as in the increase of the hemoglobin concentration in blood and aspartate aminotransferase concentration in serum.
Keywords: poultry, copper, viability, productivity, blood biochemistry, ecology

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INTRODUCTION

Copper is a common natural chemical element, which is identified in trace amounts in animals and humans in different organs and tissues, largely in the form of ion Cu^{2+}. The highest copper concentrations were noted in the liver and brain (Turnlund et al., 1998). Copper is an essential trace element involved in different physiological processes. Copper is necessary for formation of myelin – the protective layer that covers the axons, in addition it participates in the formation of melanin pigment.

Copper is a cofactor of enzymes involved in redox reactions of the cell. In particular, copper is a part of cytochrome C oxidase – a multiprotein complex, which plays a key role in the energy production in eukaryotic cells (Gaetke et al., 2014). In addition this trace element is a cofactor of enzymes (such as catalase, superoxiddismutase, etc.), involved in antioxidant protection of cells (Tchounwou et al., 2012). However, at high concentrations copper can be toxic and affect productivity and viability of animals (Afonina et al., 2003 a, b; Narozhnykh et al., 2013). In excessive concentrations, copper can directly catalyze the formation of hydroxyl radicals through Fenton's reaction (Jomova, Valko, 2011). In addition, the elevated levels of copper lead to a substantial decrease of glutathione levels (Speisky et al., 2009), which is an essential component of the antioxidant system of the cell.Therefore, an excessive intake of copper by the cell can lead to oxidative stress when accumulation of reactive oxygen species exceeds the capacity of the antioxidant system of the cell. The accumulation of active radicals can cause DNA damage, lipid peroxidation, forming part of cell membranes and modification of biological macromolecules. Currently, oxidative stress is regarded as a key mechanism in the pathogenesis of acute or chronic poisoning of the organism with heavy metals (Valko et al., 2005; Jan et al., 2015; Wu et al., 2016). In humans, the chronic intoxication with heavy metals, including copper, can lead to progressive neurodegenerative processes and cause multiple sclerosis, a disease of Parkinson, Alzheimer's disease, muscular dystrophy. In addition, heavy metals can have carcinogenic effects (Jarup, 2003; Jaishankar et al., 2014).

Due to the significant risk of copper as ecotoxicant, it is an urgent need to study the pattern of copper accumulation in organs and tissues of farm animals, particularly in poultry. Currently it is known a number of studies devoted to the influence of heavy metals on the organism and also the pattern of accumulation of heavy metals by animal organs and tissues, including poultry (Petukhova, 2012; Narozhnyh et al., 2013; Miller et. al., 2013, 2014; Petukhov et al., 2016). There was a positive correlation between a lead content in soil and its accumulation in eggs of hens breed at the farmstead (Grace, MacFarlane, 2016). The exposure of broilers to tin at a dose of 720 mg/kg resulted in a reduced feed intake and body weight, a reduced content of hemoglobin and the number of red blood cells (Sun et al., 2014). In addition, it was also found that the exposure of broilers to tin at a dose of 720 mg/kg had decreased the activity of superoxide dismutase and glutathione peroxidase in the liver, while the content of malondialdehyde increased, which served as a marker of oxidative stress. However, a comprehensive study on the impact of copper on various physiological systems of laying hens was not conducted.

The purpose of this study was to examine effects of copper on biological and productive parameters, the level of copper accumulation by various organs and tissues and pathological changes in internal organs in laying hens.

MATERIALS AND METHODS

The study was carried out on laying hens at age of 180 days, bred at the poultry farm "No 1 in Western Siberia." The control group (first group, n=30) and four experimental groups (10 animals in each of them) were formed. Within 30 days daily hens in the experimental groups by using the dispenser were drinking of 5 ml of an aqueous solution of copper chloride in concentrations exceeding the maximum permissible level (MPL) in 5 times (second group), in 10 times (the third group), in 20 times (the fourth group) and in 30 times (the fifth group).

Twice a day daily before drinking of salt solutions the eggs were gathered and their number was calculated in each group. Egg production was calculated as the number of eggs laid by one hen per day. After 30 days a body weight was determined and the slaughter of hens of the control and experimental groups was carried out. Samples of blood, muscles, liver and kidneys were taken. The number of erythrocytes was determined using the Goryaev`s camera. The number of leukocytes (WBC) was calculated visually using a hematological counter SDT-16.

Biochemical parameters were determined in the blood: hemoglobin, total protein, albumin, bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and creatinine. Total protein content was assessed by a refractometric method. The amount of hemoglobin was determined by hemoglobincyanide method using a spectrophotometer. The serum content of bilirubin was established by the method of Jendrassik-Cleghorn-Goff. The determination of creatinine was produced by a color Jaffa reaction, the content of transaminases (AST and ALT) was determined by dinitrophenilhydrazine method. The de Ritis coefficient was calculated, which represents the ratio of the serum concentrations of AST to ALT (Bortos and Sikaris, 2013).

The determination of the mass concentration of copperin feed and water, as well as in organs and tissues of hens was carried out by the inversion voltammetry method using the analyzer TA-2 in scientific and production enterprise «Technoanalit» (Tomsk).

The results were processed by methods of variation statistics. The significance of differences between mean values was determined using Student's t-test and nonparametric Mann-Whitney test. The resultswere presented as a mean and its standard error (Mean±SE).

THE RESULTS OF A STUDY

Viability, body weight and egg productivity. In the control group during 30 days of experiment the death of hens was not observed, however, in the experimental group after the exposure of copper in a dose exceeding the MPL in 5 and in 10 times, 20% of birds were dead towards the end of the experiment. After intake of copper in the dose of 20 MPL within 30 days only 30% of hens was alive, with a dose of 30 MPL all the hens died before the end of the experiment. The average life expectancy in groups 2 and 3 did not differ significantly compare to the control. Copper doses excessing the MPL in 20 and 30 times led to a significant reduction in life expectancy of hens (p<0.01, tab. 1).

Table 1. Influence of copper on the viability, body weight and egg productivity of hens

Group	Excesso fcopper limit	Weight, g	Egg productivity, egg per day	Lifetime, day	Safety of chickens, %
1	Control	1971.7±27.8	0.62±0.02	30.00	100
2	5	1900.0±18.9	0.78±0.03*	27.9±1.8	80
3	10	1975.0±25.0	0.42±0.05*	26.5±2.3	80
4	20	1500.0±152.8*	0.09±0.02*	16.8±3.2*	30
5	30	-	-	7.1±1.3*	0

Here and below * - significant differences from the control (p <0.05);
** - significant differences from the control (p <0.01)

Copper doses of 5 and 10 MPL did not have any effect on body weight (table 1), but with the intake of copper in the dose of 20 MPL the body weight of experimental birds was significantly reduced compared with the control (p<0.01). Copper doses excessing the MPL in 5 times significantly (p<0.01) increased the egg production of hens (table 1), at the same time the intake of copper at doses of 10 and 20 MPL led to a decrease of the egg productivity compared to the control (p<0.01).

*Accumulation of copper in organs and tissues.*Under copperexposurein a dose excessing the MPL in 5 times the copper content in the muscles, bones and liver was not significantly different compare to the control

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group (table 2), however, the content of a metal in the feather and kidney was significantly increased (p<0.05). The intake of copper in doses that exceed the MPL in 10, 20 and 30 times resulted in a progressive significant increase of the content of this metal in the studied organs and tissues compared to the control group. The difference between the experimental groups was also significant (p<0.01).

Table 2. Copper content (mg/kg) in the organs and tissues of laying hens

Ogran, tissure	Control	Exceeding the maximum permissible level, in several times			
		5	10	20	30
Muscles	0.53±0.03	0.87±0.21	1.02±0.08*	13.29±0.82*	51.7±6.54*
Bones	0.43±0.03	0.37±0.03	0.67±0.04*	2.08±0.11*	36.8±3.51*
Feather	3.54±0.22	47.5±3.94*	110.8±20.1*	130.3±6.62*	-
Liver	2.27±0.08	2.90±0.18	9.31±1.02*	365±36.1*	518.0±84.2*
Kidney	1.68±0.14	2.34±0.25*	6.11±0.19*	51.2±6.55*	183.7±30.3*

Hematological and blood biochemical parameters. Higher doses of copper had no impact on the number of red blood cells. The number of erythrocytes in the blood of laying hens of the control group, and also, upon the intake of copper in the dose of 5, 10 and 20 MPL was 2.52±0.04, 2.39±0.12, 2.54±0.07 and 2.44±0.32 million, respectively. Already at 5 MPL the hemoglobin level (table 3) in the blood was increased compared to the control in 2.5 times (p<0.01). Upon the intake of copper in doses of 10 and 20 MPL the hemoglobin content in the blood was higher than in the control group, but lower than that at 5 MPL.

The copper doses exceeding MPL in 5, 10 and 20 times reduced the concentration of total protein in the blood serum (table 3) compared with the control group (p<0.01). Higher doses of copper resulted in a decrease of bilirubin concentration compared to the control (p<0.01).

The serum AST concentration (table 3) was higher in experimental groups, compare to the control group (p<0.01), but the difference in this parameter between the experimental groups was not significant.

Table 3. Effect of copper on biochemical parameters of blood in laying hens

Figure	Control	Exceeding MPL in…times		
		5	10	20
Total protein, g/l	72.7±2.90	56.0±0.90**	57.8±1.1**	46.0±4,51**
Creatinine, mmol/l	46.5±1.51	42.7±1.80	45.1±0.52	45.3±3.18
Bilirubin, µmol/l	7.9±0.25	6.2±0.24**	6.4±0.37**	6.1±1.01**
AST, mmol/l	3.7±0.34	4.9±0.06**	4.9±0.14**	4.8±0.12**
ALT, mmol/l	1.3±0.09	0.83±0.08**	1.9±0.15*	0.9±0.14**
Coeff. de Ritis	2.9±0.22	6.3±0.60**	2.8±0.24	5.5±0.64**
Hemoglobin, g/l	66.03±0.25	167.86±1.66**	118.63±1.00**	115.67±2.73**

The different pattern was observed concerning the serum ALT concentration. Copper supplement in the dose of 10 MPL significantly increased of this parameter compared to the control. However, at 5 and 10 MPL the serum ALT concentration was significantly reduced compared to the control.

The de Ritis coefficient in the 5 and 20 MPL groups was higher ($p<0.01$) than in the control group, while there were not any significant differences in this index between the control and the third group (the copper supplement in the dose of 10 MPL).

The intake of high doses of copper in the organism of hens had no significant effect on the serum creatinine concentration (table 3).

*Pathological changes in the organs.*The numerous changes in the internal organs were observed in birds of all experimental groups. Salivation and gagging was observed after receiving the salt solution of copper. The mucous membranes of the mouth, craw and stomach were inflamed, ulcerated and had acquired a greenish-blue hue. After receiving of copper in doses exceeding MPL in 5 times the increase of the kidneys size observed in 30% of the birds, a blood over filling of the organ was noted in 30% of cases. After receiving of copper in the dose of 10 MPL the kidneys were increased in 80% of birds, the increased blood over filling was observed in 20% of cases. After receiving of copper in the dose of 30 MPL the kidneys of all the birds were increased, and in 20% of hens was marked a sagging of the body. Uric acid diathesis was noted in 40% of cases, and in 10% of hens foci of necrosis were observed in the kidneys.

The effect of copper on the liver already at 5 MPL was manifested by sagging of the tissue in 40% of cases. After receiving of copper in the dose of 10 MPL the sagging of the liver was found in 60% of birds and in 20% of hens foci of necrosis were discovered. After copper supplementation in doses exceeding 20 MPL in 50% of cases there was a liver sagging, in 10% of cases there were hemorrhage and foci of necrosis, and in 20% of birds a fatty degeneration of the liver was observed. After copper supplementation in doses exceeding the MPL in 30 times, flabby liver was noted in 40 % birds, in 10 % of the cases the liver had a heterogeneous structure with dotted or striped hemorrhage, and foci of necrosis in the liver was observed in 70% of the birds.

DISCUSSION OF RESULTS

In this study it was established that the copper intake in a dose exceeding the maximum permissible level in 5 times did not lead to the increase of the copper content in the muscles, bones and liver. However, interestingly, that the copper intake in the dose of 5 MPL led to a sharp (almost in 13.5 times) increase of the copper content in the feather of birds. In a previous study, it was found that the intake of high concentrations of tin in food also led to a preferential accumulation of this element in the feather of birds, but the mechanism of this selectivity remains unclear (Sun et al., 2014). The increased accumulation of copper in the feather of birds probably could be used as a good marker for copper pollution in the environment. After the intake of copper in the dose of 10 MPL the content of this element in all the studied organs and tissues increases significantly and from 20 to 30 MPL increases ten fold. And at 30 MPL the copper content in the muscles, bones and kidneys exceeded its concentration in the control group in 98, 85, and 109 times respectively. However, the content of copper in the liver at 30 MPL exceeded its concentration this figure in the control group in 228 times. Thus, our study revealed a differentiation of organs and tissues according to the degree of accumulation of copper.

The intake of copper in the dose exceeding the MPL in 5 times did not lead to significant changes in body weight, mortality, life expectancy of hens and even "stimulated" egg production. We can assume that at a given copper concentration physiological mechanisms involved in maintaining of copper homeostasis in the organism are still able to compensate for the increased intake of this trace mineral with water.

However, with the intake of copper in doses exceeding MPL in 20 and 30 times the mortality of chickens was dramatically increased (at 30 MPL the experimental hens died in 100%) with a decrease of body weight, lifespan and egg production.

It is known that 30-50% of the copper entering the body are absorbed in the small intestine mainly in the form of ions Cu^{2+}. In the blood a copper is transported by albumin and a specific protein transcuperin. Later on copper accumulates in the liver and can come in the plasma or excretes with the bile. Thus, in mammals the liver is the main organ that provides the accumulation, distribution and excretion of copper in the body (Gaetke et al., 2014).

In our study the intake of copper in doses excessing the MPL in 5 times led to a decrease of total protein and bilirubin level in the serum, which may indicate to a liver dysfunction. In addition, at high copper doses severe pathological changes in the liver and kidneys were observed. Damage of liver and kidney by heavy metals was also observed in other studies. The intake of vanadium in broilers at doses of 30 – 60 mg/kg resulted in a granular degeneration of the epithelial cells of the kidney, and the granular degeneration and fatty degeneration of hepatocytes. In addition, the authors noted a decrease in the activity of superoxide dismutase and glutathione-peroxidase in liver and kidney, and an increased content of malondialdehyde in these organs, which is a marker of oxidative stress (Liu et al., 2012). Granular degeneration and necrosis of hepatocytes are also revealed in chickens that were exposed to a copper sulphate during embryonic development (Oguz et al., 2010). The liver dysfunction due to a copper damage was also observed in humans (Uriu-Adams, Keen, 2005). Thus, the intake of high doses of copper (exceeding maximum tolerance by more than 10 times) by laying hensin our study resulted in increased copper accumulation in the liver (the content of copper in the liver already at 10 MPL increased in 4 times in comparison with the control group) and much damage of hepatocytes, probably through oxidative stress. Dysfunction of liver and kidney probably was the main cause of the sharp decline in viability, egg production and body weight of hens in our study.

In our experiments the increase in serum concentration of AST and the de Ritis coefficient was revealed at the intake of elevated concentrations of copper. The increase in serum concentration of AST and accordingly the de Ritis coefficient may occur as a result of muscular tissue destruction or hemolysis of red blood cells (Bortos, Sikaris, 2013), which can be observed under exposure to high doses of copper (Fernandes et al., 1988).

A well-known series of works demonstrated the possibility of the “in vivo” determination of heavy metals in organs and tissues of farm animals and fish (Korotkevich et al, 2014 a, b; Petukhov et al, 2007, 2010). The serum zinc concentration in laying hens can predict the accumulation of this metal in the muscles and bones (Petukhov et al, 2001). The level of heavy metals in organs and tissues of animals may also characterize the ecological situation in a certain area.

Thus, in the study it was established that the intake of copper in concentrations exceeding the MPL in more than 10 times, leads to an increase in the content of this element in all studied organs and tissues, as well as to a sharp decrease in viability, body weight and egg productivity of hens. The results of our study and literature data suggest that when the concentration of copper is 10 MPL and more the adaptive physiological mechanisms get broken, mainly as a result of disturbance of redox balance in the cells of the liver and kidney that leads to the reduced viability and productivity of laying hens.

CONCLUSION

Copper doses exceeding the maximum permissible level in 10, 20 and 30 times lead to lower viability, body weight and egg productivity of laying hens, as well as to the severe pathological changes in internal organs indicating. A disruption of physiological mechanisms that provide the copper homeostasis.

A differentiation of organs and tissues according to the degree of accumulation of copper was revealed. Under copper doses excessing of the MPL in 5 times, the copper concentration was increased only in the feather. At 10, 20, 30 of MPL the copper concentration was progressively increased in all organs and tissues with the increasing dose of copper. However, the copper accumulation in liver and kidney was more significant than in the bones and muscles.

The intake of high doses of copper had no impact on the number of red blood cells and creatinine in the blood. A toxic effect of copper was evident in the decrease in the serum total protein and bilirubin concentration and in the increase of hemoglobin in blood and AST concentration in the blood serum.

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