The effect of milk thistle, metiphen, and silimevit on the protein-synthesizing function of the liver of laying hens in experimental chronic cadmium toxicosis

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The study aimed to investigate the effect of milk thistle, metiphen, and silimevit on the liver's protein-synthesizing function of laying hens in examination chronic cadmium toxicosis. Thirty-two chickens, 78 weeks old, were selected for the study. Four groups were formed: control and three experimental. Birds of the experimental group (E1) with fodder were fed the fruits of milk thistle at a dose of 2.0 g/kg of feed once a day for 30 days. Chickens of the experimental group (E2) were fed metiphen at a dose of 0.28 g/kg of feed nutrition once a day for 30 days. Hens of the experimental group (E3) were fed silimevit at a dose of 0.36 g/kg of feed nutrition once a day for 30 days. The results showed that the total protein content and its plasma fractions of birds vary throughout the research in poultry control and experimental groups. Thus, in the blood of laying hens subjected to cadmium loading, a decrease in their blood of total protein and albumin fraction was found. Under the cadmium toxicosis of chickens, milk thistle, metiphen, and silimevit positively affect the liver's protein-synthesizing function, as indicated by an increase in the total protein level and albumin. Feeding with milk thistle and silimevit contributed to a better normalizing effect on the liver's protein-synthesizing function than the use of metiphen.

Keywords: toxicology, cadmium, poultry, blood, silimevit, metiphen, milk thistle, total protein, albumins, proteins.

Introduction

The problem of cadmium pollution, which is one of the consequences of the intensification of industrial and agricultural production, has now become especially relevant (Vorozhenko & Skalskyi, 2011; Vishchur et al., 2019; Piven et al., 2020). The increase in the content of this metal in the soils of Ukraine and other countries over the past decades is accompanied by the accumulation of Cd⁺ in agricultural products and feed, increasing the threat to human, animal, and poultry health (Uetani et al., 2005; Antoniak et al., 2010; Gutyj et al., 2016; 2017; 2019; Prysiazhniuk et al., 2019; Slivinska et al., 2020).

It is known that the intake of cadmium is associated with the environmental risk to the body due to its cumulative toxicity to organs and systems, leads to a decrease in growth rate and productivity of birds. This negatively affects the efficiency of the poultry industry. It requires an in-depth study of pharmaco-toxicological and biochemical processes underlying Cadmium-induced metabolic disorders of the bird's vital functions.

Cadmium poisoning of animals occurs when it enters the stomach or by inhalation into the respiratory tract (Al-Azem et al., 2010; Lavryshyn & Gutyj, 2019). When ingested, cadmium inhibits mitochondria's activity while increasing the sensitivity of cells to free radical oxidation (Peng et al., 2015; Slobodian et al., 2019). Cadmium has a high migration rate, biochemical activity, polytropic toxicity, and the ability to accumulate in organs and tissues (Ali et al., 1986; Lavryshyn et al., 2019). It should be noted that about 50% of absorbed cadmium accumulates in the liver and kidneys. Intensive inclusion of cadmium in the liver and kidneys tissues is associated primarily with high activity of biochemical processes occurring in these organs (Slobodian et al., 2019; Gutyj et al., 2019).

The adverse effects of cadmium cover different cell types, but their sensitivity to this element's action is different (Fregoneze et al., 1997; El-Shahat et al., 2009). The depth of cell damage is mostly determined by the accumulated dose of cadmium and the level of expression of metallothionein genes in them, which is affected by the toxic element. Thus, cadmium effects are manifested in different ways under long-term and single entry into the animals' bodies (Rodriguez et al., 2001; Salvatori et al., 2004).

Keywords: toxicity, cadmium, poultry, blood, silimevit, metiphen, milk thistle, total protein, albumins, proteins.
After oral absorption, cadmium is first transported through the portal vein to the liver, accumulates in hepatocytes, where it induces the synthesis of metallothioneins. Which, in turn, reduces the toxic effects of cadmium in the kidneys and liver (Lu et al., 2005; Liu et al., 2008). The intensity of metallothionein synthesis in hepatocytes in response to the influx of cadmium ions is higher than in other cells. Histological changes in the liver in cadmium toxicosis are represented by dystrophy manifestations, apoptosis, and inflammatory response. Dystrophic processes were manifested by hydropic dystrophy with vacuolation of the cytoplasm of hepatocytes, in places to focal colic necrosis (Guty et al., 2019).

Cadmium's indirect hepatotoxic effect is due to Kupffer cells' activation, which triggers a cascade of biochemical processes involving many cytokines and inflammatory mediators (Melnychuk et al., 2004; Khariv et al., 2017).

**The study aimed** to investigate the effect of milk thistle, methiphen, and silimevit on the liver's protein-synthesizing function of laying hens in experimental chronic cadmium toxicosis.

### Materials and methods

Thirty-two chickens, 78 weeks old, were selected for the study. Four groups were formed: control and three experimental. Birds of the experimental group (E₁) with fodder were fed the fruits of milk thistle at a dose of 2.0 g/kg of feed once a day for 30 days. Chickens of the experimental group (E₂) were fed metifen at a dose of 0.28 g/kg of nutrition once a day for 30 days. Hens of the experimental group (E₃) were fed silimevit at a dose of 0.36 g/kg of feed nutrition once a day for 30 days.

The conditions of keeping chickens and the microclimate parameters in the room for all groups were similar. The amount of feed and water consumed was taken into account during the test. All experimental interventions and slaughter of animals were carried out in compliance with the European Convention's requirements for the Protection of Vertebrate Animals Used for Experimental and Scientific Purposes (Strasbourg, 1985) and the decision of the First National Congress on Bioethics (Kyiv, 2001).

Blood from birds was collected from the vein under the wing in the periods: before administering drugs and cadmium sulfate, on the first, seventh, fourteenth, twenty-first, and thirtieth days of the test. The protein concentration and its fractions were studied according to the method (Vlizlo, 2012).

The analyses used the following drugs:

- **Cadmium sulfate** - Cadmium sulfate is an inorganic compound with the chemical formula CdSO₄. Cadmium sulfate is well soluble in water, so it is well absorbed in the digestive tract, blood and is known for its toxic effects on living organisms.

- **Milk thistle** (MT) - *Silybum marianum* is a family of compound flowers, grows wild in wastelands, along roads, abandoned fields, and it is cultivated in medicinal gardens. Milk thistle fruits are used for treatment. They contain 17-18% protein, 10-11% fat, 2-3% flavonoilignans, 0.08% essential oil, vitamins A, E, K, nutrients, and quercetin.

- **Metifen** (M) is a white crystalline powder, sweet in taste, with the smell of sulfur. Poorly soluble in cold water, better – In hot water (1:20). Thermostable. The drug contains phenarone and methionine.

- **Silimevit** (S) – feed additive, which includes the fruits of milk thistle, selenium, methiphen, vitamins A, E and C (Sobolev et al., 2017; 2020; Ostapyuk & Guty, 2020; Martynyshuk et al., 2020).

The analysis of research results was performed using the software package Statistica 6.0. The Student's *t*-test assessed the probability of differences. The results of the mean values were considered statistically significant at * - P<0.05, ** - P<0.01, *** - P<0.001 (ANOVA).

### Results and discussion

To analyze the mechanism of action of milk thistle, methiphen, and silimevit on the body of chickens of the required values, the state of metabolic processes is examined. The obtained results of biochemical studies of the blood of laying hens for cadmium loading indicate a violation of the liver's protein-synthesizing function. Our research outcomes showed that the content of total protein and its plasma fractions were performed during a general analysis in the control group and experimental groups. Thus, in the blood of laying hens that carry out cadmium loading, a decrease in their blood total protein to 39.04±1.23 g/l (Table 1).

**Table 1.** The total protein level in the blood of laying hens after the administration of milk thistle, methiphen, and silimevit in chronic cadmium toxicosis (M±m, n=8)

| Blood test time (days) | Total protein (g/l) | Groups of chickens |
|------------------------|---------------------|--------------------|
|                        | Control (cadmium)   | Experimental 1 (cadmium + MT) | Experimental 2 (cadmium + M) | Experimental 3 (cadmium + S) |
| At the beginning of the experiment | 46.58±1.06 | 46.52±1.05 | 46.60±0.90 | 46.45±1.00 |
| the first day          | 45.74±1.10        | 46.28±1.14        | 46.37±1.24        | 46.32±1.15       |
| the 7th day            | 44.62±0.96        | 45.21±1.20        | 45.05±1.11        | 45.45±1.24       |
| the 14th day           | 42.31±1.15        | 44.95±1.18        | 44.75±1.22        | 45.76±1.20*      |
| the 21th day           | 39.04±1.23        | 45.55±1.06**      | 45.34±1.18**      | 46.39±1.21***    |
| the 30th day           | 39.65±1.11        | 46.10±1.25**      | 45.93±1.20**      | 46.51±1.30***    |
In the E2 group's chickens, the total protein level on the 7th day was 46.28±1.14 g/l. Slightly higher levels were in the experimental groups of chickens fed methiphene and silimevit. In chickens of test group E2, the total protein level on the 7th day was 46.28±1.14 g/l. On the 14th day of the research, the total protein level compared with the C group of chickens increased in the group E1 by 6.2% in the E2 group – by 5.8% and in the E3 group – by 8.2%, respectively. On the 1st day of the test, the total protein level in the experimental groups of chickens fed milk thistle and methiphene was in the range of 45.55±1.06 – 45.34±1.18 g/l. The highest level of the studied indicator was in the group of hens fed silimevit. Compared to the C group of birds, it increased by 19%. On the 30th day of the research, all test groups' total protein level was within physiological values.

Similar changes were observed in the examination of albumin content (Table 2). In laying hens of the C group, the rate on the 14th day of was lower than the initial rate by 4.16%, on the 21st day by 5.62%, and on the 30th day – by 3.58%.

**Table 2.** The albumin level in the blood of laying hens after the administration of milk thistle, metiphene, and silimevit in chronic cadmium toxicosis (M±m, n=8)

| Blood test time (days) | Control (cadmium) | Experimental 1 (cadmium + MT) | Experimental 2 (cadmium + M) | Experimental 3 (cadmium + S) |
|------------------------|-------------------|-------------------------------|-------------------------------|-------------------------------|
| At the beginning of the experiment | 33.78±0.93 | 33.92±0.90 | 33.85±0.94 | 33.74±0.70 |
| the first day | 32.92±0.93 | 33.23±0.84 | 33.41±0.93 | 33.68±0.91 |
| the 7th day | 31.84±0.89 | 32.53±0.94 | 32.36±0.82 | 32.83±0.75 |
| the 14th day | 29.62±0.98 | 31.26±0.90 | 31.11±0.94 | 32.91±0.95* |
| the 21st day | 28.16±0.75 | 32.82±0.79*** | 32.64±0.95*** | 33.69±0.67*** |
| the 30th day | 29.34±0.97 | 33.26±0.90** | 33.10±0.85** | 33.80±0.84** |

The albumin level in the blood of chickens of the experimental groups at the beginning of the test ranged from 33.92±0.90 to 33.74±0.70%. On the first day, the level of albumin in all groups decreased slightly. Subsequently, on the 7th and 14th day of the research, the level of albumin in the blood of E1 group and E2 reduced compared with the beginning, but with the C group of chickens increased by 0.69 and 1.64% in the E1 group and 0.52 and 1.49% in the E2 group.

At cadmium loading of laying hens with simultaneous feeding of silimevit, the normalizing effect of the drug on the level of albumin in the blood of chickens of the E2 group was established. During the whole experiment ranged from 32.83±0.75 to 33.80±0.84%. A significant difference between the birds of the control and experimental groups was observed analyzing the indicators of total protein and protein fractions in the blood plasma of laying hens under cadmium loading and the use of drugs from 14 days of the research. In the investigation of the globulin fraction in the blood of laying hens, its progress was found only in the control group of chickens subjected to cadmium loading. On days 14 and 21, the globulins level in the blood of the C group of birds increased by 4.16 and 5.62%. When feeding laying hens of milk thistle, metiphene and silimevit, a slight increase in the globulin fraction was found on the first and seventh days. On the 14th day of the search, the globulins level in the blood of the E1 group and E2 was 68.74±1.84 and 68.89±2.06%. This figure was significantly higher in the C group and was respectively 70.38±1.85%. In the blood of laying hens fed with cadmium sulfate silimevit, a lower level of globulins was found during this experiment, namely 67.09±1.96%. On days 21 and 30 of the practice, the globulins' level in the blood of the first two experimental groups, fed milk thistle and metiphene, declined but did not reach the initial conditions. Only in chickens of the E3 group, which was provided with silymevit, the globulin fraction level was reduced to the level of initial values.

**Table 3.** The globulins level in the blood of laying hens after the administration of milk thistle, metiphene, and silimevit in chronic cadmium toxicosis (M±m, n=8)

| Blood test time (days) | Control (cadmium) | Experimental 1 (cadmium + MT) | Experimental 2 (cadmium + M) | Experimental 3 (cadmium + S) |
|------------------------|-------------------|-------------------------------|-------------------------------|-------------------------------|
| At the beginning of the experiment | 66.22±1.87 | 66.08±1.56 | 66.15±2.00 | 66.26±1.80 |
| the first day | 67.08±1.90 | 66.77±1.76 | 66.59±2.04 | 66.32±2.01 |
| the 7th day | 68.16±2.35 | 67.47±1.96 | 67.64±1.99 | 67.17±1.85 |
| the 14th day | 70.38±1.85 | 68.74±1.84 | 68.89±2.06 | 67.09±1.96 |
| the 21th day | 71.84±2.10 | 67.18±1.93* | 67.36±2.10 | 66.31±2.05* |
| the 30th day | 70.66±1.95 | 66.74±1.87 | 66.90±1.82 | 66.20±2.11* |
Diminished albumin content with a simultaneous increase in the level of globulin fraction is assessed as dysproteinemia due to impaired liver protein-necrosis function on the one hand. On the other hand, the impaired absorption of protein breakdown products from the small intestine is due to mucosal toxin damage.

**Conclusions**

Under the cadmium load of laying hens, milk thistle, metifen and silimevit have a positive effect on the liver's protein-synthesizing function, as indicated by an increase in the total level of protein and albumin. Feeding with milk thistle and silymevit food contributed to a better normalizing effect on the liver's protein-synthesizing function than the use of metifen.

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