Influence of "Geprim dlya kur" on morphobic and chemical indicators of rearing flocks and subsequent productivity of laying hens in prevention of hepatosis

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Abstract. Objective of the study: to determine the impact of "Geprim dlya kur" on the morphobic and chemical parameters of rearing flocks and the subsequent productivity of laying hens in the prevention of hepatosis. The drug was used once in the first day of life as an intramuscular injection. In the course of the experiment, blood and liver were taken for morphological, biochemical and histological studies in poultry of the experimental and control groups during the growing season at intervals of 1 month. It was found out that in the experimental group the preparation stimulates protective and respiratory functions of blood that was expressed in increase in number of leukocytes, erythrocytes and hemoglobin. In the experimental group, the Geprim increases the protein-synthetic function of the liver: in the experimental group of young animals, the level of total protein, albumins and the ratio of albumins to globulins was higher during the two months of the experiment. In the blood serum of experimental chickens, the activity of aspartate aminotransferase increased during two months of the experiment. Alanine aminotransferase activity in the experimental group was higher only in the second month of the experiment - 38.5% in relation to control group. By the end of the third month it decreased in relation to the control group by 36.3%. Histological studies have shown 1. dystrophic liver lesions of young animals are observed during the whole period of cultivation in the form of fat and protein dystrophy; 2. application of heprim reduces the accumulation of fat in the liver by 24.53-43.15%; 3. the preparation prevents protein dystrophy with the achievement of maximum effect by 2 months and further during the whole period of cultivation; 4. Geprim stimulates cellular factors of liver protection. "Geprim dlya kur" in the application of chickens allows obtaining greater productivity from laying hens for 24 weeks. The total number of eggs was higher by 29340, productivity per initial laying hen was higher by 1.6 eggs, per average laying hen - by 0.8 eggs.

1. Introduction
Poultry farming at the present stage is a complex and dynamically developing industry that uses the most advanced technologies of all represented in agriculture. Targeted selection and breeding work has allowed to create crosses of poultry with the maximum indicators of productivity, early maturity and growth intensity, as well as the ability to transform relatively cheap plant feed into a complete, nutrient-rich, and above all, protein products - eggs, meat, and by-products [1-3].
Intensive and tense level of metabolic processes creates conditions not only for obtaining high productivity, but also for the development of a large number of diseases. Among the diseases of non-communicable etiology, dystrophic liver lesions, as a biological laboratory for assessing the quality of feeding and poultry content, occupy one of the leading places [4-9].

From the numerous means of prevention of the given pathology it is not possible at present to allocate a medicine or a fodder additive, capable reliably and economically to prevent the given pathology. In this connection, the biologically active preparation "Geprim dlya kur" was developed, which is based on cytotoxic serum obtained to the tissues of healthy liver and spleen of chickens [10].

Aim of the study: to determine the impact of "Geprim dlya kur" on the morphobic and chemical parameters of rearing flocks and the subsequent productivity of laying hens in the prevention of hepatitis. The study was aimed at: 1. to determine the effect of the preparation on hematological parameters of chicken blood; 2. to study the effect of "Geprim dlya kur" on some biochemical parameters determining protein-synthetic function of the liver; 3. to study the peculiarities of chicken liver micromorphology; 4. to determine the distant indirect effect of heprimes on the egg production of adult birds.

2. Materials, conditions and methods of research
"Geprim dlya kur" is a transparent liquid with a low draft, easily broken by shaking. "Geprim dlya kur" is a biologically active drug containing both anti-reticular cytotoxic and antihepatotoxic serum. The drug is not toxic, does not have irritating and allergizing properties. The product is designed to prevent liver disease in chickens. Clinical effect of a single use of the drug remains for 3-4 months.

The research was carried out on the basis of the Chelyabinsk Poultry Farm on chickens and adult birds of the Loman-White Cross.

2 groups of daily chickens were formed in the incubator of the enterprise: an experimental group - 20320 heads and a control group - 19650 heads. The chickens of the experimental group received an intramuscular injection of the preparation "Geprim dlya kur" in the hip in a dose of 0.2 ml per chicken (in the working cultivation with physiological solution), and a vaccine against Marek's disease was injected into the opposite hip of the chickens. After that, the control and experimental groups were observed during the breeding period (up to 105 days) and egg-laying period (up to 497 days). In the course of the experiment, blood were taken for morphological and biochemical studies in poultry of the experimental and control groups during the growing season at intervals of 1 month. Erythrocytes, hemoglobin and leukocytes were determined in blood. In blood serum, the amount of total protein, the ratio of protein fractions, as well as the activity of reamination enzymes - aspartateamin transferase (AsAT) and alanineamin transferase (AlAT) - were determined. In order to study chicken liver micromorphology, a poultry slaughter and liver fragments were taken in experimental and control groups at intervals of 1 month for histological studies. Pieces of 1 cm3 were fixed with a 10% formalin solution for 72 hours, then some of them were frozen and prepared for further staining with Sudan 3, and the rest were poured into paraffin, made histological slices and stained with hematoxylin and eosin [11]. The sections were microscoped at 400x magnification, photographed with the Leica DMRXA microscope and the Leica DFC 290, and measured structures with the Image Scope M.

The following indicators were used to determine productivity: gross egg collection, number of eggs per initial laying hen and number of eggs per average laying hen.

3. Results and discussion
The results of morphological studies of chicken blood are presented in Table 1. The table shows that the respiratory (number of red blood cells and hemoglobin) and protective (number of leukocytes) functions of the blood of the experimental chickens were higher than in the control group during the whole experiment. Thus, the number of red blood cells in the chickens of the experimental group for the first month of the experiment was higher than in the control one by 80%, for the second by 73%, for the third by 53%. The amount of hemoglobin was by 51.6%, 33% and 25.8%, respectively. The number of white blood cells was 39.8%, 41.3% and 32.6%, respectively. All deviations were authentic. It should also be
noted that over time, the difference between the experimental and control groups decreased, except for leukocytes.

**Table 1.** Erythrocyte, hemoglobin and leukocyte content in chicken blood

| Index | Norm | Experiment | Control |
|-------|------|------------|---------|
| Erythrocytes, $10^{12}/l$ | 2.5-5.0 | 4.5±0.1** | 2.50±0.09 |
| Amount of hemoglobin, g/l | 90-171 | 145.0±9.2* | 95.6±3.4 |
| Leukocytes, 10^9 | 9.0-51.0 | 20.0±0.6* | 14.3±1.2 |
| Erythrocytes, $10^{12}/l$ | 2.5-5.0 | 4.5±0.1* | 2.60±0.06 |
| Amount of hemoglobin, g/l | 90-171 | 143.7±4.7** | 108.0±2.5 |
| Leukocytes, 10^9 | 9.0-51.0 | 19.5±0.8* | 13.8±0.4 |
| Erythrocytes, $10^{12}/l$ | 2.5-5.0 | 4.0±0.1** | 2.60±0.05 |
| Amount of hemoglobin, g/l | 90-171 | 141.0±0.6** | 112.0±2.5 |
| Leukocytes, 10^9 | 9.0-51.0 | 18.3±0.3** | 13.8±0.1 |

* – p<0.05, ** – p<0.01

The total protein content and the ratio of serum protein fractions are shown in Table 2.

**Table 2.** Total protein content and ratio of chicken serum protein fractions

| Index | Norm | Experiment | Control |
|-------|------|------------|---------|
| Total protein, g/l | 43-59 | 56.5±2.2* | 45.5±3.0 |
| Albumins, % | 31.4-35.1 | 40.97±1.88* | 34.62±0.72 |
| Alpha-globulins, % | 17.3-19.2 | 15.81±0.67 | 12.82±0.48 |
| Beta-globulins, % | 10.9-12.8 | 7.54±0.73 | 8.50±0.67 |
| Gamma-globulins, % | 35.1-37.1 | 36.14±0.97* | 44.08±0.50 |
| A/G | 0.4-0.5 | 0.69 | 0.53 |
| Total protein, g/l | 43-59 | 62.8±4.4 | 58.8±3.1 |
| Albumins, % | 31.4-35.1 | 31.33±0.22* | 29.32±0.66 |
| Alpha-globulins, % | 17.3-19.2 | 23.22±1.03 | 24.04±0.72 |
| Beta-globulins, % | 10.9-12.8 | 13.98±1.75 | 13.74±1.46 |
| Gamma-globulins, % | 35.1-37.1 | 32.41±2.17 | 32.9±1.5 |
| A/G | 0.4-0.5 | 0.46 | 0.41 |
| Total protein, g/l | 43-59 | 51.6±8.2 | 51.1±2.6 |
| Albumins, % | 31.4-35.1 | 26.13±0.38 | 27.5±0.6 |
| Alpha-globulins, % | 17.3-19.2 | 21.44±0.22 | 18.67±1.05 |
| Beta-globulins, % | 10.9-12.8 | 15.51±1.17 | 17.95±0.89 |
| Gamma-globulins, % | 35.1-37.1 | 36.92±0.98 | 35.48±2.28 |
| A/H | 0.4-0.5 | 0.36 | 0.38 |

* – p<0.05
As can be seen from the table, the total protein level in blood serum in experimental chickens was higher during all months of the experiment. The level was the highest with respect to control after the first month and was +24.2%, by the end of the second month this indicator exceeded the physiological norm, and after the third month of observation the amount of total protein in the experimental and control group was almost equal. The chickens of the experimental group had a gradual decrease in the content of albumins in the blood serum during three months of observation in comparison with the control group. Thus, if for the first and second months of observation the difference was +18.3% and +6.8% (p<0.05), respectively, for the third month of observation it was -5.0%. It should be noted that the alpha and beta-globulin levels increased significantly during the observation period. If for the first month of observation their values were lower than the physiological norm, for the 2nd and 3rd months their values were higher than it, except for the alpha-globulin content in the control group chicks. Gamma-globulin levels fluctuated inversely with the fluctuations in albumin content during the experiment. If by the first month of observation their content in relation to the control was 18.1% lower, by the second month by 1.5%, by the end of observation (the third month) the content of gamma-globulins in the experimental group was higher by 4.1%. The albumin to globulin ratio index changed in the same sequence. At the beginning of the experiment (1st month) this ratio in the experimental group was higher than in the control group by 30.2% in the middle of the experiment (2nd month of observation) by 12.2%; at the end of the experiment (3rd month of observation) the A/H ratio in the experimental group was lower than in the control group by 5.3%.

Thus, the amount of total protein and the ratio of serum protein fractions is of an age-related nature. Thus, the amount of total protein is higher in the experimental group than in the control group in the first and second months of the experiment. This indicates an increase in the protein synthesis function of the liver. The increase in protein synthesis by the liver is also indicated by the content of albumins.

The increased activity of reamination enzymes indicates the activation of protein metabolism in the liver. In the blood serum of experimental chickens, the activity of aspartate aminotransferase increased during two months of the experiment in comparison with the control: in the first month by 6.1%, in the second month by 28.8%. By the end of the third month, the difference in enzyme activity was insignificant at 2.4%. The difference in the activity of alanine aminotransferase during the first two months of the experiment was 0% and 38.5%, respectively, in relation to control. And only by the end of the third month it decreased in relation to the control by 36.3%.

When coloring frozen chicken liver sections, the presence of fat deposits in organ tissues of both control and experimental groups was found. However, the intensity of orange-yellow coloring of liver tissues by Sudan 3, which characterizes fatty dystrophy, attracts attention: in all liver sections of the control group of birds it is higher than in the experimental group.

The results of histological studies are confirmed by the results of measurements of the number of fat droplets per 1 µm² of cut area. They are shown in Table 3.

| Table 3. Number of fat droplets 1 µm² of cutting area, % |
|----------------------------------------------------------|
| Experiment | Control | Difference |
| 1 month | 4.37 | 28.90 | 24.53 |
| 2 month | 0.52 | 43.68 | 43.15 |
| 3 month | 0.61 | 34.79 | 34.18 |

The data in the table show a significant decrease in the amount of fat deposits in the liver tissue of the experimental chickens compared to the control group. Thus, in the first month of the experiment the difference was 24.53%, in the second month - 43.15% and the third - 34.18%.

Histological examination of liver areas with signs of fatty dystrophy revealed similar changes in both experimental and control groups of birds during the whole period of observation. Fat dystrophy of hepatocytes takes place according to the type of fat infiltration - liver cells are enlarged in size, rounded
shape, the whole area is filled with fat. Cytoplasm and the nucleus are shifted to one of the edges; the nucleus is squeezed, oval-shaped. There are large areas of cell necrosis.

In the analysis of histological structures of the liver in the age aspect in the hens of the control group, in comparison with the experimental one, a more intensive staining of hepatocyte cytoplasm and the presence of small granules in it are noted, which indicates protein dystrophy. The cytoplasm cells of the control group in all months of the experiment and the experimental group in the first month has a frothy state. In the histological study it was established that in the birds of the control group in all months of the experiment and in the birds of the experimental group in the first month a cellular reaction in the form of neutrophil-lymphocytic infiltration of perivascular and pericapillary spaces is observed. The chickens of the control group also have abnormalities in the structure of blood vessels in the form of swelling and separation of endothelium, impregnation of the wall with serous liquid, as well as formation of blood clots in the cavity.

The detected changes indicate the presence of the control group in the liver of the bird in all months of observation of the signs of granular dystrophy with simultaneous disturbance of blood circulation. In poultry of the experimental group cellular reaction and changes in the cytoplasm of hepatocytes can be caused by the stimulating effect of the drug.

Stimulating influence of "Geprim dlya kur" on protein synthesizing function of the liver, hematological indices and liver regeneration was one of the reasons for the increase in the subsequent productivity of laying hens.

Egg productivity of laying hens in the experimental group was higher than in the control group from 22 to 46 weeks of accounting. However, this distinction was continuous. In the future, the increase in productivity in the experimental group was unstable.

In quantitative terms, the difference in egg production between the experimental and control groups over the entire period of observation was as follows: the total number of eggs laid in the experimental group was higher than in the control group by 29340 eggs, the productivity per initial laying hen was higher in the experimental group by 1.6 eggs, per average laying hen - by 0.8 eggs.

4. Discussion
The liver of young laying hens is one of the most vulnerable organs of poultry due to high metabolic loads and stress. Dystrophy (hepatosis) is the most common pathology of this organ. In our studies, it has been established that they are represented by disorders of fat metabolism (fat dystrophy) and protein metabolism (granular dystrophy). In determining the total amount of fat in the liver by staining Sudan 3, the intensity of organ fat deposition in the experimental chickens was significantly lower than in the control chick liver. Histological studies have established the uniformity of liver damage with signs of fatty dystrophy in experimental and control groups, which manifests itself in the form of fat cell infiltration and hepatocyte necrosis. In terms of age, the hepatoprotective effect of the drug in protecting the organ from protein dystrophy, as well as blood circulation disorders as it was established. However, in the first month of observations "Geprim dlya kur" causes neutrophil-lymphocytic infiltration of spaces around small blood vessels, which, in our opinion, confirms the positive impact on the immune system. "Geprim dlya kur" also had a stimulating effect on the protein synthesizing the liver function and the protective and respiratory function of the blood, which increased the hepatoprotective effect.
Application of "Geprim dlya kur" to chickens allows receiving the big egg productivity from laying hens in the future: to raise productivity on initial laying hens on 1.6 eggs and on average laying hen - on 0.8 eggs.

5. Conclusion
The use of “Geprim dlya kur” on the first day of poultry life reduces the degree of dystrophic liver damage, activates the mechanisms of the immunity cellular link and accelerates the processes of organ regeneration. The drug strengthens the protein that synthesizes the liver function and stimulates the respiratory function of the blood. The final effect is to increase egg production in adult birds.
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