Immunomorphological changes in bird staphylococcus

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Abstract. Staphylococcosis in birds is everywhere and belongs to the number of ubiquitous bacterioses. The etiology and pathogenesis of this infectious disease are directly related to the immune status of susceptible individuals. In a practical aspect, the evaluation of the immune system is based on the characterization of immunocompetent organs. A special place in solving the problem of treatment of staphylococcal infection is the study of age-related morphology of immunocompetent organs. The article presents the data of histologic studies of the thymus, spleen, and bursa of Fabricius during critical periods of broiler chickens’ cultivation when exposed to them with immunocorrection drugs of plant origin. Morphological changes in the immunocompetent organs when using the antibiotic florfenicol and the echinacea purpurea root against the background of the antigenic effect of the chickens Staphylococcus aureus on the body indicate the immunotropic effect of the antibiotics of the amphenicol group, as well as the immunomodulating properties of the drug based on echinacea purpurea.

1. Introduction
The spread of bacterioses in poultry caused by antibiotic-resistant and immunotropic pathogens is a global problem. Correction of secondary immunosuppression in the industrial technology of meat poultry farming, both as a result of the pathogenesis of bacterioses and as a result of antibiotic chemotherapy, is an important task of veterinary science and practice.

2. Research status and relevance of work
The immunostimulatory effect on birds of drugs based on plant components has not been studied sufficiently. In recent years, representatives of the flora of the genus Echinacea have shown an ever-increasing interest as a cost-effective raw material for the manufacture of immunocorrective agents. Representatives of Echinacea remain one of the most studied medicinal plants. Moreover, in literary sources there is only sporadic information covering the morphology of the organs of the immune system of birds in certain diseases and the use of immunocorrective plant extracts [1, 2, 3, 4]. Some aspects of the cytomorphology of immunocompetent organs and the responses of cellular and humoral immunity in broiler chickens were highlighted in our earlier works [5, 6, 7, 8]. Noted was the immunomodulating
properties of echinacea purpurea extract and the possibility of using agents based on it in industrial poultry farming [5, 6, 7].

When growing poultry in industrial poultry farms, a wide range of antimicrobial agents are used that can have a negative effect on the immune system [1, 2, 3, 4]. Turitsina E.G. (2010) showed that a high antigenic load, formed under the conditions of an accepted massive vaccination system, leads to the emergence of immunodeficiency in birds with inhibition of lymphopoiesis in the central organs of the immune system and a decrease in the immunoreactivity of the chickens body as a whole [3]. The current epizootic situation is complicated by the widespread prevalence of immunodeficiencies, including secondary ones, caused by an inapparent and subacute infection, by the wide and unsystematic use of antibiotics, especially in industrial poultry farming [4, 5, 8]. One of the most effective ways to reduce the influence of harmful environmental factors on the body of a bird in an industrial environment and increase the natural resistance of a bird is to use immunomodulating agents that satisfy the technological and economic aspects of production.

3. Purpose of work
To study of cytomorphological changes in the immunocompetent organs of broiler chickens under experimental staphylococcosis using antibacterial therapy and immunomodulation of plant origin.

The object of the study was Ross 308 cross broiler chickens infected with a pathogenic coagulase-positive culture of Staphylococcus aureus V-16p, from the collection of FSBEI HE MSAVMB - MVA named after K.I. Scriabin in a dose of 1 million micro cells. The subject of research was the histotopograms of the immunocompetent organs of broiler chickens on days 14 and 21 of the experiment when modelling the therapeutic complex.

To achieve the goal, research tasks were determined, including modelling of experimental staphylococcosis, obtaining samples and studying histotopograms of chickens exposed to infection, including with the use of antibiotic therapy with floron and, together with it, an extract of echinacea purpurea root (Echinacea purpurea).

4. Materials and research methods
To conduct research in accordance with the intended purpose of work on the basis of the vivarium of the Lugansk National Agrarian University, 240 daily healthy broiler chickens were selected, which were divided into three groups according to the analogue principle: control (1st) and two experimental (2nd, 3th) 80 heads each. Chickens of one control (1st group) and two experimental (2nd and 3rd) groups at the age of 4 days were infected with intramuscular inoculation of a daily culture of Staphylococcus aureus (Staphylococcus aureus V-16p strain), which was isolated from a sick bird during the enzootic of staphylococcosis in one of the poultry farms in the Luhansk region in 2012. The LD50 strain constitutes 760 thousand micro cells.

The experiments were carried out as follows. Chickens in the control group were not treated for the disease, but clinical and anatomical-pathology studies were carried out with sampling to study the microscopic picture of immunocompetent organs. Chickens of the second group, with the onset of signs of the disease, were treated with an antibacterial drug, floron, to which a high sensitivity of the causative agent was established, by group drinking a dose of 1 ml of 10% solution per 1 litre of water for 5 days. Chickens of the third group with the disease were treated with floron in the same doses as the chickens of group 2, but in combination with an immunomodulator - an extract of the echinacea purpurea root.

Echinacea extract was drunk with drinking water at a dose of 0.06 g / kg live weight according to specifications. To monitor the effectiveness of treatment measures in chickens of all groups 14, 21 days after infection, the clinical condition, weight gain, and in cases of chicken death - the pathological-morphological picture was studied. Samples for histological studies were taken from the thymus, bursa of Fabricius, spleen on the 14th and 21st days, both from the fallen laboratory models and the ones subjected to slaughter.
Organ samples were fixed in a 10% solution of neutral formalin, followed by pouring in paraffin according to the standard technique. Histosections were made with a thickness of 5 - 7 μm, stained with hematoxylin and eosin, as well as Azur II-eosin.

5. Research results
The study obtained a reproducible model of an infectious disease with the development of characteristic clinical-morphological signs of staphylococcosis and death in the control group of 73.75% of the study objects by the end of the observation (21 days after infection). The waste in the treatment groups was 28.75% and 3.75% respectively. The studies revealed the effects of stabilization of blood immunological parameters with the use of echinacea, which was manifested by stimulation of the titer of immunoglobulins and the number of circulating T-helpers, T-killers, alignment of the immunoregulatory index, average cytochemical coefficient, increased phagocytic number and some other features that we described earlier [5].

Studies of histosections were performed under microscopy in three main options for increasing the lens. The obtained histotopograms were analysed by a set of structural changes for the subject of research.

Histotopograms of thymic lobules are presented in figure 1.

As can be seen in the histotopograms shown in figure 1, the thymus of chickens in the studied groups has a typical organ structure. In the lobules, the cortical and medulla are clearly distinguishable. A comparative visual analysis of the size of the thymus lobes of chickens of all three groups shows its different reactivity to the acting agents. Moreover, in all groups, a slight increase in thymic lobes was noted by the 21st day of the experiment.

The largest size in both studied periods was lobules of the thymus of chickens of the 1st group. In chickens treated with an antibiotic (2nd group and 3th group), the lobule size in the corresponding periods of the experiment (14th and 21st days) did not differ significantly between the groups. Multiple petechiae were noted in the medulla-cortical area of the thymus with a predominance in infected chickens, this symptom was significantly reduced in the treatment groups.

More significant differences were found in the structure of thymic lobules.
On histotopograms of the thymus of group 1 chickens experimentally infected with S. aureus and not receiving treatment, a predominance of the brain substance over the cortical was observed on the cut area of the lobule, both on the 14th and 21st days of the experiment. At the same time, deep structural cuts appear on the 21st day, characteristic of the process of fragmentation of the lobules, while maintaining the relative position of the cortical and medulla.

In chickens of groups 2 and 3 infected and treated with an antibiotic, signs of fragmentation of thymus lobes, with varying degrees of severity, occur on both the 14th and 21st days of the experiment. In this case, the most pronounced fragmentation of lobules in chickens of the second group on the 21st day. In chickens, against the background of antibiotic therapy and immunocorrection with the extract of echinacea purpurea root during the same period of the experiment, signs of fragmentation of lobules were less pronounced, both in comparison with the previous period of the study, and with the same period in infected chickens of the first groups. In the thymus segments of chickens of all groups, an increase in the area of the brain zone by the 21st day was visually observed. The migration of eosinophils into unicellular and multicellular young thymic bodies (Hassall’s) was noted, which is expressed in samples of the first group and less of the second group. On histo-slides of the thymus of chickens of groups 1 and 2 on the 21st day of the experiment in the arteries, the folding of the intima increases, as a result of which the latter acquires a “scalloped” appearance. This picture indicates increased vasoconstriction. On the surface of the endothelium of the folds of intima, aggregation of blood cells is noted. The perivascular spaces of the vessels of the microvasculature channel were dilated, which, in turn, indicates degenerative processes in the thymus lobes.

In the group using the antibiotic floron and echinacea purpurea extract, there were no perforated enlightenments. Drops of a hyaline-like substance formed due to transudate at the initial stage of the disease were contained in the brain substance of the lobules of chickens of the 3rd group in a significantly smaller amount compared to the comparison groups.

Bursa Fabricius reacted to influencing factors by changing the size of the organ in both studied periods. The histotopograms of Fabricius bursa are presented in figure 2.

![Histotopograms of the thymus of broiler chickens in the studied groups. Histo-specimen (azu II and eosin, ×40).](image)

The most variable reaction of bursomegaly was on the 14th day of research. The smallest size of the cross-sectional area was observed in an organ in chickens of the second group, and the largest in chickens of the third group, which indicated the immunotropic nature of the effects of both florfenicol and echinacea. The folds of the mucous membrane of the factory bag were well developed. Their own
plate was mostly uniformly filled with lymphoid tissue. Moreover, even at a macroscopic level in the bursa, experimentally infected chickens on the 14th day showed folds partially free of lymphoid nodules, which was also confirmed microscopically.

By the 21st day of the experiment, all chickens showed a significant increase in Fabricius bursa. At the same time, the largest organ size was observed in chickens during combination therapy using immunomodulation. In some chickens of this group, a characteristic feature was a local decrease in the thickness of the organ wall due to a decrease in the height of the folds and the thickness of the mucous membrane. In these areas, bending of the bursa wall and the presence of illumination between the lymphoid nodules were observed. Their functional zones and the basal membrane forming the folded contour were well defined. The formation of new follicles is marked by the formation of loop-like folds of the basement membrane and “gemmation” of the medulla, around which the cortical zone is further organized. Follicles formed in this way, as a rule, do not have contact with the integumentary epithelium and the lumen of the bursa.

In chickens experimentally plunged into bacteriosis and treated with the Floron antibiotic, immediately after the termination of antibiotic therapy, as was noted, on the 14th day of the experiment, a significant decrease in the size of the Fabricius bursa was noted. At the same time, interfollicular layers of loose connective tissue were increased in the folds of the mucous membrane. The general structure of most follicles had a typical structure, however, at the base of the folds, found were a few follicles with delymphotization of the cerebral zone and visualization of its epithelial reticular base. In such cases, a decrease in the width of their cortical zone was also recorded. The observed changes indicated a decrease in lymphoid tissue in the mucous membrane of the bursa with preservation of the immunopoietic function of most follicles.

Another organ participating in hematopoiesis is the spleen. In continuation of the immunohematological studies that we described earlier [5], to reveal immunotropic changes in the competent organs, histotopograms of spleen samples obtained by us from chickens of all study groups in similar periods were studied. Histotopograms are presented in figure 3.

Figure 3. Histotopograms of the spleen in broiler chickens in the studied groups. Histo-specimen (azur II and eosin, ×40).
The histotopograms of the spleen of chickens on the 21st day show half of the longitudinal section of the organ in its median plane.

When comparing the areas of the spleen slices, it was found that on the 21st day of the experiment splenomegaly develops, more pronounced in chickens of the second experimental group with monochemotherapy with simulated staphylococcal bacteriosis. For the spleen of chickens of this group, the uneven colouring of its parenchyma with signs of red pulp infiltration is characteristic. In combinative treatment of chickens (group 3), red pulp has a more uniform coloration, against which elements of the white pulp are visible in the form of irregularly rounded formations of different sizes.

The models of the first group during the period from the 14th to the 21st day of the experiment under the antigenic effect of Staphylococcus aureus did not show significant differences in the size of the spleen (section area).

By the 14th day of the experiment, in the chickens of the control group against the background of septic inflammation in the spleen from the microvasculature there were signs of endothelial dysfunction, venous stasis, accompanied by sludge in the pulp veins and venous sinuses, plasmorrhagia. As an indicator of dynamically developing hemocirculation disorders in the organ, the presence of extracellularly located crystals of hematoidin was noted, both in areas of red pulp impregnated with blood plasma and red blood cell lysis products, as well as in intravascular cell aggregates. The absence of anastomoses between the cystic arterioles caused the insufficiency of intra-organ collateral circulation, which led, if closed, to ischemia, and in continuation, to pulp necrosis. We observed such sections at both the macroscopic and microscopic levels. In the white pulp of the spleen of chickens on the 14th day after infection, all its functional components were determined: periarterial lymphatic vagina and lymphoid follicles (spleen bodies). As revealed in the studies, acute septic inflammation, which during these periods of the experiment was accompanied by the death of 17.5% of the livestock, by the 21st day received a chronic tendency, which adequately affected the morphofunctional state of the spleen. Microcirculation conditions were partially improved by reducing the effects of intravascular aggregation of red blood cells, restoring the morphofunctional state of vascular endothelium, and normalizing hemodynamic conditions in the arteriolar bed.

Our studies indicate that the spleen, in conditions of reducing the pathogenic effect of the infecting culture of Staphylococcus aureus, showed high adaptive and plastic properties aimed at restoring organ function and maintaining the body's immune status.

Studies of the spleen when using the antibiotic floron for the treatment of broiler chickens have shown mixed results at different periods after the end of antibiotic therapy. So, at the first stage of histological studies in the spleen of chickens of the second group, we noted a decrease in hemolytic and hemostatic effects. Intravascular cell aggregates were observed singly in small pulp veins. In general, the venous bed, according to morphological characteristics, showed the ability to perform collector function, which reduced the level of hypoxia of the spleen tissue. On histo-specimens, this, respectively, was manifested by an almost complete absence of hematoidin crystals, which were found in small quantities locally in the pulp of the organ and in the lumen of the veins. At the same time, we found the effect of floron on the morphology of red blood cells, which was manifested by the presence in the vascular blood of a large number of round-shaped cells instead of the oval characteristic of birds.

At the second stage of the study (21 days of the experiment, which corresponds to 12 days after the termination of antibiotic therapy), the bird was noticeably stunted, in most chickens there were extensive necrosis in both white and red pulp, cell conglomerates at the stage of lysis in the venules, a large number of crystals of hematoidin in the plasma, impregnated with the red pulp of the organ. These observations suggest that functional disorders of the endothelium are the trigger mechanism for tissue homeostasis disorders.

In the group with the combined use of floron and echinacea purpurea, the revealed characteristics of histo-specimens, in general, indicated that the use of the extract of the root of echinacea purpurea simultaneously with the antibiotic floron had a positive effect on the spleen, with the persistent tendency of a delayed immunosuppressive effect after the end of antibiotic therapy, levelling echinacea. If on the 14th day in infected chickens receiving the Echinácea purpúrea root extract simultaneously with an
antibiotic, the spleen specimens differed on histo-specimens by a high degree of population of T- and B-dependent zones of white pulp, well-defined reactive centres along the pulp arteries, and the absence of hematoidin crystals in red pulp, then on the 21st day recorded an increase in the spleen to a lesser extent than in chickens of the 2nd group. Macroscopically, foci of necrosis on the surface of the organ were not visible, with the exception of single small-sized subcapsular ischemic sections. In addition, it was noted that lymphocyte apoptosis in the B-dependent zones of the white pulp of the spleen was significantly less pronounced, and it was not combined with necrosis of reticular nodular cells.

6. Conclusion
Staphylococcal infection in poultry causes great damage, is a second immunosuppressive pathogen and, in connection with the multiple resistance of the pathogen, is difficult to treat. Topical antibacterial drugs, as a rule, have an immunotrophic effect, as shown by the example of floron - a florfenicol drug from the class of amphenicol (immunotropity and hematotoxicity - established side effects and known analogues - chloramphenicol drugs). The search for soft and cheap immunocorrection agents for use in the industrial technology of meat poultry farming justifies the relevance and practical significance of the results.

The established morphological and immunohematological changes in broiler chickens during experimental staphylococcosis were characterized by signs of hemodynamic impairment in the thymus and spleen, as well as increased proliferation of lymphocytes, increased secretory activity of integumentary epithelial cells and cerebral region of the follicles of Fabricius bursa.

In the results obtained, it is noteworthy that the fact that the degenerative processes in the thymus bodies in chickens of the third group are noticeable reduced, compared with analogues, which indicates the levelling of the extract of echinacea purpurea hemodynamic disorders caused by exposure to Staphylococcus aureus.

The revealed structural changes at the macro and microscopic levels indicate that the use of the antibiotic floron inhibits the immunopoietic function of the Fabricius bursa, which is accompanied by a decrease in the amount of lymphoid tissue, leading to a decrease in the size of the organ as a whole.

The use of echinacea extract together with the antibiotic floron allowed to increase the effectiveness of therapeutic measures for staphylococcal infection in broiler chickens: an 8.3 times mortality reduction and a significant increase in live weight gain (shown by us in previous works [5]). The results of histological studies indicate a positive effect of echinacea root extract as a cell protector in conditions of necrobiotic processes in tissue hypoxia of organs, which is realized by stabilization of cell membranes of endothelial cells of immunocompetent organs. The use of echinacea root extract against the background of a model of staphylococcal bacteriosis in poultry chickens and treatment with the florfenicol drug provided normal morphogenesis of the thymic bodies in contrast to the single use of the antibiotic, the formation of new secretory cells in the bursa of fabricius, the absence of sludge and signs of intravascular aggregation in the spleen.

The data obtained allow us to expand our knowledge in the pathogenesis of avian staphylococcosis, the immunotrophic effects of the fluorinated synthetic analogue of thiamphenicol of veterinary use (floron) and the immunohematoprotective properties of echinacea purpurea root extract. The presented results confirm the available information, including obtained by us about the immunomodulatory effect of extracts of Echinacea purpurea and allow us to recommend these herbal remedies for widespread use in poultry farming.

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