Clinical and morphological substantiation of the use of glycyrizzic acid in poultry farming

Ildar Gatiyatullin[1*], *George* Bazekin[1], and *Ilgiz* Dolinin[1]

[1]FSBEI of Higher Education "Bashkir State Agrarian University", Ufa, 450001, Russian Federation

Abstract. The paper presents a clinical and morphological justification for the use of glycyrrhizic acid in poultry farming. It was found that the use of glycyrrhizic acid in broiler chickens enhances the histological picture of the thymus with a pronounced image and differentiation of the cortical layer. Increased mitotic activity of thymocytes and improved blood supply to the organ were determined. Revealed, Fabricius's bag of broiler chickens, which were given glycyrrhizic acid, has a fairly high level of functional dynamics. Installed, the thickness of the cortical layer of the thymus lobule in broiler chickens in the control group was 1.2 times lower than after the use of glycyrrhizic acid in broiler chickens in the experimental group, and the cerebral layer was 1.8 times less than in the experimental group. It was revealed that glycyrrhizic acid has a beneficial effect on the body of birds.

1 Introduction

To date, significant experimental data have been accumulated on the use of biological stimulants in poultry and animal husbandry [3, 5-8]

Natural resistance under adverse environmental conditions is of particular importance in the conditions of industrial poultry farming and is one of the determining factors for the preservation of poultry stock and increasing its productive qualities. The interest in glycyrrhizic acid derivatives is caused by high and diverse biological activity and low toxicity [1, 2, 4].

Thus, the need for immunotropic drugs in medicine and veterinary medicine is extremely high. Currently, a wide search is underway for drugs that have an activating effect on non-specific and specific protective reactions of the body.

There is a wide range of means of non-specific stimulation of the immune response, differing in structure and mechanism of action: polysaccharides of bacterial and yeast origin, preparations of nucleic acids, vitamins, derivatives of nitrogenous bases, etc. . Many products used in veterinary medicine have undesirable properties: high toxicity, pyrogenicity, allergenicity, etc.

As a result of improper use of immunosuppressive drugs, an extremely high susceptibility to infections develops in the animal body. Considering the above, the interest

* Corresponding author: gatiyatullinildar@yandex.ru

of immunopharmacologists in the search for new nonspecific immunostimulants becomes understandable [9-10].

2 Materials and methods

The work was carried out in the conditions of the biochemical laboratory of the Bashkir State Agrarian University. The experiment was conducted on 60 broiler chickens of the Ross 308 cross. 20 heads each in the control and experimental groups. The birds were kept in equal conditions. A balanced feed was used. Hematological (counting of erythrocytes, leukocytes, hemoglobin) and immunological (lysozyme, BAS, beta-lysines, Ig G, Ig M) blood tests were performed. Blood sampling was carried out in the morning, from the axillary vein into vacuum tubes "Vacuette": for hematological studies - with EDTA-K2. Hematological studies were performed using an automatic hematology analyzer Abacus Junior 5 Vet. The main triterpene glycoside of licorice roots, glycyrrhizic acid, was used as the main pharmacological substance under study. Glycyrrhizic acid was given to the broiler chickens under study in the form of aqueous solutions.

The thymus and bursa (central organs of immunity) were the material for research. Organs were weighed using scales and the mass of the thymus and bursa of birds was calculated.With the use of 10% formalin, organs (thymus and bursa) were fixed and dehydrated in alcohol. Next, they were immersed in paraffin. Histosections were made on a Leica RM 2145 microtome and stained according to the generally accepted Van Gieson technique with hematoxylin and eosin.

3 Results

According to the research results, it is clear that glycyrrhizic acid has a beneficial effect on the body of birds. Table 1 shows the data on the content of shaped elements and hemoglobin in the blood of broiler chickens. From the tabular data, it can be seen that when using glycyrrhizic acid, an increase in the number of red blood cells was observed in the chickens of the experimental group. At 14 days of age, the number of erythrocytes in the control group chickens was 2.11±0.01x1012/l, in the experimental group - 2.14±0.02x1012/l. At 28 days of age, this indicator in the experimental group chickens was higher than in the control group by 4.19%. At 42 days of age, the number of red blood cells in the chickens of the experimental group exceeded the control values by 4.26%. In the experimental group, the amount of hemoglobin in chickens was higher than the control values.

The number of white blood cells varied in broiler chickens throughout the experiment. Moreover, the increase in the number of leukocytes in chickens of the experimental groups was more pronounced at all age periods. As a result of the conducted studies, it was found that the beta-lytic activity of blood serum in chickens under the influence of glycyrrhizic acid changed slightly throughout the experiment. Table 2 shows that lysozyme activity in chickens on day 7 of the study in the experimental group significantly exceeded the values of control birds by 19.77%. By the end of the experiments, lysozyme activity in the chickens of the experimental group remained at a high level. The use of glycyrrhizic acid led to an increase in this indicator relative to the values of control chickens by 15.55% ($p<0.05$).

Table 1. The content of shaped elements and hemoglobin in the blood of broiler chickens (n=20).

Age of chickens, day	Indicator		
	Erythrocytes, 10^{12}/l,	Leukocytes, 10^{9}/l	Hemoglobin, g/l
I control – intact chickens			
1	1.91±0.12	22.40±0.93	70.03±1.32
7	2.06±0.02	28.20±1.24	74.02±2.24
14	2.11±0.01	25.00±1.08	78.04±1.37
28	2.15±0.01	30.00±0.55	82.11±2.37
42	2.58±0.03	30.20±0.37	84.12±2.24
II experiment – glycyrrhizic acid			
1	1.91±0.03	22.80±0.80	68.01±1.37
7	2.05±0.01	30.80±0.47*	76.05±2.60
14	2.14±0.02	29.60±2.36**	84.09±1.93
28	2.24±0.14*	32.40±0.75	96.02±2.87
42	2.69±0.02	31.80±0.86	96.05±1.51

Note: * – $p<0.05$

By their nature, beta-lysines are close to the antidiuretic hormone. An increase in their activity indicates an increase in compensatory and adaptive processes in the body. A decrease in the beta-lytic activity of the blood is associated with autoimmunization of the body and is observed in pathological processes of moderate and high severity.

Table 2 shows that the bactericidal activity in experimental blood samples, where glycyrrhizic acid was used, significantly expressed a stimulating effect. On day 7, when using the drug, bactericidal activity in blood samples increased by 7.64% ($p < 0.05$) in relation to the (intact) control group. The following changes were observed during morphological examination after the use of glycyrrhizic acid. In the control group, the organ has a typical lobular structure characteristic of birds. The anatomical thymus is located correctly, covered from the outside with a shell of connective tissue. It passes deep into the parenchymal organ, dividing into incomplete lobules. The lobule consists of a gray cortical substance. Location on the periphery of the lobule and contains mainly small T lymphocytes, epithelioretic cells and macrophages (Figure 1).

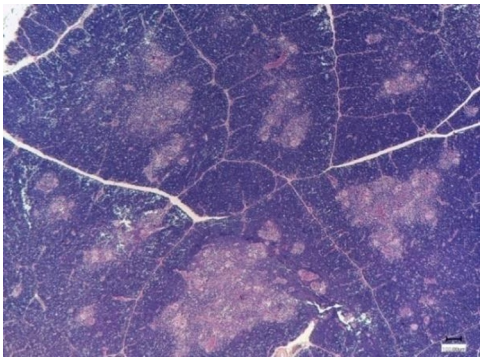

Fig. 1. Thymus of broiler chickens in the control group. Stained with hematoxylin and eosin.

Table 2. Immunological blood parameters of broiler chickens.

Group	Age of chickens, day (n=20)				
	1	7	14	28	42
Lysozyme, %					
Control	55.40±1.7	35.40±2.56	38.60±1.03	43.8±1.9	47.6±2.7
experience	55.20±1.7	42.40±2.5*	39.40±0.8	45.20±2.0	55.0±2.5*

BAS, %					
Control	54.60±1.66	62.80±1.59	65.20±1.7	70.0±1.5	74.60±1.2
experience	55.60±1.6	67.60±1.93*	66.20±1.96	70.00±2.0	78.4±1.8*
Beta-lysines, %					
Control	53.60±1.7	59.60±1.08	61.00±1.0	62.40±1.2	65.20±1.9
experience	53.40±1.7	58.60±0.60	61.00±0.9	62.20±0.8	64.60±1.8
Phagocytic activity of leukocytes, %					
Control	48.60±2.7	53.00±2.74	56.80±3.5	61.40±2.9	67.00±3.1
experience	49.00±2.2	60.40±2.7*	67.80±5.2*	67.60±1.1*	69.00±2.5
Ig G, g/l					
Control	1.94±0.07	2.64±0.11	2.90±0.07	3.46±0.14	4.48±0.06
experience	1.94±0.09	2.72±0.11	3.00±0.08	3.90±0.1*	4.54±0.09
Ig M, g/l					
Control	0.24±0.07	1.18±0.04	0.98±0.07	1.32±0.9	1.34±0.5
experience	0.26±0.08	1.20±0.04	1.08±0.09	1.36±0.6	1.36±0.4

Note: * – $p<0.05$

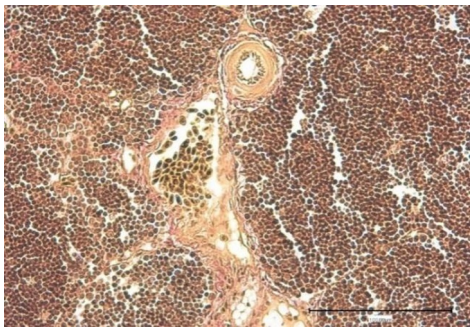

Fig. 2. Thymus of broiler chickens in the control group. Blood vessels. Painting by Van Gieson.

In the experimental group, after the use of glycyrrhizic acid, no distinctive features were found in the histological picture of the thymus of broiler chickens compared with the control. There was a lobular structure of the thymus, each of these lobules had a cortical layer and a medulla. The structure was different, the image was good. In the perenchyma of the septa, loose fibrous connective tissue formed trabeculae, which are represented by thin layers (Figure 3).

Thus, the thickness of the cortical layer of the thymus lobule in broiler chickens in the control group was 1.2 times lower than after the use of glycyrrhizic acid in broiler chickens in the experimental group, and the cerebral layer was 1.8 times less than in the experimental group.

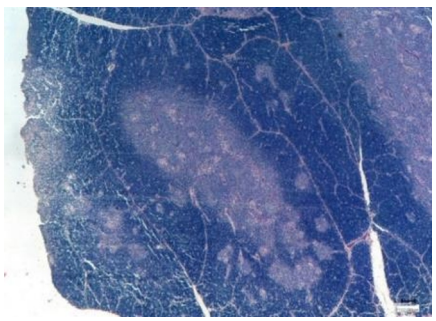

Fig. 3. The thymus of chickens in the experimental group. Stained with hematoxylin and eosin.

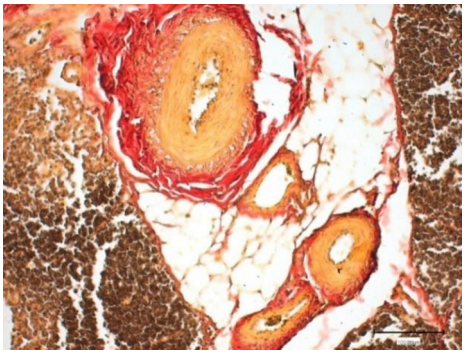

Fig. 4. The thymus of chickens in the experimental group. The growth of loose fibrous connective tissue. Painting by Van- Gieson.

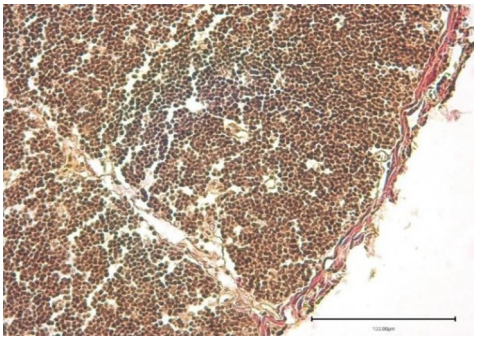

Fig. 5. The thymus of chickens in the control group. Connective tissue capsule. Painting by Van-Gieson.

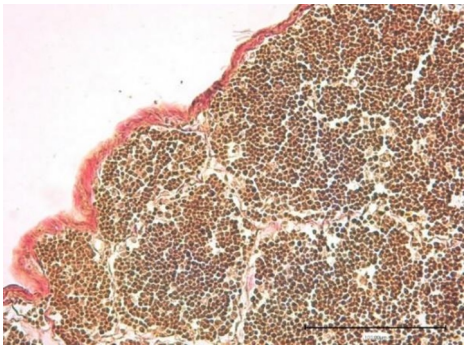

Fig. 6. The thymus of chickens in the experimental group. Connective tissue capsule. Painting by Van-Gieson.

Consequently, hypertrophy of thymus lobules was observed in broiler chickens in the experimental group after the use of glycyrrhizic acid.

During the experiments, it was found that the use of glycyrrhizic acid in broiler chickens has and confirms an immunomodulatory effect, which is expressed in normal hypertrophy and hyperplasia of the thymus.

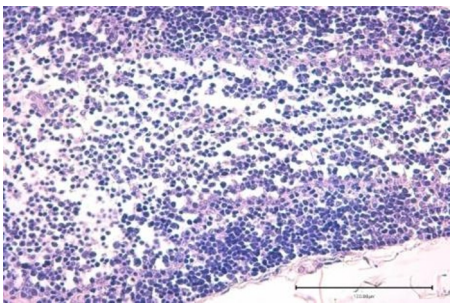

Fig. 7. Lobule (lymphoid follicle) of the bursa of broiler chickens in the control group. Stained with hematoxylin and eosin.

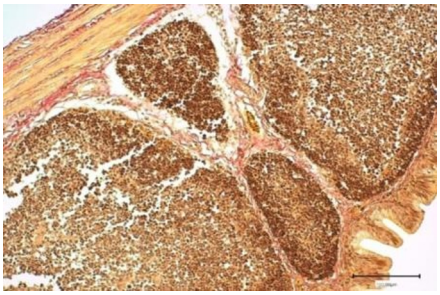

Fig. 8. Stroma of bursa folds of ROSS308 cross broiler chickens in the experimental group. Painting by Van-Gieson.

Massive accumulations of pseudoeosinophils were found in the cortical substance of lymphoid follicles, which could be a reflection of the course of immuno-inflammatory processes.

The condition of the bloodstream is without peculiarities. Venules are moderately dilated, full-blooded, endothelial cells are aligned. The lumen of the arterioles is free and dilated. Fabricius's bag (bursa) of broiler chickens, which were given glycyrrhizic acid, was characterized by the following morphological pattern.

In broiler chickens, which glycyrrhizic acid was given, the lymphoid nodules were tightly adjacent to each other, were more often arranged in two rows, the cortical zone was expressed more intensively and with a high cellular density. Multiple germinate centers with high infiltrative and proliferative activity were detected in the lymphoid nodule. The number of germination centers the number of lymphoid follicles in the experimental group was increased 2.36 times compared with the control group.

Unlike broiler chickens of the experimental group, the bursa of broiler chickens in the control group demonstrates a progressive increase in involutive processes, expressed in thinning of the cortical substance, predominance of cerebral rarefaction, increase in the relative area of the stroma of the folds, desolation and deformation of nodules, restructuring of the single-row cubic epithelium of the lateral surfaces of the folds into a pseudomolayer.

4 Conclusions

The administration of glycyrrhizic acid to broiler chickens had a positive effect on the phagocytic activity of blood leukocytes. An increase in the number of leukocytes in the blood of chickens of the experimental group indicates the mobilization of the immune system and the strengthening of specific immunity in response to the use of glycyrrhizic

acid, since leukocytes are the main executive link in the manifestation of cellular and humoral protection of the body.

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