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Nonspecific Resistance Of Broilers On The Background Of Application Of A Herbal Complex Of Biologically Active Compounds Under The Conditions Of Industrial Technology.

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ABSTRACT

In this manuscript we present data on the determination of the age-related dynamics of the nonspecific resistance of broiler chickens in the industrial poultry farming on the background of application of a herbal complex with biologically active compounds. Four groups of broilers of cross-breed "Smena-2" with 200 chickens in each group were selected in order to conduct the research on the basis of a typical Ural farm, according. Age of the broilers, their body weight, health condition and cross-breed were taken into account in the research. The chickens of experimental groups 1, 2, and 3, which were 5 days old, were fed with the herbal complex in amount of 10, 20 and 40 mg per kg of body weight, respectively. At 5, 21 and 42 days of age, ten broilers from each group were blood tested. To monitor the non-specific resistance of chickens, a morphological analysis of the blood was performed. To identify the adaptogenic and bacteriostatic properties of the herbal complex, the animals were studied on a model of depleting physical activity. It was established that the herbal complex of biologically active compounds can be referred to chelating complexes of transition metals with polydentate ligands of plant extracts. It has a bacteriostatic effect on pathogenic microflora, stimulating and adaptogenic action in conditions of increased functional load on the organism of laboratory animals. The herbal complex had a correcting effect on metabolic processes and it stimulated cellular immune defense factors of the organism in early postnatal ontogenesis, which is confirmed by an increase in the number of lymphocytes by 1.8-3.6%; pseudo-eosinophils - by 2 times; monocytes - by 3 times. The immunomodulating effect of the drug was also noted. This was manifested as an increase in the total number of leukocytes, and their redistribution to the increasing number of more mature forms of immunocompetent cells, which is of great importance in the process of increasing the anti-infection protection of the organism.
Keywords: broilers, nonspecific resistance, herbal complex of biologically active compounds, bacteriostatic and adaptogenic activity, immunomodulating effect.

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INTRODUCTION

Modern poultry farming is characterized by the introduction of industrial technologies: high concentration of livestock in limited areas, year-round housing of birds in enclosed areas with cages, use of highly concentrated fodder. Intensive growth and production are the basis of this industry, which leads to weakening of the constitution and health of the bird, which is accompanied by a decrease in physiological reactivity and natural resistance of the organism, metabolic disorders, decreased productivity, increased aggression and stress hormones that adversely affect the human[1,9].

Technology of industrial poultry farming requires veterinarians that have a deep knowledge on the peculiarities of biochemical processes occurring in the birds organisms in the early postnatal period. Veterinarian also must find ways to improve the adaptive capabilities of the birds by using “soft” medications (adaptogens, antioxidants, hepatoprotectors, immunostimulants), which improve the condition of functional systems that increase the resistance, productivity and safety of birds without any disturbances in digestion and metabolism.

An advanced area in these studies is the use of herbal medications that are able to be metabolized to the natural products of the biotope. These herbal medications are extracts of plants that contain a balanced set of microelements, phytoncides and other biologically active compounds[2-8, 9-12].

The purpose of our work was a comprehensive study of the age-related dynamics of nonspecific resistance of broiler chickens in industrial poultry farming pn the background of application of a herbal complex of biologically active compounds (complex).

EXPERIMENTAL PROCEDURE

Four groups of broilers of cross-breed “Smena-2” with 200 chickens in each group were selected for the experiement. The farm was free from infectious and invasive diseases during the experiment.

All the experimental chickens received a starter feed ration during the preparatory period. From the age of 5 days, chickens of the 1, 2, and 3 experimental groups were fed the herbal complex in amount of 10, 20 and 40 mg per kg of body weight, respectively. The whole experimental poultry was kept in one house; the conditions of feeding and keeping were the same for all groups of birds.

At 5, 21, and 42 days of age, ten broilers from each group were blood tested. To obtain blood in large quantities, the chickens were slaughtered by decapitation and all the blood was collected in a test tube using a funnel. The blood was delivered to the department of biochemical analysis of the regional veterinary laboratory.

To monitor the nonspecific resistance of chickens, a morphological analysis of the blood was performed.

To identify the adaptogenic and bacteriostatic properties of the herbal complex, the studies were conducted on laboratory animals on a model of depleting physical activity.

Processing of the results was carried out with the Microsoft Excel programme. The reliability of the studies was determined with the help of the Student-Fisher test, the difference was considered reliable at $P < 0.05$. The effect of the herbal complex was evaluated during a single-factor analysis of variance.

COMPOSITION AND PROPERTIES OF THE HERBAL COMPLEX OF BIOLOGICALLY ACTIVE COMPOUNDS

This herbal medication is a multivitamin complex from extracts of plant raw materials with the addition of a certain set of microelements.

The medication was obtained by treating the herbal raw material of grass or hay with an extractant containing a mixture of trace elements. The relation of plant raw material to extractant is at 1: 13.5. The plant raw materials and the extracting mixture was previously treated with cold extraction at a temperature of 50 °C for 60 minutes, and then heated to a temperature of 120 -130 °C under a pressure of 6.0 x 10 Pa for 20

minutes. The extract was dried in a "gentle" mode at a temperature of 40-50 °C. This treatment allowed the most complete extraction of biologically active substances from the raw material and avoid loss of their valuable properties.

The extracting mixture is balanced in microelement composition and contains a mixture of trace elements of iron, copper, manganese, cobalt, zinc, chromium, vanadium, and additionally enriched with titanium and molybdenum salts. In comparison with other herbal medications has a lower content of iron, zinc and manganese; but an increased content of vanadium, cobalt and copper salts. When choosing a trace element, we proceeded from the ability of the elements to participate in oxidation-reduction processes and their ability to complex with the chelating organic substances of plant raw materials; we took into account the synergistic and antagonistic action of the elements.

Introduction of titanium elements is due to the fact that titanium ions can form strong complex compounds with organic part of plant raw materials and contribute to the preservation of the enzymatic status of the organism. Elements of molybdenum and cobalt, in a certain ratio, affect the activity of xanthine oxidase of the liver and kidneys, which gives the basis for the introduction of the above named elements in the extractant.

Biogenic trace elements, which were added to the extractable solution, form organometallic complexes with the organic part of the plant material - that is amino acids, hydroxy acids, flavonoids, hexoses, humic substances, in which the metal ion occupies a central position. The metal ions with the addends around them form the inner sphere of the complex. Counterions of metal and an addend display an external coordination sphere.

When an organometallic complex is formed, the addends are often grouped around the complexing ion so that the central ion complements the outer electron shell to a stable configuration. The reaction occurs between the metal and the organic compound, one molecule of which contains several groups - electron donors (addends). In this case, due to one organic molecule, one can saturate a number of coordination sites of the metal. Nitrogen, oxygen and sulfur are responsible for complex formation. These elements are located by electronic pairs, through which a coordination interrelation and filling of the electronic configuration of the central ion is carried out. Divalent iron, copper, and cobalt ions have a pronounced tendency to bond with nitrogen-containing groups. Trivalent iron is usually combined with oxygen, divalent manganese and zinc - with nitrogen-containing and sulfur-containing donors.

Amino acids form complexes with a large number of metals by coordination through the carboxyl and amine groups. In carbohydrates, electron donors (addends) can be primary and secondary alcohol groups, as well as keto groups. The addition of a metal ion will occur to that oxygen atom, which has two free electron pairs. The greatest biological activity is phenolic compounds with two or more hydroxyl groups in the benzene nucleus, acting as "traps" of free radicals, actively involved in the neutralization of oxidants, proton donors. Humic substances have a complexing ability with relation to toxic metals, pesticides, etc. Data on the microelement composition of the plant complex of biologically active compounds are given in Table 1.

Table 1: Chemical composition of the herbal complex of biologically active compounds, in mg/kg

Element	Content
Iron	300.0
Zinc	200.0
Titanium	5.0
Copper	5.0
Manganese	5.0
Molybdenum	5.0
Cobalt	5.0
Vanadium	2.0
Chromium	1.0

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The herbal complex of biologically active compounds can be referred to chelating complexes of transition metals with polydentate ligands of plant extracts.

BACTERIOSTATIC AND ADAPTOGENIC ACTION OF THE HERBAL COMPLEX OF BIOLOGICALLY ACTIVE COMPOUNDS

To evaluate the bacteriostatic effect, the E. coli, Staphylococcus aureus and Salmonella antaricidis were used as the test cultures. These cultures were chosen not by chance, since they are the most common microflora of the intestines of chickens.

Bacteriostatic action is an action of a chemical that impedes germination of microorganisms under certain conditions, but does not lead to their death.

Bacteriostatic action was determined by testing the survival of the test cultures in solutions of the herbal complex with different concentrations. The results were observed after three days of incubation in a thermostat at 37 °C. The results are shown in Table 2.

Table 2: Bacteriostatic activity of the herbal complex of biologically active compounds

Test cultures	Solution concentration, in %			
	Colony growth		Absence of colony growth	
E. Coli	1.0	3.0	5.0	7.0
Staphylococcus aureus	1.0	3.0	5.0	7.0
Salmonella antaricidis	1.0	3.0	5.0	7.0

Studies have shown that 1.0% and 3.0% solutions of the herbal complex have no inhibitory effect on test cultures. Bacteriostatic properties appear at a solution concentration of 5.0% and higher. The first colonies in the test tube appeared 48 hours after incubation in a thermostat at 37 °C, and in control tubes (without the herbal complex solution) the growth of bacteria in the form of a ring was observed after 18 to 20 hours under the same incubation conditions. Bacteriostatic action of the complex directly depends on its concentration in the solution. The growth of the bacterial ring decreased as the percentage of the solution increased. So 5.0 and 7.0% solutions of the herbal complex completely suppressed the growth of bacteria, and the microscopy showed the presence of lysis of microbial cells.

The bacteriostatic action of the complex increases with an increase of its concentration, the activity (35 units) accepted in the Technical Conditions (TU) at a concentration of 5.0% completely inhibits the growth of the tested bacterial cultures.

The study of adaptogenic action of biologically active additives (BAA) was carried out on standard models under various extreme conditions. We studied the adaptogenic effect of the herbal complex of biologically active compounds, for this purpose we used a model of exhausting physical activity - the swimming of animals.

For animals (mice) of the experimental groups, the herbal complex was given intragastrically once 40 minutes before the first physical activity load in doses of 10.0; 20.0; 40.0 mg / kg of body weight. The physical activity was carried out three times: the first - 40 minutes after the introduction of the herbal complex, the rest - 24 and 48 hours after the first one. The duration of swimming sessions was estimated in seconds. The results are shown in Table 3.

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Table 3: Influence of the herbal complex of biologically active compounds on the depleting physical activity, ($\overline{X} \pm S\overline{x}$; n=10)

Index	Group number				Influence of the herbal complex, in %
	Control group	1st experimental group	2nd experimental group	3rd experimental group	
1st swimming session, in sec.	246.83±11.44	362.66±11.25*	378.16±15.73*	388.17±14.30*	78.44***
% in relation to control group	100.0	146.92	153.21	157.25	-
% in relation to initial data	100.0	100.0	100.0	100.0	-
2nd swimming session, in sec.	262.23±10.81	391.87±6.78*	463.73±14.09**	490.3±19.18***	89.54***
% in relation to control group	100.0	149.43	176.84	186.97	-
% in relation to initial data	106.24	108.05	122.66	126.31	-
3rd swimming session, in sec.	227.50±8.07	368.02±7.87*	390.97±8.69*	380.55±5.56*	93.47***
% in relation to control group	100.0	161.76	171.85	167.27	-
% in relation to initial data	93.16	101.42	100.73	100.63	-

*P<0,05; ** P<0,01;*** P<0,001

This analysis shows that at the first swimming session of animals in the 1st, 2nd and 3rd experimental groups, the endurance increased in relation to the control group by 46.92; 53.21 and 57.25%, respectively. The most effective dose of the herbal complex was of 40.0 mg/kg, which significantly (P <0.05) increased the duration of physical activity by 1.57 times.

In the second session, the use of the complex in all doses had a similar effect, significantly increasing endurance in relation to the control group by 49.43 (P <0.05), 76.84 (P <0.01), and 83.97% (P <0.001), respectively. At the same time, the duration of the activity increased in groups 1, 2 and 3, compared with the control group, at 1.49, 1.77, and 1.87 times, respectively. This is confirmed by the dose-dependent effect of the complex.

The third swimming session was also characterized by an increase in the endurance of animals by 61.76, 71.85 and 67.27%, respectively, in relation to the control group (P <0.05), with the maximum efficiency demonstrated when the herbal complex was used at a dose of 30.0 mg/kg of body weight. Compared with the previous session, the decrease in endurance of mice was noted at doses 10.0 and 40.0 mg/kg.

In conditions of increased physical activity, 24 hours after the introduction of the herbal complex, its high biological activity was established in a different dose range. The solution of the herbal complex of biologically active compounds introduced according to this scheme has the ability to increase the endurance of animals, both at the first session and at the further sessions, in relation to the initial parameters and the control group. The maximum increase in the parameters is observed at the second session (24 hours after the herbal complex intake) at a dose of 40.0 mg/kg. It must be noted that a high degree of stimulation is depleting in nature and is naturally accompanied by some decrease at subsequent sessions. The effectiveness of the complex at a dose of 20.0 mg/kg is maintained 48 hours after its intake, which is characterized by persistent preservation of endurance of animals.

The one-way analysis of variance showed that the herbal complex has an impact on depleting physical activity ($P < 0.001$).

According to the results of the study, a regularity was discovered: an increase of the dose of the herbal complex provides an increase of the endurance of the animals of the experimental groups in relation to the control group.

This experiment made it possible to establish the bacteriostatic action of the herbal complex on the pathogenic microflora, which stimulates and adaptogeneously influences the organism of laboratory animals under conditions of increased physical activity.

These results open the possibility to study this herbal complex on a living organism, which was one of the tasks set before us.

INFLUENCE OF THE HERBAL COMPLEX OF BIOLOGICALLY ACTIVE COMPOUNDS ON NONSPECIFIC RESISTANCE OF BROILERS

Housing of broiler chickens under conditions of industrial technology affects the dynamics of hematological and biochemical indicators and they might change. This leads to a change in metabolism, but clinically it may not be shown.

In recent years the medicine of herbal origin is more and more preferable, because it is capable of being metabolized in the body to the natural biotope products.

The content of white blood cells was analyzed, during evaluation of the herbal complex effectiveness, since they perform antitoxic and protective function, have the ability to synthesize globulins, specific immunoglobulins and antibodies, to absorb and destroy microbial cells and inactivate toxins. This data is presented in Table 4.

The quantity of leukocytes of 5-day-old chicks is in limits from 25.60 up to 28.65 10^9/l.

The leukocyte formula of broilers had corresponding age-related changes in the period of postnatal development[11]. In 5-day-old chickens the qualitative composition of white blood was of lymphoid nature and it was represented mainly by granular leukocytes (segment nucleated pseudo-eosinophils), the content of which was in the range from 62.2 to 68.2%.

The number of leukocytes tended to increase and amounted to 26.7-30.0 10^9/l in the blood of 21-day-old broiler chickens.

The change in the number of leukocytes influenced the dynamics of white blood cells composition. The number of basophils was higher in the blood of the control group chicks than in the 1, 2 and 3 experimental groups by 11.6; 8.83 and 10.25%, respectively, at the 21st day if the study; the number of segmented pseudo-eosinophils is lower by 7.5 and 6.0%, in groups 2 and 3, respectively. The number of eosinophils in groups 1, 2 and 3 is lower than in the control group by 4.06; 15.13 and 9.9%, respectively.

A clear tendency of lymphocytes and monocytes growth was observed on the 21st day under the influence of herbal medicine in experimental group; with an apparent increase in the total number of

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lymphocytes, which indicates the stimulation of the hematopoietic organs. The number of lymphocytes in the blood of broilers of the 1, 2 and 3 groups increased in comparison with the control group by 0.4, 4.24 and 3.5%, respectively; monocytes - by 13.64, 22.72 and 16.0%.

Blood test of the broiler at the age of 42 days showed that the total number of leukocytes in chickens receiving different doses of the herbal complex exceeded the control group value by 0.37% in the 1st experimental group; by 6.29% in the 2nd group; and by 7.52% in the 3rd group. The differences were not reliable.

Table 4: Dynamics of indicies of the blood protective function of broiler chickens ($\overline{X} \pm S\overline{x}$; n=10)

Group number	Leukocytes, 10^9/l	Leukogram, in %						
		Basophils	Eosinophils	Pseudo-eosinophils			Lymphocytes	Monocytes
				Immature cells	Band cells	Segmented cells		
5th day								
Control group	28.65±0.82	2.70±0.06	3.20±0.12	1.60±0.02	5.50±0.10	62.20±0.59	23.6±0.50	1.20±0.03
1st experimental group	28.56±0.61	2.65±0.07	3.00±0.08	1.55±0.01	5.20±0.10	66.80±1.05	19.4±0.40	1.40±0.03
2nd experimental group	26.52±0.33	2.60±0.09	3.00±0.05	1.60±0.03	5.40±0.10	64.50±0.96	21.5±0.60	1.40±0.04
3rd experimental group	25.60±0.29	2.75±0.09	3.10±0.06	1.60±0.2	5.60±0.10	62.20±0.76	23.55±0.30	1.20±0.06
Medicine effect. in %	0.57	0.61	0.74	0.05	0.19	0.09	0.05	0.02
21st day								
Control group	29.80±0.12	3.51±0.05	2.71±0.10	-	1.48±0.09	30.0±0.48	60.1±1.00	2.20±0.06
1st experimental group	28.40±0.21	3.10±0.08**	2.60±0.10	-	1.35±0.07	30.10±0.54	60.35±0.7	2.50±0.04*
2nd experimental group	30.00±0.16	3.20±0.07***	2.30±0.06***	-	1.40±0.04	27.75±0.74*	62.65±0.6	2.70±0.05**
3rd experimental group	28.20±0.17	3.15±0.05**	2.45±0.07*	-	1.45±0.04	28.20±0.95	62.2±0.8	2.55±0.03**
Medicine effect. in %	53.47***	0.72	0.11	-	0.04	0.14	0.16	40.11*
42nd day								
Control group	26.60±0.04	2.00±0.02	7.00±0.30	-	0.50±0.02	28.50±0.63	56.0±0.59	6.00±0.16
1st experimental group	26.70±0.04	2.45±0.1	6.00±0.4	-	0.50±0.02	27.50±0.61	56.05±0.61	7.50±0.09
2nd experimental group	28.30±0.08	2.5±0.06	5.00±0.35*	-	0.50±0.02	26.50±0.38	58.00±0.54*	7.50±0.08*

3rd experimental group	28.60±0.02	3.0±0.08	5.50±0.41*	-	1.00±0.03**	26.00±0.25*	57.00±0.60*	7.50±1.01*
Medicine effect. in %	31.30	16.31	27.22		0.6	0.12	49.61**	56.61**

The leukogram analysis shows that the number of basophils in the experimental groups has increased insignificantly compared to the control group.

With an increase of the dose of the herbal complex, the number of eosinophils significantly decreased from a control group value of 7.0 to 5.0-5.5% (P <0.01.) The use of the complex also affected the number of pseudo-eosinophils. Stab cells increased in the experimental groups from a control group value of 0.5% to 1.0% in the 3rd group at P <0.01, and the number of segmented cells, on the contrary, decreased from 28.5% to 26.5% in 2nd group and to 26.0% in 3rd group (P <0.05).

An increase in the functional activity of lymphoid tissue was noted, which is characterized by a significant increase in the number of lymphocytes at 42nd day of age. The level of lymphocytes in the 2nd and 3rd experimental groups was higher than in the controls group by 3.6 and 1.8%, respectively (P <0.05).

The monocytes were activated as well during the intake of the herbal complex. The number of monocytes increased by 3 times at 42nd day compared to the initial indices in all experimental groups from 6.0 to 7.5% (P <0.01). These values correspond to physiological norms.

According to the results, the parameters of the protective function of blood are formed in the period of postnatal development of broilers. By the 42nd day of age, there was a decrease in the number of segmented and stab cells, the number of lymphocytes and monocytes increased, so by the end of the incubation, the composition of the white blood cells became more stable, and was similar to the composition of an adult bird.

The use of different doses of the herbal complex of biologically active compounds positively influenced the protective function of blood, as evidenced by a moderate increase in the number of leukocytes, monocytes and stab cells by the 42nd day of broilers' age, which led to an increase in the immunocompetence of the organism. When estimating the effect of this complex on the leukocyte composition of the blood as a whole, we can note not only the stimulating, but also the immunomodulating effect of the medicine. This was manifested as an increase in the total number of leukocytes, as well as their redistribution to the ranks of increasing the number of more mature forms of immunocompetent cells, which have great importance in the process of increasing the anti-infection protection of the poultry organism.

The broiler chickens that received the complex at a dose of 20.0 mg/kg, had an increase in the number of lymphocytes compared to the broilers of 1st and 3rd groups by 3.36 and 1.72%, respectively, although the differences between the groups were not reliable.

During the one-way analysis of variance, a significant effect of the herbal complex of biologically active compounds on the 21st day on the number of leukocytes and monocytes (P <0.01) and on the 42nd day on the number of lymphocytes and monocytes (P <0.001) was established.

A definite regularity has been established in the action of the complex - the stimulating effect on cellular immunity factors, depending on the dose of the drug. The maximum effect was achieved using a dose of 20.0 mg/kg of body weight. This data were confirmed in the study of postvaccinal immunity.

INFERENCE

1. The herbal complex of biologically active compounds can be referred to chelating complexes of transition metals with polydentate ligands of plant extracts.
2. There have been established a bacteriostatic effect of the herbal complex on the pathogenic microflora; a stimulating and an adaptogenic action in conditions of increased physical activity on the organism of laboratory animals.

3. Introduction of the herbal complex of biologically active compounds into the diet of broiler chickens in early postnatal ontogenesis had a correlative effect on metabolic processes in the body and stimulated cellular immune defense factors of the organism, which is confirmed by an increase in the number of lymphocytes by 1.8-3.6%, pseudo-eosinophils by 2 times, monocytes by 3 times.

CONCLUSION

The use of different doses of the herbal complex of biologically active compounds positively influenced the protective function of blood of broiler chikens, and it is evidenced by a moderate increase in the number of leukocytes, monocytes and stab cells to the 42nd day of age, which led to an increase of the immunocompetence of the organism.

When estimating the effect of the herbal complex on the leukocyte composition of the blood as a whole, we can note not only the stimulating, but also the immunomodulating effect of the complex. This was manifested as an increase in the total number of leukocytes, as well as their redistribution to the ranks of increasing the number of more mature forms of immunocompetent cells, which have great importance in the process of increasing the anti-infection protection of the poultry organism.

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