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Effectiveness of the Study of Plant-Contaınıng Immunostımulants in Poultry Industry

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Abstract

The effect of the plant-derived preparation (*Tilia cordata* Mill., *Thymus kotschyanus* Boiss and Hohen., *Matricaria chamomilla* L.) Imuneo on the immunity indices in chickens vaccinated against avian infectious bronchitis was studied, and the immunostimulatory efficacy was evaluated. In the group of chickens treated with immunostimulants, a significant increase in their immune status was recorded. The immunostimulating effect of Imuneon was determined. Microbicidal and immunostimulating properties of plant-derived Hedalin preparation were studied. The expediency of using immunostimulating drugs in poultry complexes is presented.

Keywords: Plant-derived immunostimulants, vaccines, infectious bronchitis of chickens, Immuneo and Hedalin preparations

INTRODUCTION

Respiratory infections are one of the most important problems that cause great economic damage to poultry farming in many countries of the world, including Azerbaijan. In recent years, as a result of this problem, modern poultry industries have seen an increase in the number of infections with infectious diseases against the background of a weakened immune system. A number of studies conducted towards ethnoveterinary show the effective therapeutic effect of plant mixtures in the treatment of domestic animals. In ethnobotanical studies of Azerbaijan, studies were carried out on the use of infusion of *Persica vulgaris* - the leaf of the peach tree, *Quercus castanifolia* – bark of chestnut-leaved oak, *Punica granatum* –bark of the pomegranate tree and its fruit, *Arctostaphylos uvaursi* – leaves of barren myrtle plant in the national ethnobotanical recipes prepared for the treatment of poultry (Aghayeva, Ibadullayeva, 2012).

The spread of secondary immunodeficiencies depends on the accumulation of birds in a certain area, stress factors, and food quality (Gorbach *et al.*, 2018). The emergence of secondary immunodeficiencies in poultry farming is explained by many scientists by the large-scale use of antibiotics and hormonal preparations for infections, mutational and recombinational changes in microorganisms, the formation of heterogeneous populations, and other factors. Thus, the prophylactic use of a number of plant-derived immunomodulators can prevent respiratory infections (Oksana Sytar *et al.*, 2021). Basically, immunodeficiencies occur in birds in which the mechanism of the immune response is not yet fully developed. In this regard, it is important to use immunostimulants in poultry farming. As a result of study, it was found that the virus isolated from vaccinated birds differs from other serological types included in the vaccine due to its antigenic determinants. For this purpose, when using vaccines, it is necessary to take into account the antigenic characteristics of the serotypes of the causative agent. In order to determine the serotypes of the virus, a vaccination program should be designed, at this time, the presence of IBP preparations that enhance the immune activity of the vaccine should be taken into account.

For the specific prevention of coronavirus infection in birds, live and inactivated vaccines have been developed, which have their own advantages and disadvantages, often not fully protecting vaccinated birds (Apatenko, 2003; Alvarado *et al.,* 2003; Dantal, 2015). Taking into account the fact that in the conditions of industrial poultry farming, the technogenic and microbiological load on the bird's body increases significantly, rapid variability of viruses and bacteria is recorded, resistance to antibiotics develops, it is necessary to use immunostimulants, nutritional supplements. In this regard, the use of immunostimulants in a complex of therapeutic and preventive measures is considered important. On the other hand, in folk medicine, in addition to an effective plant, other auxiliary plants or substances are used in the treatment of birds.

Immunostimulants are preparations that have immunotropic activity, capable of restoring the state of the immune system, up to a "normal" level (Vorobyev, 2002; Karput, 2005; Maksyutina *et al.,* 1985). There is a huge arsenal of immunostimulating drugs of biological and synthetic origin. However, much attention has been paid to plant-derived immunostimulants in recent years [Patet9]. Plant immunostimulants, unlike others, have the following advantages: mild immunostimulating effect, low toxicity, stimulation of immune, endocrine and nervous systems (Manyak *et al.,* 2004). From the foregoing, it can be considered that the use of plant immunostimulants is more promising.

The main purpose of the study was to study methods for the prevention of coronavirus infection in birds using plant-derived immunostimulating preparations and the immunobiological feasibility of their use in poultry farming. It provides a large yield of biologically active substances that contribute to the maximum effect. The use of dry extracts ensures and maintains its stability and value, dose accuracy (Aghayeva, Ibadullayeva, 2012). For this purpose, the preparation, selection of plant-derived preparations with immunostimulating properties is a timely and urgent task.

MATERIALS AND METDHODS

The studies were carried out in expeditionary, stationary and laboratory conditions in 2021-2022. The plants distributed in the flora of Azerbaijan and tested in folk medicine were selected. Small-leaved lime tree *(Tilia cordata Mill.),* Kochi thyme *(Thymus kotschyanus* Boiss and Hohen.*)* and wild chamomile (*Matricaria chamomilla* L.) species were collected under natural conditions from different botanical-geographical regions (Greater Caucasus-Guba, LC-north-east and Talysh). Extracts prepared from them at 60^{O}C were sent to the laboratory. The flowers of *Tilia cordata* and *Matricaria chamomilla* species, and the aerial grass part of *Thymus kotschyanus* species were used to obtain the extracts. The aerial parts of *Thymus kotschyanus* were usually collected with a sickle a little before flowering or at flowering, while other plant flowers were collected in full flowering phase. The research material was personally collected by the authors in the course of surveys conducted among the older generation in various regions of Azerbaijan using the method of frequency of use of plants (Guber, 2001; Martin, 2001).

The preparation of the new drug was carried out according to the generally accepted method (Kaukhova, 2016). Primary vaccination of broilers and breeding birds was carried out with CeVak I Bird vaccine. CeBak I Bird vaccine is a live attenuated vaccine derived from the 1/96 strain belonging to the 793B group (produced in bottles of 500, 1000, 2500 doses). Immunity is formed 3 weeks after vaccination and lasts up to 5 weeks. 350 broiler chickens of the ROSS 308 breed were used as the main object to study the indicators of the immune status during the use of an immunostimulant against the background of IB vaccination. The birds for the experiment were selected according to the analogue principle. Plant-containing Imuneo and plant-containing complex preparation (dry extract of linden flowers, dry extract of thyme grass, dry extract of chamomile flowers, dry extract of orange peel, xanthan resin, potassium sorbate) were used as immunostimulants. It was previously used together with the vaccine at a dose of 13 liters / ton in the first 3 days before vaccination. The toxic properties and harmlessness of these preparations have been studied on laboratory animals. The antimicrobial properties of Hedalin syrup with a new composition were first studied by the disk-diffusion method.

Experimental part

An experiment was conducted on 30-day-old broiler chickens in Neftchala, Ujar and Shabran districts of the Republic. Initially, vaccines against infectious bronchitis of chickens were used. The main goal was to evaluate the immunostimulating effectiveness of the new complex plant-derived preparation Imuneo and Hedalin syrup. According to the generally accepted rule, as a test culture the following were taken from the opportunistic pathogenic microbiota agents that cause infectious diseases in poultry farming: MRSA - methicillin-resistant *Staphylococcus aureus* as a representative of gram-positive bacteria - (700699) (golden staphylococci), *Echerichia coli* (25922) - gram-negative bacteria (intestinal bacterium), *Pseudomonas aeruginoza* (1022) - the causative agents of nosocomial infections (blue pus bacillus) and *Candida albican*s (2024) (candida)- yeast-like fungus as a representative of fungi *Acinetobacter* spp., *Bacillus anthracoides* - as a representative of spore-forming gram-positive rod-shaped bacteria, *Klebsiella pneumonie* (505562) – as a representative of capsuliferous bacteria (Table).

Table 1: Study of the presented substance by disk-diffusion method

Test - culture	The studied substance Hedalin- syrup	Control substance Physiological solution
Staphylococcus aureus	14mm	0mm
Echerichia coli	18mm	0mm
Pseudomonas aeruginoza	7mm	0mm
Candida albicans	12mm	0mm
Klebsiella pneumoniae	3mm	0mm
Bacillus anthracoides	6mm	0mm
Acinetobacter spp	16mm	0mm

Note: The numbers indicate the diameter of abacterial zones in mm. Experiments were repeated 3 times

Considering the spread of new serotypes, the high immune reactivity of vaccines should be taken into account when vaccinating birds. In order to study the effect of Imuneo and Hedalin syrup on the immune system during vaccination, the birds were divided into 5 groups according to the analogue principle, with 50 birds in each group. Group I - chickens are vaccinated against IBV with CeBak I Bird vaccine by drinking water, group II - birds are vaccinated together with Imuneo, in this group the drug is given for 3 consecutive days before vaccination, then vaccination is done, and on the 5th, 6th and 7th days after vaccination, the immunostimulant is given again. Group III - to study the effect of Hedalin syrup on the immune system, the birds were fed by drinking water. Group IV - birds were given Hedalin syrup along with CeBak I Bird vaccine. In this group, the syrup was used for 3 consecutive days, then vaccination was done and the immunostimulant was re-introduced on days 5, 6 and 7 after vaccination.

Group V was the control group - birds were not vaccinated and were not given Imuneo and Hedalin syrup. 3 weeks later, after vaccination in all groups, blood was taken from the subclavian vein of the birds and immunological studies were performed. A significant increase in the number of leukocytes and lymphocytes was observed when assessing the immunological status of chickens in all experimental groups. In compared to control group, the number of leukocytes in group II increased to 45.2±1.0 min/µl, in control - 24.2±0.5 min/µl, in group I - 32.9±0.8 min/µl, in group III - 23.2 ±0.5 min/µl, and in group IV to 34.9±0.8 min/µl. Also, an increase in the number of lymphocytes was recorded in all experimental groups.

Table 2: Results of experimental studies

Experimental groups		The amount of leukocytes (min/µl)	Lymphocytes	
			T- lymphocytes (min/µl)	B- lymphocytes (min/µl)
Group I	CeBak I Bird vaccine	32,9 ± 0,8	7,9 ± 0,5	5,9 ± 0,20
Group II	Imuneo + CeBak I Bird vaccine	45,2 ± 1,0	8,7 ± 0,15	6,8 ± 0,16
Group III	Hedalin	23,2 ± 0,5	5,1 ± 0,42	2,9 ± 0,18
Group IV	Hedalin + CeBak I Bird vaccine	to 34,9 ± 0,8	6,5 ± 0,15	3,35 ± 0,8
Group V	Control	24,2 ± 0,5	1,75 ± 0,20	1,31 ± 0,15

As can be seen from the table, in birds treated with *Imuneo* simultaneously with vaccination, a significant increase in the number of lymphocytes was recorded compared to the control group. Thus, compared to the control group, the number of T lymphocytes was 1,75 ± 0,20 against 8,7 ± 0,15, the number of B lymphocytes was 1,31 ± 0,15 against 6,8 ± 0,16. Administration of *Hedalin* drug together with *CeBak I Bird* vaccine caused a relative increase in the number of lymphocytes. Thus, the number of T lymphocytes was recorded as 6.5 ± 0.15, and the number of B lymphocytes as 3.35 ± 0.8. However, despite that, the preparation had an active bactericidal effect, as a result of which no opportunistic infections were found in the birds during the entire study period, and no antibiotics were used.

The combined use of Imuneo and Hedalin syrup in birds led to a noticeable increase in bactericidal and phagocytic activity. Thus, after the introduction of Imuneo, the phagocytic activity of macrophages increased by 22.5% in group II and by 15.3% in group IV. Also, in experimental groups 2 and 4, the bactericidal activity of blood serum was recorded as 29.1% and 20.5% compared to the control group. Thus, immunostimulating Imuneo has a strong immunostimulating effect. Hedalin, in addition to the immunostimulating effect, has a bactericidal effect, in this regard, no opportunistic infections were found in these groups during the experiments.

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