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APITHERAPY AND IMMUNOSTIMULATION OF CHICKEN WITH DRONE BROOD EXTRACT IN VACCINATION AGAINST NEWCASTLE DISEASE UNDER DIFFERENT SCHEMES OF APPLICATION

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Abstract. *In the presented article, the study of the immunostimulating effect of the developed immunostimulant - drone brood extract (DBE) on the body of chickens under different application schemes was carried out. Immune status was calculated based on serological and immunomorphological indicators. To determine the optimal scheme of DBE application, 3 experimental and control groups of 6 two-week-old chickens were formed. The second group was given the extract together with the vaccination, the third - 5 days before the vaccination, and the fourth - 5 days after the vaccination. The first group remained the control group. Blood sera were studied with the help of Hemagglutination inhibition assay (HI). Immunomorphological studies were carried out at the macroscopic and microscopic levels and indices of the thymus, bursa of Fabricius and spleen were calculated.*

According to the results of the study, it was established that the titer of antihemagglutinins in HI was higher in the 3rd group, where the immunostimulant was administered 5 days before vaccination and was 7.4±0.13 log_2, this indicator was slightly lower in the fourth group 7.3±0.12 log_2 and in the second group 7.2±0.12 log_2. In the first group, the level of antihemagglutinins was 5.3±0.3 log_2. The absolute mass of the thymus increased in the experimental groups when using the vaccine and DBE compared to the control. Positive changes were noted in the thymus index, which in the 2, 3, and 4th experimental groups equaled 5.56±0.014, respectively; 5.63±0.13 and 5.51±0.15. In the control, this indicator was lower and corresponded to 4.58±0.01. The Bursa of Fabricius index in the control group of chickens was equal to 4.26±0.012, and in the 2nd, 3rd and 4th experimental groups it reached 5.42±0.01, respectively; 5.46±0.01 and 5.50±0.01. Bursa indices also demonstrate the superiority of this indicator in experimental groups that received an immunostimulant. Mass indicators and spleen indices differed in the control and experimental groups. If in control chickens this indicator corresponded to 412.4±42.4 mg, then in subjects in the 2nd, 3rd and 4th groups it increased to 532.4±15.30; 598.2±17.2 and 542.8±33.4 mg. The same trend was observed in the change of the spleen index.

Key words: apitherapy, drone brood extract, immunostimulation, chickens, bee products, Newcastle disease.

Every year, among scientists, the interest in beekeeping products is growing due to their therapeutic potential. In the scientific literature, there are works dedicated to the study of the therapeutic effect of propolis, royal jelly, bee venom and pollen [4, 9, 10]. However, there are much fewer works on the use of beekeeping products in veterinary medicine, although data indicate their effectiveness in the treatment of various diseases in animals (Stevanović, 2024).

Thus, in studies on various species of animals, it was proved that propolis has antifungal, antimicrobial and anti-inflammatory properties (Stevanović, 2024). In the form of an aqueous extract, propolis has good adjuvant and antioxidant effects (Liu, 2020; Mendonça, 2020). Bee venom in studies on dogs, broiler chickens, mice and pigs has proven an immunostimulating,

antibacterial, antifungal and antiviral effect. Melittin, the dominant component of bee venom, is responsible for its anti-inflammatory effects and also exhibits anti-cancer potential. The positive therapeutic effect of melittin in animals infected with methicillin-resistant *Staphylococcus aureus* (MRSA) opens the possibility of its use for the treatment of MRSA infections (Wilson, 2022). During the use of bee pollen in broiler chickens, an increase in body weight and an increase in the immune response were noted (Petričević, 2022). Regarding royal jelly, there is little data on its use in animals. But, in studies on rabbits, it has been shown to alleviate neurological disorders by increasing estrogen levels and the activity of the cholinergic and antioxidant systems (Pan, 2019).

Our attention was drawn to a product of bee origin, namely drone brood, since there are very few publications in the scientific literature on its use in veterinary medicine and they only concern the study of the androgenic effect and the effect on reproductive capacity in various animal species (Sawczuk, 2019; Kistanova, 2020).

We developed an immunostimulator made from the larvae of drone breed - (ETR), for which a utility model patent was obtained (Burdeinyi, 2023b). This immunostimulant is available, inexpensive, and can be produced under farm conditions. In previous studies, we determined the optimal dosage and determined the optimal method of introducing drone brood extract into the body of chickens (Burdeinyi, 2023a).

In this study, we conducted a study on the comparison of schemes for the use of drone brood extract (DBE) in vaccination against Newcastle disease.

The aim of study. To study the immunostimulating effect of the developed DBE immunostimulator on the body of chickens under different application schemes.

The task of the research: to investigate the immunostimulating properties of DBE for chickens under different application schemes.

Materials and methods. In order to determine the immunostimulating properties of the DBE immunostimulator on the body of chickens under different application schemes, the immune status was calculated by serological and immunomorphological indicators.

To determine the optimal scheme of DBE application, 3 experimental and control groups of 6 two-week-old chickens were formed. The second group was given the extract simultaneously with the vaccination, the third - 5 days before the vaccination, and the fourth - 5 days after the vaccination. The first group remained the control group, which was not given an immunostimulant.

Blood sera were studied according to the generally accepted method - Hemagglutination inhibition assay (HI). Immunomorphological studies were carried out at the macroscopic and microscopic levels and indices of the thymus, bursa of Fabricius and spleen were calculated.

The immunostimulating properties of DBE immunostimulant were determined 14 days after vaccination against Newcastle disease.

During the experimental work with chickens, the requirements of the Law of Ukraine "On the Protection of Animals from Cruelty" were observed; the Law of Ukraine "On Veterinary Medicine"; By order of the Ministry of Education and Science, Youth and Sports of Ukraine No. 249 dated 01.03.2012 "On approval of the Procedure for carrying out experiments and experiments on animals by scientific institutions"; WHO Handbook: good laboratory practice (GLP): quality practices for regulated non-clinical research and development (2009); European Convention on the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, March 18, 1986); Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes.

Research results. It can be seen from the above table that according to the indicators of serological studies, the titer of antihemagglutinins in HI was higher in group 3, where the immunostimulant was administered 5 days before vaccination and was 7.4±0.13 log2 ($P<0.001$), this indicator was slightly lower in the fourth group 7.3±0.12 log2 ($P<0.001$) and in

the second group 7.2±0.12 log2 (P<0.001). In the first group, the level of antihemagglutinins was 5.3±0.3 log2.

Table 1

Comparative data with different schemes of immunostimulation and vaccination against Newcastle disease (M±m, n=6)

Evaluation criteria	Vaccination with La Sota virus vaccine			
	Control	DBE simultaneously with vaccination	DBE 5 days before vaccination	DBE 5 days after vaccination
	1st gr.	2nd gr.	3rd gr.	4th gr.
Titer of antihemagglutinins, log_2.	5,3±0,3	7,2±0,12*	7,4±0,13*	7,3±0,12*
Absolute mass of the thymus, mg	1458±624	1642,2±364,2*	1631,6±435*	1656,4±264*
Thymus index	4,58±0,01	5,56±0,14*	5,63±0,13*	5,51±0,15*
The absolute mass of the bursa of Fabricius, mg	1018,3±432,8	1137±371*	1169,2±237*	1175±212*
Bursa of Fabricius index	4,26±0,012	5,42±0,01*	5,46±0,01*	5,50±0,01*
Absolute mass of the spleen, mg	412,4±42,4	532,4±15,30*	598,2±17,2*	542,8±33,4*
Spleen index	1,46±0,001	1,59±0,01*	1,66±0,02*	1,74±0,025*

Note: * -P<0,001.

The absolute mass of the thymus increased in the experimental groups when using the vaccine and DBE compared to the control. Positive changes were noted in the thymus index, which in the 2nd, 3rd, and 4th experimental groups equaled 5.56±0.014 (P<0.001), respectively; 5.63±0.13 (P<0.001), and 5.51±0.15 (P<0.001). In the control, this indicator was lower and corresponded to 4.58±0.01.

The Bursa of Fabricius index in the control group of chickens was equal to 4.26±0.012, and in the 2nd, 3rd and 4th experimental groups it reached 5.42±0.01 (P<0.001); 5.46±0.01 (P<0.001) and 5.50±0.01 (P<0.001). Bursa indices also demonstrate the superiority of this indicator in experimental groups that received an immunostimulant.

Mass indicators and spleen indices differed in the control and experimental groups. If in control chickens this indicator corresponded to 412.4±42.4 mg, then in subjects in the 2nd, 3rd and 4th groups it increased to 532.4±15.30 (P<0.001); 598.2±17.2 (P<0.001) and 542.8±33.4 (P<0.001) mg. The same trend was observed in the change of the spleen index. The conducted immunomorphological studies at the histological level revealed positive changes when using DBE. In the second group of chickens, which were given an immunostimulant together with vaccination, the area of the bursa of Fabricia slightly decreased after stimulation. On histological examination, this correlated with a decrease in the size of the lymphoid nodules and some reduction in the width of the cortex of the lymphoid nodules.

In the third and fourth groups, where the stimulant was administered 5 days after vaccination and 5 days before vaccination, respectively, in the bursa of Fabricius, there was a slight tendency to increase its area, the size of the lymphoid nodules, and the width of the cortical substance in comparison with the second group. The bursa of Fabricius of the chickens of the control group were similar to those of the experimental group, but were more reductions, and evenly filled with monomorphic cells of the type of medium lymphocytes.

In the thymus of experimental chickens of the 2nd, 3rd and 4th groups, no histological signs of weakening of the morphofunctional state of the lobules were found. As a rule, the cortical substance was clearly defined and was densely filled with cells. In the cerebral layer, a sufficient number of lymphocytes was noted, which were evenly located in the territory of the cerebral substance.

Thus, according to the results of serological and immunomorphological studies, the immunostimulating effect of DBE was established with different application schemes. The method of simultaneous administration of an immunostimulator together with vaccination of chickens should be considered the most affordable.

Conclusions

1. The use of beekeeping products in veterinary medicine is quite promising, as they are able to stimulate an immunocompetent system, as demonstrated in studies on chickens, in which, especially at day-old age, the immune system is not yet sufficiently formed and, accordingly, it cannot fully function, especially for the preservation of young birds from the action of various pathogens.

2. According to the results of serological studies, the scheme of using the DBE immunostimulator was quite effective, where the immunostimulator was administered 5 days before vaccination and was 7.4±0.13 log2. This indicator was slightly lower in the group where the immunostimulant was administered together with the vaccination and was 7.2±0.12 log2. In the group of chickens where the immunostimulant was administered 5 days after vaccination, this indicator was 7.3±0.12 log2. Thus, DBE immunostimulant showed sufficient immunostimulating properties even after its use 5 days after vaccination.

3. According to the results of immunomorphological studies, the indices of lymphoid organs increased in all studied schemes of immunostimulant use. Thus, positive changes were noted in the thymus index, which in the 2nd, 3rd and 4th experimental groups was equal to 5.56±0.014, respectively; 5.63±0.13 and 5.51±0.15. In the control, this indicator was lower and corresponded to 4.58±0.01.

4. The conducted immunomorphological studies at the histological level revealed positive changes when using DBE. In the second group of chickens, which were given an immunostimulant together with vaccination, the area of the bursa of Fabricius slightly decreased after stimulation. On histological examination, this correlated with a decrease in the size of the lymphoid nodules and some reduction in the width of the cortex of the lymphoid nodules.

5. The most rational in terms of availability and ease of implementation should be considered the method of simultaneous use of DBE immunostimulator together with vaccination of chickens against Newcastle disease

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АПІТЕРАПІЯ ТА ІМУНОСТИМУЛЯЦІЯ КУРЧАТ ЕСТРАКТОМ ТРУТНЕВОГО РОЗПЛОДУ ПРИ ЩЕПЛЕННІ ПРОТИ НЬЮКАСЛСЬКОЇ ХВОРОБИ ЗА РІЗНИХ СХЕМ ЗАСТОСУВАННЯ

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***Резюме**. В представленій статті проводилося вивчення імуностимулюючого впливу розробленого імуностимулятора – екстракту трутневого розплоду (ЕТР) на організм курчат за різних схем застосування. Імунний статус вираховували за серологічними та імуноморфологічними показниками. Для визначення оптимальної схеми застосування ЕТР було сформовано 3 піддослідних та контрольна групи по 6 курчат двотижневого віку. Другій групі екстракт давали разом зі щепленням, третій – за 5 діб до щеплення, а четвертій – через 5 діб після щеплення. Перша група залишалася контрольною. Сироватки крові досліджували за допомогою РЗГА. Імуноморфологічні дослідження проводилися на макроскопічному та мікроскопічному рівнях та розраховували індекси тимуса, бурси Фабриціуса та селезінки.*

За результатами проведеного дослідження встановили, що титр антигемаглютининів в РЗГА був вищий у 3 групі, де імуностимулятор було введено за 5 діб до вакцинації і становив 7,4±0,13 log_2, незначно нижчим цей показник виявився у четвертій групі 7,3±0,12 log_2 та у другій групі 7,2±0,12 log_2. У першій групі рівень антигемаглютинінів складав 5,3±0,3 log_2. У піддослідних групах при застосуванні вакцини та ЕТР абсолютна маса тимусу збільшувалась порівняно з контролем. Позитивні зміни відзначено в індексі тимуса, який у 2-й, 3-й та 4-й піддослідних групах дорівнював відповідно 5,56±0,014; 5,63±0,13 та 5,51±0,15. У контролі цей показник був

нижчим і відповідав 4,58±0,01. Індекс бурси Фабриціуса в контрольній групі курчат дорівнював 4,26±0,012, а у піддослідних 2-й, 3-й та 4-й групах він досягав відповідно 5,42±0,01; 5,46±0,01 та 5,50±0,01. Індекси бурси також демонструють перевагу цього показника в піддослідних групах, які одержували імуностимулятор. Показники маси та індекси селезінки відрізнялись у контрольній та піддослідних групах. Якщо у контрольних курчат цей показник відповідав 412,4±42,4 мг, то у піддослідних в 2-й, 3-й та 4-й групах він збільшувався відповідно до 532,4±15,30; 598,2±17,2 та 542,8±33,4 мг. Така ж тенденція спостерігалась у зміні індексу селезінки.

Ключові слова: апітерапія, екстракт трутневого розплоду, імуностимуляція, курчата, продукти бджільництва, хвороба Ньюкасла

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