Influence of the nanophytopreparation "Betulin in carbon microsphere" on the state of pathogenic and persistent factors of the joints of enteromicrobiocenosis of broiler chickens

Lysko Svetlana¹, Valentina Pleshakova², Nadegda Lescheva², Marina Zadorozhnaya¹, and Tatyana Lorengel²

¹Siberian Scientific Research Institute of Poultry Farming – Branch of the Federal State Budget Scientific Institution "Omsk Agrarian Scientific Center", 60 let Pobedy street, s. Morosovka, Omsk region, Russia, 644555
²Omsk State Agrarian University named after P.A. Stolypin, 1 Institutskaya Ploshchad, Omsk, Siberian Federal District, Russia, 644008

E-mail: vet@sibniip.ru, vi.pleshakova@omgau.org

Abstract. The current development stage of the poultry industry is characterized by a rapid consolidation of the role of pathogenic and opportunistic microorganisms. It was found that some phytopreparations have a diverse impact on the persistent characteristics of microorganisms. This work aims to study the influence of the nanophytopreparation “Betulin in carbon microsphere” on the manifestation of pathogenic (hemolytic, adhesive, proteolytic, catalase activities) and persistent (antilysozyme, anti-complementary activities) characteristics of microorganisms isolated from broiler chickens. Studied were microorganisms (Escherichia coli, Citrobacter freundii, Staphylococcus aureus, Salmonella spp., Pseudomonas aeruginosa) isolated from broiler chicken droppings. The nanophytopreparation “Betulin in carbon microsphere” was prophylactically added to the diet of chickens. As a control, considered were cultures of microorganisms isolated from broiler chickens, who did not receive the preparation in the diet. It was established that 37.5% of E. coli cultures isolated from droppings of the control broiler group exhibit hemolytic activity, while E. coli isolates from the experimental group did not have this activity. The percentage of isolated cultures of microorganisms that showed adhesive, proteolytic and catalase activity was lower for broiler chickens of the experimental group compared to the control one. This tendency was observed when studying persistent factors, in particular, antilysozyme and anti-complementary characteristics. The nanophytopreparation "Betulin in carbon microsphere" can be applied not only to increase the general resistance of the poultry body but also to target the main types of microbiocenosis joints, reducing their pathogenic and persistent potential.

1. Introduction
At the present stage of development of both veterinary and medical science and practice, a fairly extensive array of theoretical and experimental data has been accumulated, which testifies about the rapidly growing spread of antibiotic-resistant microorganisms [1-5]. So, the vast majority of previously successfully used antibiotic drugs do not have the required prophylactic and therapeutic effect [6-8].

The search for new effective drugs for the treatment and prevention of infectious pathology of poultry of bacterial etiology is relevant and in demand in veterinary science and practice [9-11]. In this
regard, phytopreparations are of certain interest not only as a basis for the construction of antibacterial agents, but can also act as ingredients with multidirectional activity towards the persistent characteristics of pathogenic microorganisms.

Scientists have studied the inhibitory activity of phytopreparations against the antilysozyme and anti-interferon signs of some microorganisms, and in particular enterobacteria. It was also found that tinctures of several medicinal plants have a pronounced unidirectional inhibitory effect on the antilysozyme activity of clostridia, enterobacteria, and several fungi. At the same time, other plant extracts have a diverse impact on persistent characteristics. Thus, along with the inhibitory effect, some tested microorganisms showed an increase in the level of expression of certain persistent characteristics [12-16].

The new Russian preparation "Betulin in carbon microsphere" is a microspherical nanoporous carbon carrier made from natural fossil raw materials sapropel and betulin - one of the main components of birch bark. The technology of obtaining the new-generation phytopreparation with a nanodispersed bioactive component is based on the transition of betulin from a crystalline state to a soluble one, followed by its integration into a nanoporous carbon matrix.

It should be noted that several scientific works consider the influence of this drug on the hematological, biochemical, and immunological parameters of the animal body blood and its therapeutic efficacy [17, 18]. However, its impact on the potentially pathogenic and persistent properties of microorganisms isolated from the biotope of the gastrointestinal tract of poultry has not been studied.

This work aims to study the influence of nanophytopreparation "Betulin in carbon microsphere" on the manifestation of pathogenic and persistent characteristics of microorganisms isolated from broiler chicken droppings.

2. Materials and methods

The studied material was bacteria of the Enterobacteria genus isolated from samples of droppings from broiler chickens. The nanophytopreparation “Betulin in carbon microsphere” was prophylactically introduced into the diet of chickens. As a control, we took cultures of microorganisms isolated from broiler chickens, who did not receive the preparation in their diet. Bacteriological studies were carried at the department of poultry diseases of Omsk Regional Veterinary Laboratory.

Isolation and identification of microorganism cultures were carried out using the generally accepted methods, taking into account the cultural-morphological, tinctorial and biochemical characteristics. The STAPHY test, ENTERO test (LA-Chema, Czech Republic), and RapiDTM ONE System 20 (USA) systems were used to identify microbial cultures.

The hemolytic activity of bacteria was determined using blood agar at 37 °C for 24 hours. The results were accounted by the appearance of zones of clearing (hemolysis) around the colonies of microorganisms. Between the studied cultures, a comparison was made of the hemolytic activity of the studied strain divided by the diameter of the zone of grown colony. The calculated coefficients for various cultures were compared.

The quantitative assessment of the adhesive activity of the isolated cultures was carried out according to V.I. Brilis. The experiment was as follows: a suspension of the studied microorganisms and washed standard formalized erythrocytes was suspended to a buffer solution (pH=7.2), and a drop of it was added to a glass slide. The degree of adhesive activity was assessed using the average adhesion index (AAI), which took into account the average number of microorganisms attached to one erythrocyte. AAI from 0 to 1 shows a zero adhesiveness; low adhesiveness is from 1.04 to 2.0; medium adhesiveness is from 2.04 to 4.00, and AAI exceeds 4.0 at high adhesiveness.

The proteolytic activity of the isolated cultures of microorganisms was determined using the gelatinase test. To detect catalase activity, a drop of 3% hydrogen peroxide solution was added to the bacterial cell colonies. The antilysozyme, anti-complementary activities were determined using the recommendations of O.V. Bukharin. The experimental data obtained was statistically processed.
3. Results and discussion
The performed studies on the use of nanophytopreparation showed that enteromicrobiomes of broiler chickens of the experimental group were characterized by a variety of basic microorganisms. It was also found that potentially pathogenic bacteria of various taxonomic groups were isolated to a greater extent for the broiler chickens of the control group (Table 1).

Table 1. Taxonomic profile of isolated enteromicrobiome joints in broiler chickens.

| Isolated microorganisms (n) | Control group | Experimental group |
|----------------------------|---------------|-------------------|
|                            | Number of     | %                 | Number of     | %                 |
|                            | strains       |                   | strains       |                   |
| Escherichia coli (n=12)    | 8             | 66.7              | 4             | 33.3              |
| Citrobacter freundii (n=16)| 9             | 56.3              | 7             | 43.7              |
| Staphylococcus aureus (n=16)| 10           | 62.5              | 6             | 37.5              |
| Salmonella spp. (n=8)      | 8             | 100               | -             | -                 |
| Pseudomonas aeruginosa (n=9)| 9             | 100               | -             | -                 |

Thus, Escherichia coli cultures in poultry of the control group were isolated in 66.6% of cases, which is 2 times more than for the experimental one. This tendency was observed for other joints of the enteromicrobiome. It should be noted that several microorganisms, in particular Salmonella spp. and Pseudomonas aeruginosa, were not isolated from the fecal samples of broiler chickens of the experimental group.

When studying the pathogenicity factors of the joints of the enteromicrobiome, it was found that hemolytic activity was presented in 37.5% of Escherichia coli cultures isolated from the droppings of broiler chickens of the control group. Meanwhile, the isolates obtained from the poultry of the experimental group did not have the indicated activity. It should be noted that the largest number of cultures with hemolytic activity (62.5%) belongs to the species Citrobacter freundii, and the smallest number of cultures belongs to the species Salmonella spp. At the same time, in the experimental group of broiler chickens, only 28.5% of Citrobacter freundii cultures exhibited hemolytic activity, while other microorganisms did not (Table 2).

Table 2. Factors of pathogenicity of cultures of bacteria isolated from samples of broiler chickens droppings.

| Microorganisms                  | Positively reacting cultures, % |
|---------------------------------|---------------------------------|
|                                 | Control group | Experimental group |
|                                 | h   | a   | p   | c   | h   | a   | p   | c   |
| Escherichia coli                | 37.5| 50.0| 62.5| 12.5| -   | 25.0| -   | 25.0|
| Citrobacter freundii            | 62.5| 33.3| 44.4| 55.5| 28.5| 14.2| -   | -   |
| Staphylococcus aureus           | 40.0| 50.0| 30.0| -   | -   | 16.6| -   | -   |
| Salmonella spp.                 | 25.0| 50.0| 62.5| -   | -   | -   | -   | -   |
| Pseudomonas aeruginosa          | 44.4| 77.7| 88.8| -   | -   | -   | -   | -   |

Designations:
- h - hemolytic activity,
- a - adhesive activity,
- p - proteolytic activity,
- c - catalase activity

It was found that for broiler chickens of the control group, the highest percentage of microorganisms with positive adhesion was found in the culture of Pseudomonas aeruginosa, and the lowest percentage was in Citrobacter freundii. Moreover, the results obtained showed that for the broiler chickens of the experimental group, the percentage of isolated cultures of microorganisms that showed adhesive activity was significantly lower.
This pattern was also registered when testing the proteolytic characteristics of the isolated cultures. Thus, only 25.0% of Escherichia coli cultures isolated from droppings of the experimental group broilers had proteolytic activity, while in the control group all isolated microorganisms exhibited proteolytic properties.

It should be noted that not a single culture of microorganisms isolated from the droppings of broilers from the experimental group showed catalase activity. Meanwhile, 100% of the isolates from the control group could decompose peroxide to one degree or another.

Analysis of the research results showed that in the control group of broiler chickens the ability of isolated bacterial cultures to specifically inactivate lysozyme was manifested in 5.0% of Escherichia coli and Salmonella spp. microorganisms, in 50.0% of Staphylococcus aureus cultures and in 66.6% of Pseudomonas aeruginosa isolates (Table 3). At the same time, in this group, the minimum percentage was accounted for by Citrobacter freundii cultures (22.2%). It should be noted that cultures of microorganisms isolated from poultry droppings from the experimental group had a lower percentage of isolates with antilysozyme activity. So, in particular, 25.0% of Escherichia coli cultures exhibited antilysozyme activity, which is 3 times less than that in the control group.

**Table 3.** Persistent characteristics of microorganisms isolated from broiler manure samples.

| Microorganisms          | Positively reacting cultures, % | Control group | Experimental group |
|-------------------------|---------------------------------|---------------|--------------------|
|                         | ALA    | ACA    | ALA    | ACA    |
| Escherichia coli        | 75.0   | 50.0   | 25.0   | 25.0   |
| Citrobacter freundii    | 22.2   | -      | 14.2   | -      |
| Staphylococcus aureus   | 50.0   | 60.0   | 16.6   | -      |
| Salmonella spp.         | 75.0   | 37.5   | -      | -      |
| Pseudomonas aeruginosa  | 66.6   | 33.3   | -      | -      |

Designations: ALA - antilysozyme activity, ACA - anti-complementary activity

A similar pattern was observed when studying the anticomplementary activity of microorganisms isolated from broiler chicken droppings. Thus, the largest percentage of bacteria having this persistent factor was recorded in poultry of the control group. For broiler chickens of this group, 50.0% of the isolated Escherichia coli cultures had the indicated property, while in the experimental group the amount of such cultures was 2 times less. This tendency was observed for other isolated microorganisms, in particular, Staphylococcus aureus, Salmonella spp. and Pseudomonas aeruginosa.

4. Conclusions
Thus, the analysis of the results obtained allows us to state that the nanophytoreparation "Betulin in carbon microsphere", when introduced into the diet of broiler chickens, has the ability to change a number of biological characteristics of the joints of the enteromicrobiome. It was found that the preparation has a suppressive impact on some potentially pathogenic factors of isolated microorganisms and, in particular, on hemolytic, proteolytic, adhesive and catalase activity.

The results obtained show that the tested nanophytoreparation, in addition to the known immunomodulatory activity, can to a certain extent inhibit a number of persistent factors of the joints of the poultry enteromicrobiome.

So, the nanophytoreparation “Betulin in carbon microsphere” can be applied not only to increase the general resistance of the poultry's body, but also to target the main types of pathogenic and opportunistic microorganisms, reducing their pathogenic and persistent potential.

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