Effect of Bacillus cereus IP 5832 and coumarin in the diet on the general state of the broilers

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Abstract. Currently, there is an interest in complex biological preparations based on probiotic strains and plant extracts. Probiotic preparations, together with plant extracts, are natural growth stimulants and can become a promising replacement for antibiotic growth stimulants in poultry farming, the latter stimulate the production of endogenous enzymes, improving the digestibility and absorption of nutrients in feed. Technological prospects for the probiotics and plant extracts practical joint use in poultry farming touch upon a wide range of problems related to the correction of intestinal biocenosis, hormonal and enzymatic systems, stimulation of immunity, prevention and treatment of dysbacteriosis. The purpose of this study is to assess the effect of the complex introduction of the probiotic strain Bacillus cereus and a coumarin derivative in the diet of broiler chickens on hematological parameters. In the studied broiler chickens, changes in hematological and biochemical parameters are noted in connection with the processes of their growth and development. All parameters in the blood are accompanied by changes, which indicates an intense metabolic rate, an additional load in the metabolism that requires the participation of protective blood elements.

1. Introduction
Feed antibiotics have a direct effect on the microbiocenosis of the gastrointestinal tract of poultry, as a result - an increase in pathogenic microorganisms and inhibition of intestinal microflora, and for that reason, antibiotic resistance develops. In this case, probiotics are considered as an integral component of the pharmacological support of industrial livestock and poultry [1-3].

One of the priority tasks in poultry farming today is healthy young growth, its safety from diseases and death. Recently, plant extracts have been used in practice to reduce mortality and reduce the consumption of biological products used. As is well known, the latter have a positive effect on the immune status of poultry [4], productivity [5], growth and development [6], have antibacterial properties [7-8].

At present, there is interest in complex biological preparations based on probiotic strains and plant extracts [9]. Unlike probiotics, the general effect of complex preparations on the farm animals and poultry organism is associated not only with antimicrobial effect, but also with their positive effect on digestion processes. Probiotic preparations, together with plant extracts, belong to natural growth stimulants and can become a promising replacement for antibiotic growth stimulants in poultry [10], the latter stimulate the production of endogenous enzymes, improving the digestibility and absorption of nutrients in feed. Many of them serve as natural flavoring agents that stimulate feed intake, which has
a positive effect on the productivity of animals [11]. As a rule, complex preparations have a more pronounced effect than each component separately.

The combined use of probiotic strains and plant substances improves the morphological and biochemical parameters of the blood in laying hens [12], increases the egg production [13]. Phytobiotic preparations have a special effect on the microbiological composition of the intestine, maintaining the microflora in an optimal state [14-15]. Technological perspectives of practical probiotics and plant extracts joint use in poultry farming touch upon a wide range of problems associated with the correction of intestinal biocenosis, hormonal and enzymatic systems, stimulation of immunity, prevention and treatment of dysbacteriosis [16-18]. The purpose of this study is to assess the effect of the complex introduction of the probiotic strain Bacillus cereus and a coumarin derivative in the diet of broiler chickens on hematological parameters.

2. Materials and methods

Substances
- 7-Hydroxycoumarin 99% AC12111-0250 (Acros);
- Bacillus cereus strain IP 5832 (ATCC 14893), which is part of probiotic preparations.

The primary isolation of microorganisms was carried out on LB agar (Sigma-Aldrich, United States); further cultivation was carried out in LB broth at 37 ± 1 ° C with dynamic growth monitoring at 545 nm (OD545). After 72 hours, the bacterial biomass was separated by centrifugation for 10 minutes at 12000 g (MiniSpin, Eppendorf, Germany), and the resulting supernatants were sterilized through a sterile syringe filter attachment, CA (cellulose acetate) (Membrane Solutions, USA) with a pore diameter of 0.22 μm.

Experimental studies were carried out on 120 heads of 7-day-old broiler chickens (4 groups, n = 30). Control group - BD; Experimental I - BD + probiotic strain Bacillus cereus (4 ml / kg FM / day); Experimental II - BD + coumarin (9 μg / kg / day); Experimental III - BD + Bacillus cereus strain + coumarin. Feeding and watering of poultry was carried out by a group method.

Blood samples were collected for biochemical studies in vacuum tubes with a coagulation activator (thrombin). Blood biochemical analysis was carried out on a CS-T240 automated biochemical analyzer (DIRUI Industrial Co., Ltd, China) using commercial biochemical kits for veterinary medicine DiaVetTest (Russia).

Statistical processing was carried out using the Statistica 10.0 application (Stat Soft Inc., USA). Analysis of poultry live weight included the determination of the arithmetic mean (M), standard error of the mean (m). Differences were considered significant at P≤0.05. For bioinformatic processing of sequencing results, the PEAR program (Pair-End AssembleR, PEAR v0.9.8) was used.

3. Results and discussion

In the study of blood serum in broiler chickens in the control and experimental groups, with the introduction of a probiotic strain and a coumarin derivative into the diet, changes in amino acid metabolism enzymes in the age aspect were established (table 1).

| Table 1. Indicators of blood serum of broilers when used in Bacillus cereus IP 5832 and a coumarin derivative diets. |
|-------------------|-----------------|-----------------|-----------------|
| Index             | Control         | I               | II              |
|                   | Age 16 days     |                 |                 |
| ALT, U / l        | 5.33±1.49       | 5.33±0.98       | 7.95±1.55*      |
| AST, U / l        | 230.3±18.3      | 246.8±8.23      | 136.5±29.5*     |
| Direct bilirubin, μmol / l | 0.41±0.05 | 0.34±0.07 | 0.36±0.04 |
| g-GT, U / l       | 15.7±2.19       | 17.0±5.00       | 16.0±6.00       |
| Alkaline phosphatase, U / l | 28.7±4.33 | 53.0±12.2* | 36.0±13.0 |

* Differences were considered significant at P≤0.05.
At the age of 16 days, the number of leukocytes in broiler chickens is at the level of 38.8*10^9 cells/l, and a significant increase was noted in group I - 1.4 times (p≤0.05) and a decrease in group II - in 1.7 times (p≤0.05), compared with the control group. During the growth period, when the highest average daily weight gain is noted, the number of leukocytes increases by 10.0-54.9%. The maximum saturation of blood with leukocytes was recorded in chickens at the age of 43 days, when their number increased compared with the previous age by 10.0 - 32.0% (table 2).
Table 2. Morphological parameters of broiler blood when used in diets of Bacillus *cereus* IP 5832 and a coumarin derivative, 10^9 cells / I.

| Index          | Control | I            | II            | III           |
|----------------|---------|--------------|---------------|--------------|
|                | Age 16 days |             |               |              |
| Leukocytes     | 28.4±4.35 | 38.8±0.25*   | 16.9±1.15*    | 32.1±2.65    |
| Lymphocytes    | 58.9±1.45 | 61.0±1.00    | 61.9±1.15     | 60.3±0.40    |
| Monocytes      | 6.45±0.65 | 5.35±0.05    | 7.00±0.20     | 5.35±0.25    |
| Granulocytes   | 34.7±0.80 | 34.9±0.15    | 33.8±1.25     | 34.4±0.65    |
| Platelets      | 67.0±1.00 | 78.0±2.00    | 56.3±1.30     | 62.5±8.50    |
|                | Age 29 days |             |               |              |
| Leukocytes     | 42.1±4.60 | 43.1±14.4    | 37.5±2.40     | 50.5±12.1    |
| Lymphocytes    | 57.9±1.61 | 64.2±3.35    | 60.3±0.49     | 65.0±4.75    |
| Monocytes      | 6.43±0.66 | 5.35±0.75    | 5.17±0.33     | 5.40±0.10    |
| Granulocytes   | 35.7±1.15 | 30.5±2.60    | 34.5±0.20     | 29.6±4.80    |
| Platelets      | 105.7±6.36| 102.5±4.50   | 114.7±7.31    | 98.3±12.0    |
|                | Age 43 days |             |               |              |
| Leukocytes     | 59.2±12.1 | 47.9±0.67    | 55.3±2.38     | 59.7±5.87    |
| Lymphocytes    | 56.6±4.43 | 51.7±1.33    | 52.2±1.27     | 54.4±5.72    |
| Monocytes      | 8.30±0.10 | 8.50±0.45    | 7.80±0.40     | 7.30±1.05    |
| Granulocytes   | 35.1±4.33 | 45.4±6.54*   | 45.9±6.50*    | 38.6±4.41    |
| Platelets      | 101.7±11.9| 102.3±6.17   | 116.0±4.62    | 109.3±6.96   |

Note: * – p≤0.05; ** – p≤0.01; *** – p≤0.001 when comparing the control groups with the experimental groups.

Monocytes have phagocytic and bactericidal activity, the level of monocytes at the age of 29 days in the experimental groups was lower than in the control group, but all changes were unreliable, at the end of the experiment a similar picture was observed.

Lymphocytes perform the function of immune surveillance, carry out response to the penetration of pathogenic microorganisms, are responsible for the formation of specific immunity. At the age of 16 and 29 days, an increase in the level of lymphocytes in the experimental groups was revealed, but the changes were insignificant. At the age of 43 days, on the contrary, the decrease was noticed, however, the indicators fit into the framework of the accepted reference values. Against the background of an increase in lymphocytes, there is a decrease in granulocytes at the age of 16 and 29 days and the opposite picture at the end of the experiment, so in I and II experimental groups a significant increase in the latter by 1.3 times (p≤0.05), relative to the control was revealed.

4. Conclusion
The additional inclusion of a coumarin derivative in the diet of broiler chickens helps to reduce aspartate aminotransferase, as in our case, as well as the level of alanine aminotransferase, but on the 29th day of the experiment [19], a similar picture was observed in [20].

Coumarin, which is a part of citrus waste [21], can have a positive effect on the biochemical profile of laying hens, in particular the level of gamma-glutamyltransferase, thereby indicating the activity of enzymes such as ALT and AST.

In [22], it is presented that the introduction of only one plant extract of thyme has a positive effect on the biochemical parameters of the blood of broiler chickens, in comparison with the group of combined use of the probiotic strain *Bacillus subtilis* PB6 and plant extract.
The combined use of the probiotic strain *Bacillus adolescentis* and the oak burk extract will increase the antioxidant activity of the body and antimicrobial components of blood plasma compared to broiler chickens with similar growth rates, but without adding this combination [23], similar results were obtained in [24], in our case, the introduction of *Bacillus cereus* and a coumarin derivative gives a similar result.

Thus, in the studied broiler chickens, changes in hematological and biochemical parameters are noted in connection with the processes of their growth and development. All parameters in the blood are accompanied by changes, which indicates an intensive metabolic rate, an additional load in the metabolism that requires the participation of protective blood elements.

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