UDC 636.087.52 HAC Code 4.2.4 DOI: 10.32417/1997-4868-2023-231-02-53-61

Intestinal microbiocenosis and fodder digestibility in broiler chickens when using probiotics

S. S. Aleksandrova¹²⁷, A. A. Bakharev², A. P. Duktov³, N. A. Sadomov³

¹Research Institute of Agriculture of the Northern Trans-Urals – a branch of the Tyumen Scientific

Center of the Siberian Branch of the Russian Academy of Sciences, Moskovskiy, Russia

² State Agrarian University of the Northern Trans-Urals, Tyumen, Russia

³ Belarussian State Agricultural Academy, Gorki, Republic of Belarus

[™]*E-mail: aleksandrova*977@mail.ru

Abstract. Research objective was to study the intestinal microbiocenosis in broiler chickens. Laboratory test was conducted at Research and Development Center for Agriculture of the North-Western Branch of Tyumen Research Center of SB RAS. Methods. Cobb 500 chickens breed was used. The main part of experiment began at the age of 14 days. By this time, chickens had reached an average body weight of 365 grams. The control group of chickens was fed basic diet, consisting of total mixed ration fodder and pure water. Chickens of the 1st experimental group during the main stage of experiment were fed with "Vetom 2" probiotic additive in drinking water at 50 mg / 1 kg of poultry weight, whereas chickens of the 2nd experimental group were fed "Lactobifadol" added to their fodder at the rate of 0.2 g per 1 kg of poultry weight. Research results have showed that prior to feeding probiotic formulations no pathogenic microflora was observed in the microbiocenosis of the droppings of broiler chickens. Conditionally pathogenic bacteria, such as Proteus mirabilis, Klebsiella oxytoca, Klebsiella pneumoniae, Staphylococcus aureus were present. Bacillis spp. was present in all of the samples. After feeding the formulations, no pathogenic microflora was detected in the droppings of chickens in experiment groups. Conditionally pathogenic bacteria Proteus mirabilis were present in the droppings of control group chickens, the 1st experimental group, 2nd experimental group. Concentrations of Bifidobacteria and Lactobacteria in chickens' droppings fed with "Lactobifadol" were significantly higher than in the rest of the groups. Bacillus spp. was present in all samples of the 1st experimental group fed with "Vetom 2". It was also present in the droppings of chickens in other groups. Scientific novelty. Laboratory experiment involving broiler chickens aimed at researching intestinal microbiocenosis and its effect on digestibility of mixed fodder was conducted in Northern Trans-Urals conditions for the first time.

Keywords: broiler chickens, Vetom, Lactobifadol, intestinal microbiocenosis, digestibility coefficients.

For citation: Aleksandrova S. S., Bakharev A. A., Duktov A. P., Sadomov N. A. Intestinal microbiocenosis and fodder digestibility in broiler chickens when using probiotics // Agrarian Bulletin of the Urals. 2023. No. 02 (231). Pp. 53–61. DOI: 10.32417/1997-4868-2023-231-02-53-61.

Date of paper submission: 10.10.2022, date of review: 03.11.2022, date of acceptance: 18.11.2022.

Introduction

Organic agriculture is quickly permeating enterprises, producing food products, as well as markets of European countries.

In EU countries, environment friendly animal husbandry and crop farming regulations form a legislative framework for environmental agriculture, being the constituent part of the documentation of Organics International (International Federation of Organic Agriculture Movements), formed in 1972. The laws of this body define that within the EU the environmental agriculture products are notable for monitoring their production process, rather than probing a number of controlled components. In EU countries, the use of fodder antibiotics was banned as of January 1, 2006.

The problem of finding biologically active substances capable of preventing gastrointestinal disorders, increasing immunity and improving the digestibility of fodder is front and center as on this day. Therefore, world practice is focusing close attention to developing and testing of probiotics, prebiotics, biopolymers and synbiotics.

Based on data obtained from numerous authors, probiotic formulations are used when restoring intestinal symbiosis of bacteria by introducing bacteria of normal intestinal microflora.

лександрова С.

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Бахарев А.

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2023

Аграрный вестник Урала № 02 (231), 2023 г.

Биология и биотехнологии

Probiotics are live cultures of bacteria used in veterinary medicine and animal nutrition to treat and increase their productivity. They appear antagonistic to pathogenic and conditionally pathogenic microflora in intestines, exert a positive effect on immunity, increase digestibility of fodder nutrients.

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Probiotics stimulate symbiotic intestinal microflora, are harmless, have no contraindications, in combination with veterinary and sanitary measures have a positive effect on microbiocenosis of gastrointestinal tracts of animals [1–9].

One instance of such formulations are "Vetom 2" and "Lactobifadol". "Vetom 2" contains dry biomass of live spore-forming bacteria Bacillus amyloliquefaciens strain RNCIM B-10642 (DSM 24614) and Bacillus amyloliquefaciens strain RNCIM B-10643 (DSM 24615) [10].

"Lactobifadol" is a dry mass of lactic acid bacteria Lactobacillus acidophilus, as well as bifidobacteria Bifidobacterium adolescentis [10].

Bacterial formulations are used to maintain animals' immunity, reduce the burden of antimicrobial substances, reduce various stresses induced by production process, restore the normoflora of the intestine of animals and poultry [12–18].

The objective of this research was to study the intestinal microbiocenosis in broiler chickens. In this regard, the following goals were set:

- to probe intestinal microflora of chickens in experimental groups;

to conduct hematomancy to monitor physiological condition of chickens;

- to determine digestibility of fodder nutrients in chickens depending on formulations used.

#### Methods

Studies of intestinal microbiocenosis in broiler chickens have been conducted in Northern Trans-Urals conditions. Laboratory experiment was conducted at Research and Development Center for Agriculture of the North-Western Branch of Tyumen Research Center of SB RAS. Cobb 500 chickens breed was used. The chickens were distributed based on the principle of balanced equivalent groups consisting of 35 heads per group. The chickens rearing period was 34 days. The experiment's primary stage began by the time the chickens had reached an average body weight of 365 grams, at the age of 14 days.

Based on research design, the control group of chickens was fed industrially produced all-in-one complete feed and pure water.

Chickens of the 1<sup>st</sup> experimental group were fed with industrially produced all-in-one complete feed, with water, as well as "Vetom 2" formulation at the rate of 50 mg / 1 kg of poultry weight.  $2^{nd}$  experimental group of chickens received the same feed with "Lactobifadol" at the rate of 0.2 g per 1 kg of poultry weight. "Vetom 2" is a white powder for oral use, finely dispersed, odorless, soluble in water, forming a white sediment. Dry biomass of live spore-forming Bacillus amyloliquefaciens strain RNCIM B-10642 (DSM 24614) and Bacillus amyloliquefaciens strain RNCIM B-10643 (DSM 24615). Manufacturer: NPF Research Center LLC (Russia).

"Lactobifadol" is a powder for oral use. International non-proprietary name: lactobifadolum. Contains, dried by sorption method, microbial mass of lactic acid bacteria Lactobacillus acidophilus LG1-DEP-VGNKI, bifidobacteria Bifidobacterium adolescentis B-1-DEP-VGNKI and filler agent – wheat flour.

In terms of its appearance, "Lactobifadol" is a homogeneous loose powder of beige color. One gram of the formulation contains at least 1.0x106 CFU (colonyforming units) of living cells of lactic acid bacteria Lactobacillus acidophilus LG1-DEP-VGIKI and 8.0x107 CFU of live cells of bifidobacteria Bifidobacterium adolescentis B-1-DEP-VGNKI.

The intestinal microbiocenosis was probed in group's three chickens at the start and four chickens at the end of experiment in the bacteriological laboratory of the State Educational Institution for Higher Professional Education The Tyumen State Medical Academy under Federal Service on Surveillance in Healthcare by applying laboratory methods of inoculation of nutrient media as provided for by the Industry Standard OST 91500.11.0004-2003 (enacted by the Order of the Ministry of Health of the Russian Federation No. 231 of 09.06.2003. The calculation of the quantity of microorganisms was accepted as CFU/g based on generally acknowledged methodology. Material for the analysis was collected from intestines into sterile containers. Feces were analyzed on day 12 and day 34 of rearing period.

At the age of 12 and 34 days, blood serum was collected from experimental poultry for research. The blood serum was analyzed to determine: total protein, albumin, creatinine, urea, cholesterol, triglycerides. The analyses were carried out in the laboratory of the State Educational Institution for Higher Professional Education The Tyumen State Medical Academy of Federal Service on Oversight in Healthcare.

#### Results

The microbial community is divided into obligate microflora, which inhabits it permanently, and optional, ingressed from external environment. The main instances of beneficial microflora are: bifidobacteria, lactobacilli, E. coli with normal enzymatic activity, Bacillus subtilis. Bifidobacteria represent numerous instances of intestinal microflora. They inhibit the development of pathogenic microflora. Bifidobacteria contribute to normal functioning of intestine. During their life processes, volatile fatty acids, such as lactic, acetic, formic and succinicare formed. Formation of acids helps in reducing pH levels of intestinal mucosa down to 4.0 –

Table 1

### Agrarian Bulletin of the Urals No. 02 (231), 2023

3.8. As a result, the growth of pathogenic microflora inside intestine is inhibited. An important biological effect of bifidobacteria is expressed in synthesis of amino acids, proteins, a number of vitamins – thiamine, riboflavin, nicotinic, pantothenic, folic acids, pyridoxine, cyanocobalamin, vitamin K, which are absorbed in the intestine and used in metabolic processes. Synthesized vitamins B exert a positive effect on development of an immune response in animals.

Lactobacteria are also instances of a normal intestinal microbiota. They are antagonists to pathogenic microorganisms. They participate in forming immunity. They exhibit antibacterial activity associated with their ability to form lactic acid during fermentation. In addition, they produce lysozyme, lactoline, lysine, lactocidin and others.

Lactic acid bacteria, in the digestive tract, prevent excessive reproduction of a number of bacteria that periodically enter gastrointestinal tract with fodder or belong to the category of concomitant flora and capable of causing development of endogenous infection as a result of a decrease in resistance of microorganism. High levels of lactobacteria contribute to the development of high resistance of animals to experimental infection after infecting them with Staph. aureus and Klebs. pneumonie. Lactic acid bacteria are able to suppress reproduction of putrefactive and pyogenic microbes: Pseudomonas, Aeromonas, E. coli, Salm. Cholera suis, Klebs. pneumonie and many others.

E. coli – Escherichia coli with normal enzymatic activity, is also a competitor to pathogenic flora. Symbiotic action with bifido- and lactobacteria, which are involved in synthesis of vitamins K and B. Contributes to normal functioning of immunity. An important function of escherichia is hydrolysis of lactose, synthesis of vitamins, antibiotic-like substances that inhibit the growth of pathogenic E. coli. A powerful immunomodulatory effect, activating humoral and local immunity. Escherichia colonize the colon and lower parts of the small intestine.

Bacillus subtilis (senna bacillus) is a gram-positive, spore-forming, aerobic type of soil bacteria. Contributes to synthesis of polypeptide antibiotics, as well as industrially produced enzymes such as amylase, protease. They exert an active biological effect on bodies of animals due to synthesis of various enzymes, antibiotics, lipopolysaccharides, as well as act as immunostimulants. In addition to antibacterial action of spore-forming aerobic bacteria, many authors have established antiviral action. Sarcinaspp – a type of grampositive non-pathogenic cocci. They are found in air, water, living organisms.

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|------------------------------------------------|---------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------|-------------------------|---------------------------------|---------------------------------|------------------------------------|---------------------------------|---------------------------------|--|--|
| Microorganisms                                 | Control group                   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |                         | I <sup>st</sup> exp     | erimental                       | l group                         | 2 <sup>na</sup> experimental group |                                 |                                 |  |  |
|                                                | 1                               | 2                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              | 3                       | 4                       | 5                               | 6                               | 7                                  | 8                               | 9                               |  |  |
| Pathogenic enterobacteria                      | none                            | none                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           | none                    | none                    | none                            | none                            | none                               | none                            | none                            |  |  |
| Typical E. coli                                | 10 7                            | 107                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            | 107                     | 107                     | 107                             | 107                             | 107                                | 107                             | 107                             |  |  |
| Lactose negative E. coli                       | none                            | none                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           | none                    | none                    | none                            | none                            | none                               | none                            | none                            |  |  |
| Hemolytic E. coli                              | none                            | none                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           | none                    | none                    | none                            | none                            | none                               | none                            | none                            |  |  |
| Other conditionally pathogenic enterobacteria: |                                 |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |                         |                         |                                 |                                 |                                    |                                 |                                 |  |  |
| 1) Proteus mirabilis                           | none                            | 107                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            | none                    | none                    | 107                             | none                            | none                               | none                            | 107                             |  |  |
| 2) Klebsiella oxytoca                          | none                            | 107                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            | none                    | none                    | none                            | none                            | none                               | none                            | 107                             |  |  |
| 3) Klebsiella pneumoniae                       | none                            | none                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           | none                    | none                    | none                            | 10 6                            | none                               | none                            | none                            |  |  |
| Non-fermenting bacteria                        | none                            | none                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           | none                    | none                    | none                            | none                            | none                               | none                            | none                            |  |  |
| Enterococci                                    | 106                             | 106                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            | 106                     | 106                     | 106                             | 107                             | less<br>than<br>10 <sup>5</sup>    | 105                             | 106                             |  |  |
| Staphylococcus aureus                          | $10^{3}$                        | 104                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            | $10^{2}$                | none                    | $10^{3}$                        | 104                             | 104                                | none                            | 103                             |  |  |
| Other staphylococci                            | none                            | none                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           | none                    | none                    | none                            | none                            | none                               | none                            | none                            |  |  |
| Bifidobacteria                                 | less<br>than<br>10 <sup>8</sup> | less<br>than<br>10 <sup>8</sup>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | 108                     | 109                     | less<br>than<br>10 <sup>8</sup> | 109                             | 109                                | 1010                            | 108                             |  |  |
| Lactobacteria                                  | 107                             | less<br>than<br>10 <sup>5</sup>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | 106                     | 106                     | 106                             | 107                             | less<br>than<br>10 <sup>5</sup>    | 106                             | 106                             |  |  |
| Lactotreptococci                               | 107                             | 107                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            | 106                     | 106                     | 106                             | 108                             | 106                                | 106                             | 107                             |  |  |
| Fungi p. Candida                               | none                            | none                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           | none                    | none                    | none                            | none                            | none                               | none                            | none                            |  |  |
| Clostridia                                     | over<br>10 <sup>5</sup>         | over<br>10 <sup>5</sup>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        | over<br>10 <sup>5</sup> | over<br>10 <sup>5</sup> | less<br>than<br>10 <sup>5</sup> | less<br>than<br>10 <sup>5</sup> | less<br>than<br>10 <sup>5</sup>    | less<br>than<br>10 <sup>5</sup> | less<br>than<br>10 <sup>5</sup> |  |  |
| Other microorganisms                           |                                 |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |                         |                         |                                 |                                 |                                    |                                 |                                 |  |  |
| Bacillis spp.                                  | 105                             | 106                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            | 105                     | 105                     | 105                             | 105                             | 105                                | 104                             | 105                             |  |  |

#### Microbiological analysis of chickens' droppings at the start of experiment

## Аграрный вестник Урала № 02 (231), 2023 г.

Enterococci, staphylococci, streptococci, fungi, proteus, Klibsiella pneumonieal, Serratia marcescens, Citrodacter freundii – are instances of conditionally pathogenic microflora, which, in moderate quantities, do not cause diseases. Under unfavourable conditions, they can multiply and cause intestinal disorders. This occurs when their number increases in relation to the normal microbiota, as well as with a decrease in functioning of immunity.

Clostridia is a type of gram-positive, anaerobic bacteria that produce endospores. They are constituent instances of a normal intestinal flora.

Hemolytic and salmonella E. coli are pathogenic microorganisms that cause gastrointestinal tract diseases in poultry, animals and humans. Microbiological snapshot of the chickens' intestines contents at the start and at the end of the main period of experiment are shown in Tables 1 and 2.

The Table 1 data show that no pathogenic microflora was observed in microbiocenosis of the droppings of broiler chickens prior to drinking probiotic formulations. Conditionally pathogenic bacteria Proteus mirabilis were present in 3 cases out of 9, Klebsiella oxytoca in 2 cases, Klebsiella pneumoniae in 1, Staphylococcus aureus in 7 cases out of 9. There was no difference between the groups observed. Bacillis spp was present in all of the samples.

Table 2 presents a snapshot of microflora of chickens' droppings after drinking formulations. No pathogenic microflora was detected in the droppings. Conditionally pathogenic bacteria Proteus mirabilis in the droppings of control group chickens were present in 2 cases out of 4, 1<sup>st</sup> experimental group also in 2 cases,  $2^{nd}$  experimental group in 1 case out of 4. No staphylococcus aureus was detected in the droppings of control group chickens, while in the 1<sup>st</sup> experimental group it was present in 2 samples out of 4, in 2<sup>nd</sup> experimental group in 3 samples. Concentrations of Bifidobacteria and Lactobacteria in the droppings of chickens of the 2<sup>nd</sup> experimental group, where the chickens were fed with "Lactobifadol", were much higher than in other groups. Bacillissppwas present in all samples of the 1<sup>st</sup> experimental group, control group – in 2 samples out of 4, 2<sup>nd</sup> experimental group – in 3 samples. Thus, adding of bacterial formulations allows to increase concentrations of corresponding bacteria in intestinal microbiocenosis of broiler chickens.

Intestinal microbiocinosis can affect digestibility of nutrients in the ration, because microbes have cellulolytic, proteolytic activity, secrete enzymes and synthesize vitamins, proteins and other nutrients used in the metabolism of the hosting body.

In our study, the nutrients digestibility coefficients of the fodder were determined. As well as the balance of protein, calcium and phosphorus in broiler chickens bodies. Digestibility of fodder was studied during a physiological experiment that was conducted by using a group method on 12 heads of a group (6 hens and 6 roosters) of broiler chickens at the age of 30 days.

The protein digestibility coefficient in chickens of control group was 87.4 %, in the 1<sup>st</sup> experimental group -86.8 %, in the 2<sup>nd</sup> experimental group -87.3 %; fiber -20,7; 24,4; 27.0 % respectively. Calcium -51,2; 55,3; 60,5 %. Phosphorus -81,3; 83,1; 87,1 %. Fat -75,6; 74,0; 73,0 %.

Table 2

| microbiological analysis of chickens aroppings at the end of experiment |                                 |                                 |                                 |                         |                                    |                         |                         |                         |                                    |                                 |                                 |                         |
|-------------------------------------------------------------------------|---------------------------------|---------------------------------|---------------------------------|-------------------------|------------------------------------|-------------------------|-------------------------|-------------------------|------------------------------------|---------------------------------|---------------------------------|-------------------------|
| Microorganisms                                                          | Control group                   |                                 |                                 |                         | 1 <sup>st</sup> experimental group |                         |                         |                         | 2 <sup>nd</sup> experimental group |                                 |                                 |                         |
|                                                                         | 1                               | 2                               | 3                               | 4                       | 5                                  | 6                       | 7                       | 8                       | 9                                  | 10                              | 11                              | 12                      |
| Pathogenic enterobacteria                                               | none                            | none                            | none                            | none                    | none                               | none                    | none                    | none                    | none                               | none                            | none                            | none                    |
| Typical E. coli                                                         | 106                             | 106                             | 106                             | 106                     | 106                                | 106                     | 107                     | 106                     | 106                                | 107                             | 107                             | 106                     |
| Lactosenegative E. coli                                                 | none                            | none                            | none                            | none                    | none                               | none                    | none                    | 10 3                    | none                               | none                            | none                            | none                    |
| Hemolytic E. coli                                                       | none                            | none                            | none                            | none                    | none                               | none                    | none                    | none                    | none                               | none                            | none                            | none                    |
| Proteus mirabilis                                                       | none                            | 106                             | none                            | 105                     | 105                                | none                    | 107                     | none                    | none                               | none                            | none                            | 107                     |
| Non-fermenting bacteria                                                 | none                            | none                            | none                            | none                    | none                               | none                    | none                    | none                    | none                               | none                            | none                            | none                    |
| Enterococci                                                             | 105                             | less                            | less                            | 105                     | 105                                | less                    | 106                     | less                    | less                               | 105                             | 106                             | 106                     |
|                                                                         |                                 | than<br>105                     | than<br>10 <sup>5</sup>         |                         |                                    | than<br>10 <sup>5</sup> |                         | than<br>105             | than<br>105                        |                                 |                                 |                         |
| Staphylococcus aureus                                                   | none                            | none                            | none                            | none                    | none                               | 102                     | none                    | $10^{3}$                | none                               | 103                             | 103                             | 105                     |
| Other staphylococci                                                     | none                            | none                            | none                            | none                    | none                               | none                    | none                    | none                    | none                               | none                            | none                            | none                    |
| Bifidobacteria                                                          | 109                             | 109                             | $10^{8}$                        | 108                     | 108                                | $10^{8}$                | 118                     | 109                     | 1010                               | 109                             | 109                             | 108                     |
| Lactobacteria                                                           | 107                             | 105                             | 106                             | 106                     | 106                                | 107                     | 106                     | 106                     | 107                                | 107                             | 107                             | 107                     |
| Lactotreptococci                                                        | 107                             | less<br>than<br>10 <sup>5</sup> | 106                             | 106                     | less<br>than<br>10 <sup>5</sup>    | 107                     | 106                     | 106                     | 107                                | less<br>than<br>10 <sup>5</sup> | 107                             | 108                     |
| Fungi p. Candida                                                        | none                            | none                            | none                            | none                    | none                               | none                    | none                    | none                    | none                               | none                            | none                            | none                    |
| Clostridia                                                              | less<br>than<br>10 <sup>5</sup> | over<br>10 <sup>5</sup>         | less<br>than<br>10 <sup>5</sup> | over<br>10 <sup>5</sup> | over<br>10 <sup>5</sup>            | over<br>10 <sup>5</sup> | over<br>10 <sup>5</sup> | over<br>10 <sup>5</sup> | over<br>10 <sup>5</sup>            | over<br>10 <sup>5</sup>         | less<br>than<br>10 <sup>5</sup> | over<br>10 <sup>5</sup> |
| Bacillis spp.                                                           | none                            | $10^{3}$                        | $10^{4}$                        | none                    | $10^{2}$                           | $10^{4}$                | $10^{3}$                | 105                     | $10^{3}$                           | none                            | 105                             | 104                     |

## Microbiological analysis of chickens' droppings at the end of experimen

#### Agrarian Bulletin of the Urals No. 02 (231), 2023

Thus, coefficients of calcium and phosphorus digestibility in chickens of the first experimental group were higher by 4.1 and 1.8 %, and in the second experimental group by 9.3 and 5.8% higher than in the control group. Fiber was better digested in chickens of the  $2^{nd}$  experimental group by 6.3 %, whereas in the  $1^{st}$ experimental group – by 3.7 % compared to the control group. Thus, "Lactobifadol" and "Vetom 2" formulations improve absorption of calcium, phosphorus and fiber in broiler chickens.

Physiological condition of the poultry was monitored by hematological and biochemical analyses, because blood composition and its serum reflect biochemical processes occurring in a body.

Blood serum was collected from experimental poultryat the ages of 12 and 34 days to run biochemical analyses.

The intensity of broiler poultry rearing imprints biochemical composition of chickens' blood (Fig. 1, 2).

Biochemical analyses (Fig. 1, 2) of chickens' serum showed that comparing with the start of experiment, at the end of the experiment, triglyceride indicators in the control group decreased by 0.37 mmol/ l, whereas in the 1<sup>st</sup> experimental group by 0.52, and in the 2<sup>nd</sup> experimental group by 0.13 mmol/l and were lower than the physiological norm both at the start and at the end of the experimental period. At the same time, the amount of cholesterol was higher in all samples, both at the start and at the end of the experiment. There is an inverse correlation between the concentrations of triglycerides and cholesterol here - the lower the level of triglycerides, the higher are cholesterol concentrations. In this case, we observe a metabolic disorder based on the above-mentioned indicators. This is not resulting from intake of formulations involved in the study, because the ratio of triglycerides to cholesterol was not optimal both before and after exposure to formulations, as well as between groups.

Urea is formed during metabolism of proteins in the liver. As a final product of such metabolism, it is delivered from the liver to the blood and to the kidneys for excretion from the body. Increased concentrationsof urea in the blood serum of more than 20 mmol/l causes swelling of organs and heart muscle, because the compound has hydrophilic capabilities.

The quantity of urea in the serum of our experimental poultry decreased by 0.52 mmol/l in the control group, by 0.64 in the 1<sup>st</sup> experimental group, and by 0.73 mmol/l in the 2<sup>nd</sup> experimental group compared to the start of experiment and was within the normal range.

Creatinine levels were lower by 10  $\mu$ mol/l in the control group, by 5.33 in the 1<sup>st</sup> experimental group, and by 4.92  $\mu$ mol/l in the 2<sup>nd</sup> experimental group compared to the start of experiment and were slightly below normal.

Low levels of creatinine and triglycerides in broiler chickens may be associated with the process of establishing metabolic processes, high growth rate, muscle gain and increased energy demand to maintain these processes.

#### **Discussion and Conclusion**

Many publications dedicated to poultry's gastrointestinal diseases list intensive poultry rearing technologies as a factor limiting the contact of birds with the environment and available sources of normal microflora. This has a negative impact on microecology of gastrointestinal tract, changing the evolutionarily formed structure of intestinal microbiotope.

The data of S. A. Glaskovich, P. P. Krasochko indicate that the bacterial formulation "Bifidofloran liquid" populates poultry's gastrointestinal tract and stimulates formation of lacto- and bifidoflora in gastrointestinal tract of chickens. The use of this probiotic leads to inhibition of intestinal-paratyphoid bacteria in gastrointestinal tract of chickens. The formulation improves poultry's intestinal digestion, it normalizes liver condition



Fig. 1. Results of biochemical analysis of blood samples of broiler chickens at the start of experiment time frame

Аграрный вестник Урала № 02 (231), 2023 г.



and metabolic processes within the body, especially metabolism of protein and minerals [13].

Probiotics "Prolam", "Levisel SB Plus" and "Profort" have a positive effect on meat efficiency, slaughtering performance indicators and safety of broiler chickens [14].

The use of "Lactobifadol" probiotic increases poultry's gains of live weight, survival rates and it is economically justified. The formulationcan be recommended for use in rations when rearing broiler chickens [11].

The research of E. R. Rafikova, G. A. Nozdrin was aimed at studying the growth-stimulating effect of the "Vetom" formulation based on adaptogenic fungus Dudingtonia flagrans. A positive effect of the formulation on intensity of live weight gains in broiler chickens was recognized [18].

The "Vetom" formulation microorganisms culture successfully inhibited the growth of Enterobacter cloacae microorganisms over entire surface of a petri dish with MPA in an in vitro experiment [19].

In our studies, it was found that the use of "Vetom 2" and "Lactobifadol" bacterial formulations contributed to the increase of corresponding microorganisms in intestinal microbiosis of chickens, improved digestibility of nutrients and minerals, in particular, fiber, calcium and phosphorus.

In the microbiocenosis of broiler chickens' droppings pathogenic microflora was not observed prior to feeding probiotic preparations. Conditionally pathogenic bacteria Proteus mirabilis were present in 3 cases out of 9, Klebsiella oxytoca in 2 cases, Klebsiella pneumoniae in 1 case, Staphylococcus aureus in 7 cases out of 9. No difference was identified between the groups. Bacillis spp. were present in all samples. After feeding the formulations, no pathogenic microflora was detected in the droppings of chickens of experimental groups. Conditionally pathogenic bacteria Proteus mirabilis in the chickens' droppings of control group were present in 2 cases out of 4, in the 1<sup>st</sup> experimental group also in 2 cases, in the 2<sup>nd</sup> experimental group in 1 case out of 4. Staphylococcus aureus was not detected in droppings of control group's chickens, in the 1<sup>st</sup> experimental group was present in 2 samples from 4, in the 2<sup>nd</sup> experimental group in 3 samples. Concentrations of Bifidobacteria and Lactobacilli in droppings of chickens of the 2<sup>nd</sup> experimental group, where the poultrywere fed with "Lactobifadol" formulation, was much higher than in other groups. This is logical, because "Lactobifadol" contains live lactobacteria and bifidobacteria. Bacillis spp was present in all samples of the 1<sup>st</sup> experimental group, because "Vetom 2" contains biomass of live Bacillus bacteria. In the control group, Bacillus was present in 2 samples out of 4, in the 2<sup>nd</sup> experimental group – In 3 samples.

Thus, addiing of bacterial formulations allows to increase concentration of corresponding bacteria in intestinal microbiocenosis of broiler chickens, which has a positive effect on physiological conditionof poultry.

The coefficients of calcium and phosphorus digestibility in chickens of the first experimental group were higher by 4.1 and 1.8 %, and in the second experimental group by 9.3 and 5.8 % higher than in the control group. Fiber digestion in chickens of the 2<sup>nd</sup> experimental group was better by 6.3%, while in the 1<sup>st</sup> experimental group it was better by 3.7% compared to the controlgroup. Thus, the "Lactobifadol" and "Vetom 2" formulations improve absorption of calcium, phosphorus and fiber in broiler chickens.

Low serum creatinine and triglyceride levels in broiler chickens may be associated with metabolic processes, high rate of growth, muscle gain, and higher than normal energy consumption to support these processes.

The work of many scientists is devoted to research in the field of intestinal microbiology of animals and poultry. It was found that the adding of aerobic bacteria (Bacillus subtillis, Bacillus licheniformis) to the digestive tract of animals and poultry enhances the phagocytic activity of leukocytes in blood. Bacteria of this type induce formation of endogenous interferon [8].





## Agrarian Bulletin of the Urals No. 02 (231), 2023

The bacterial formulation "Vetom", containing Bacillus subtilis bacteria, has an antibiotic effect on bird's body whenever bacterial infections occur, as well as an immunoregulatory effect in cases of viral diseases. The bacterial strain, based on which the formulation was developed, has high efficiency and capability to produce interferon, as it carries the gene for human leukocyte interferon. This cytokine is one of the key factors in the nonspecific resistance of the body in viral diseases. Thus, further research in this area has an important significance and prospects for further development.

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#### Authors' information:

Svetlana S. Aleksandrova<sup>1</sup>, candidate of agricultural sciences, researcher, ORCID 0000-0002-6436-4124, AuthorID 759266; +7 919 947-83-94, aleksandrova977@mail.ru

Aleksey A. Bakharev<sup>2</sup>, doctor of agricultural sciences, professor of the department of production technology and processing of livestock products, ORCID 0000-0002-0604-4157, AuthorID 270467; *salers@mail.ru* Aleksandr P. Duktov<sup>3</sup>, candidate of agricultural sciences, associate professor of the department of veterinary hygiene, ecology and microbiology, ORCID 0000-0002-8910-7669; (+375) 29 747-28-00, *duktov@mail.ru* Nikolay A. Sadomov<sup>3</sup>, doctor of agricultural sciences, professor, head of department of veterinary hygiene, ecology and microbiology, ORCID 0000-0001-6528-3505; (+375) 29 398-83-60, *sadomovnikolai@mail.ru* <sup>1</sup>Research Institute of Agriculture of the Northern Trans-Urals – a branch of the Tyumen Scientific Center of the Siberian Branch of the Russian Academy of Sciences, Moskovskiy, Russia

<sup>2</sup> State Agrarian University of the Northern Trans-Urals, Tyumen, Russia

<sup>3</sup> Belarussian State Agricultural Academy, Gorki, Republic of Belarus

# Микробиоценоз кишечника и переваримость кормов у цыплят бройлеров при использовании пробиотиков

С. С. Александрова<sup>1</sup><sup>∞</sup>, А. А. Бахарев<sup>2</sup>, А. П. Дуктов<sup>3</sup>, Н. А. Садомов<sup>3</sup>

<sup>1</sup>НИИСХ Северного Зауралья – филиал ТюмНЦ Сибирского отделения Российской академии наук, Московский, Россия

<sup>2</sup>Государственный аграрный университет Северного Зауралья, Тюмень, Россия

<sup>3</sup>Белорусская государственная сельскохозяйственная академия, Горки, Республика Беларусь ⊠*E-mail: aleksandrova977@mail.ru* 

Аннотация. Целью исследований являлось изучение микробиоценоза кишечника у цыплят-бройлеров. Лабораторный опыт был проведен в НИИСХ Северного Зауралья – филиале ТюмНЦ СО РАН. Методы. Использовались цыплята кросса Кобб 500. Основной период опыта начался в возрасте птицы 14 дней. К этому времени цыплята достигли средней живой массы 365 г. Контрольная группа цыплят потребляла основной рацион, состоящий из полнорационного комбикорма и чистой воды. Цыплята 1-й опытной группы в основном периоде опыта получали пробиотическую добавку «Ветом 2» в питьевой воде по 50 мг / 1 кг массы цыплята 2-й опытной группы – «Лактобифадол» в корм по 0,2 г на 1 кг массы птицы. Результаты исследований показали, что в микробиоценозе помета цыплят-бройлеров до выпойки пробиотических препаратов патогенной микрофлоры не наблюдалось. Присутствовали условно-патогенные

## Agrarian Bulletin of the Urals No. 02 (231), 2023

бактерии Proteus mirabilis, Klebsiella oxytoca, Klebsiella pneumoniae, стафилококк золотистый. Bacillus spp. присутствовали во всех пробах. После выпойки препаратов, в помете цыплят подопытных групп патогенной микрофлоры не выявлено. Присутствовали условно-патогенные бактерии Proteus mirabilis в помете цыплят контрольной группы, 1-й опытной группы, 2-й опытной группы. Содержание бифидобактерий и лактобактерий в помете цыплят, получавших подкормку препаратом «Лактобифадол», было значительно больше, чем в остальных группах. Bacillis spp. присутствовали во всех пробах 1-й опытной группы, получавшей «Ветом 2», а также в помете цыплят остальных групп. **Научная новизна**. Лабораторный опыт на цыплятах-бройлерах по изучению микробиоценоза кишечника и влияния его на переваримость комбикормов был впервые проведен в условиях Северного Зауралья.

*Ключевые слова:* цыплята-бройлеры, Ветом, Лактобифадол, микробиоценоз кишечника, коэффициенты переваримости.

Для цитирования: Александрова С. С., Бахарев А. А., Дуктов А. П., Садомов Н. А. Микробиоценоз кишечника и переваримость кормов у цыплят бройлеров при использовании пробиотиков // Аграрный вестник Урала. 2023. № 02 (231). С. 53–61. DOI: 10.32417/1997-4868-2023-231-02-53-61.

Дата поступления статьи: 10.10.2022, дата рецензирования: 03.11.2022, дата принятия: 18.11.2022.