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POULTRY DIETS WITHOUT ANTOBIOTICS. I. INTESTINAL MICROBIOTA AND PERFORMANCE OF BROILER (*Gallus gallus* L.) BREEDERS FED DIETS WITH ENTEROSORBENT POSSESSING PHYTOBIOTIC AND PROBIOTIC EFFECTS

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Abstract

Recent trend of rejection of the in-feed antibiotics in animal and poultry production launched the search for reliable alternative growth stimulators, primarily probiotics, or phytobiotics (most commonly essential oils) rendering antimicrobial and antioxidant properties to improve the digestibility of dietary nutrients, suppress the growth of pathogens, etc. Another important problem is the contamination of feeds with mycotoxins which can negatively impact the productive performance in poultry. The growth efficiency and composition of intestinal microbiota were studied in growing broiler (*Gallus gallus* L.) breeders of preparental lines B5 and B9 (selected at the Center for Genetic Selection "Smena", Moscow Province) fed vegetable diets supplemented with complex preparation Zaslon 2+ (JSC Biotrof+, Russia), containing an intestinal adsorbent, a mixture of essential oils, and a strain of *Bacillus* sp. (10^5 CFU/g). Zootechnical and physiological experiments were carried out in in 2017 (Smena Center, Zagorsk EPH, Sergiev Posad, Moscow Province). Control poultry fed the same vegetable diets with dietary antibiotics Bacitracin 30 (42 IU/mg, a dosage of 100 g/t). Test and control groups contained 50 birds each. There were no significant differences between the control and experimental treatments in live bodyweight at 21 weeks of age: 3168 g in males and 2317 g in females in B5 line (vs. 3171 and 2307 g in control), 2592 and 1930 g in B9 line (vs. 2574 and 1924 g in control), in the development of the reproductive organs (testicles in males, ovary and oviduct in females), and in the digestibility of dietary nutrients. In the duodenal microbiota from 18 to 110 bacterial phylotypes, with statistically significant differences from control for several taxonomic groups ($p < 0.05$), were found using terminal restriction fragment length polymorphism (T-RFLP) analysis. The calculated indices revealed taxonomic diversity and complexity of the intestinal bacterial communities in both control treatments (B5 and B9); in both experimental treatments more unidentified bacterial phylotypes were found in compare to the respective control treatments. An increase in the number of bacteria of the *Bacillaceae* and cellulolytic bacteria of the *Clostridiaceae* in the duodenum of the B9 line birds and an increase in the number of bacteria of the genus *Bifidobacterium* and the order *Bacteroidales*, along with a decrease in genus *Campylobacter* counts, in the duodenum of the B5 line birds occurred as a result of administration of the Zaslon 2+ preparation. Therefore, an increase in the number of bacteria in these groups and a decrease in the proportion of pathogenic microorganisms may indicate correction of dysbiotic disorders in the intestines of birds.

Keywords: preparental lines, broiler chicken, phytobiotics, intestinal adsorbent, live bodyweight, intestinal microbiota.

Providing food, in particular, high-value dietary meat is based on use of

high-productive poultry crosses. The genetic potential of broiler productivity is quite high and corresponds to average daily gain of 60-70 g, feed consumption of 1.4-1.8 kg per 1 kg of live weight, and 97-98% safety of livestock during the growing. The maximum realization of genetic potential under the production conditions depends on the norms and modes of feeding, management of broilers, as well as on the quality of the breeding bird and the initial lines [1, 2].

A macroorganism and its microflora represent a single ecological system in a dynamic equilibrium. Microorganisms are involved in metabolic processes, so the composition of the organism's microbiome is relatively constant. Meanwhile, the microbiota colonizing the digestive tract of poultry is influenced by such factors as age, feed composition, antibiotics, mycotoxins, and other substances [3, 4]. Mycotoxins are the most dangerous ones, which are secondary metabolites of mold fungi *Aspergillus* sp., *Fusarium* sp., and *Penicillium* sp. (aflatoxins, ochratoxins, zearalenone, fumonisins, and deoxynivalenol). Potential threats to poultry from mycotoxins include the high probability of contamination of grain with mycotoxins, the absence of standards for maximum permissible concentrations (MPCs) for most of them, and synergies of action [5]. To prevent mycotoxicosis in poultry, specialized feed additives are used (mycotoxin sorbents, probiotics, immunomodulators, enzymes, etc.) [6].

At present, the method of enterosorption is used to reduce the concentration of mycotoxins in animal and poultry feeds. However, the used sorbents (activated carbon, hydrated sodium-calcium aluminosilicate) have significant drawbacks. New sorbents with high and irreversible sorption capacity are sought, which lack a binding capacity with respect to essential micro- and macroelements, vitamins, and other nutrients. The modification of sorbent carriers with biologically active substances is used to correct the immune reactivity of animals, as well as to introduce probiotic properties [7, 8].

It was established that the natural resistance of birds to mycotoxins always depends on the state of the microbiota of the gastrointestinal tract. With its maximum stabilization and high resistance, the pathological effect of the toxins of the fungal microflora can be significantly decreased and practically reduced to zero. Also, the competing normal microflora produces a set of its own enzymes acting at pH characteristic of the host organism. These enzymes can biotransform groups of mycotoxins of different mycotoxin producers [9].

Because of the prohibition of antibiotics inclusion in feed in most European countries, feed additives that could replace antibiotics are studied [10]. It was proved that essential oils and probiotic strains of bacteria can be a worthy alternative to antibiotics [11, 12]. Essential oils are a variety of biologically active substances, i.e. terpenes and terpenoids, aromatic compounds, saturated and unsaturated hydrocarbons, aldehydes, organic acids and alcohols, their esters, as well as heterocyclic compounds, amines, ketones, flavones, phenols, quinones, organic sulfides, and oxides [13-15]. The potential therapeutic use of essential oils in poultry is due to their immunomodulatory properties, antimicrobial activity against pathogens, the ability to enhance the production of nutritional secretion, stimulate blood circulation, exert an antioxidant effect, and increase nutrient absorption of feed [16-18]. In this case, the microflora of the digestive tract acts as a highly sensitive indicator system that responds to occurring changes [19]. Using molecular genetic methods, up to 140 genera of bacteria are found in the intestinal microbiome of poultry, of which only 10% are identified by the 16S rRNA gene, and the rest belong to new species and even genera [20-22].

Due to the success of genetic selection, metabolic processes in modern meat poultry crosses intensifies, so that the ability of the poultry digestive system

to ensure the physiological efficiency of nutrient metabolism of diets becomes the limiting factor for the development of poultry farming. Over the past 50 years, significant progress has been made in the world in terms of the growth rate of broilers and their effective use of feed [23]. Moreover, genetic progress has led to changes in the physiology of nutrition of modern meat birds and the composition of the microflora of its gastrointestinal tract (GIT) [24]. To realize the genetic potential of poultry productivity, adequate functional support of the digestive tract is required, in particular, regulation of microflora composition.

The role of gastrointestinal microorganisms in the digestion and metabolism is of increased interest in the context of substantiating more rational and complete feeding, increasing productivity and improving the health status of poultry as a whole [25-28]. The intestinal microbiome of poultry is one of the first barriers to pathogens, coming with feed [29], and plays a huge role in the functioning of the macroorganism.

A significant part of the research on the intestinal microflora of poultry was conducted by sowing cultured strains on artificial nutrient media, which made it possible to study less than 20% of the actual number of microorganisms. Since the 1990s, the understanding of the composition of cicatricial microbiota has significantly expanded, molecular and biological methods for studying microorganisms have evolved, based on information about their genome [30-32].

In the present work, for the first time, the data are presented comparing the productivity of young females of the initial lines B9, B5 of a new domestic meat cross, as well as the effectiveness of their use of feed nutrients in connection with the composition of the intestinal microbiota when using the in-feed antibiotics and the complex preparation Zaslon 2+, consisting of sorbent, a mixture of essential oils and probiotic bacteria.

The goal of the paper was to assess the effect of the complex preparation of enterosorbent, containing bioactive substances and probiotic bacteria, on live weight, the development of reproductive organs, digestibility and the use of nutrient components of feed, as well as the composition of the microbiome in the intestines of meat chickens.

Techniques. The studies were conducted on two initial lines of meat chickens (*Gallus gallus* L.) (obtained at the Smena Center for Genetic Selection, Moscow Province) [33]. B5, the paternal line of paternal parental breed of Cornish form, is quick-growing with feed conversion, higher growth rate, and meat quality as the main selectable traits. B9 is the maternal line of maternal parental breed of Plymouth Rock form selected for egg production, hatchability, growth rate, feed conversion, and viability.

During the period of zootechnical and physiological experiments (Centre for Genetic Selection Zagorsk Experimental Breeding Economy, Sergiev Posad, Moscow Province, 2017), 1-day old to 21-weeks old birds were kept in special cages of 50 birds each in a group. The light, temperature and humidity regimes, the front of the feed and watering corresponded to the recommendations of the All-Russian Research and Technological Institute of Poultry [34].

Up to 1 week of age, the poultry of all groups consumed combined feed from vegetable components ad lib; from week 2, the daily amount of feed was limited. The control group received combined feeds of the vegetable type, balanced in all nutrients in accordance with age periods, with the addition of the in-feed antibiotics Bacitracin-30 (activity 42 IU/mg) in the amount of 100 g/t throughout the raising. The poultry of the experimental group instead of the in-feed antibiotics received 1000 g/t feed of complex enterosorbent Zaslon 2+ (Technical Specifications 9291-028-50932298-2016 of November 18, 2016, JSC Biotrof +, St. Petersburg).

The poultry of the initial lines were fed with crumbled combined feed as follows: during days 1-21 of raising the 280 kcal/100 g exchange energy, 20% crude protein, 1.0% calcium, 0.7% phosphorus, 1.15% total lysine, 0.95% available lysine, 0.45% total methionine, and 0.39% available methionine; during days 22-35 — 275 kcal/100 g, 18, 1.0, 0.7, 0.9, 0.76, 0.38, and 0.32%, respectively; during days 36-105 — 265 kcal/100 g, 14, 1.0, 0.65, 0.65, 0.58, 0.30, and 0.26%, respectively; and during days 106-147 — 270 kcal/100 g; 15, 1.5, 0.7, 0.64, 0.57, 0.30, and 0.26%.

The poultry of all groups were weighed weekly. At 4 and 7 weeks of age, balance experiments were conducted, for which 3 birds from each group with an average live weight were placed in special balance cages. The preliminary period of the experiment lasted 5 days, the test period lasted 3 days. The consumption of feed, and during the test period also the amount of poultry manure were recorded. The live weight of the bird at the beginning and the end of the experiment was recorded. Ammonia in the average sample of the manure was fixed by 0.1 N oxalic acid solution (4 ml per 100 g manure). At the end of the balance experiment, the samples were dried at 60-70 °C and stored in a container with a ground lid in a refrigerator. According to the data of daily accounting of the feed and manure weight and composition, the number of released and assimilated substances was counted.

After the birds were slaughtered at 21 weeks of age, the testicles of males, ovaries, and oviducts of females were weighed ($n = 3$ from each group). The composition of the microbiome in the intestines of these birds was also studied.

The composition of the microflora of the blind processes of the intestine was investigated by the T-RFLP method (terminal restriction fragment length polymorphism). Total DNA from the samples was isolated using the DNA Purification Kit (Fermentas, Inc., Lithuania), following the manufacturer's recommendations. PCR was performed with 63F 5′-CAGGCCTAACACATGCAAGTC-3′ eubacterial primers labeled at the 5′-terminus (D4 WellRED fluorophore, Sigma-Aldrich, Inc., USA) and 1492R 5′-TACGGHTACCTTGTTACGACTT-3′ (Verity DNA Amplifier; Life Technologies, Inc., USA). These primers allow amplifying a 63 to 1492 fragment of the 16S rRNA gene (the positions are numbered according to 16S rRNA *Escherichia coli* gene) in the mode: 3 min at 95 °C (1 cycle); 30 s at 95 °C, 40 s at 55 °C, 60 s at 72 °C (35 cycles); 5 min at 72 °C. Fluorescently labeled amplicons were purified by standard methods [35]. Concentration of purified 16S rRNA gene fragments was determined on a Qubit 2.0 fluorometer (Invitrogen, Germany). The amplicons (30-50 ng) were restricted with HaeIII, HhaI, and MspI endonucleases (Fermentas, Lithuania). The obtained fragments were analyzed (a CEQ 8000 sequencer, Beckman Coulter, USA). The phylogenetic groups of bacteria were determined using Fragment Sorter software and a database (http://www.oardc.ohiostate.edu/trflpfragsort/index.php).

The results were statistically processed in the Microsoft Excel program. The mean values (M) and standard errors of the mean (±SEM) were determined. The significance of differences was assessed by Student's *t*-test. The differences were considered statistically significant at $p < 0.05$. When estimating taxonomic diversity, Shannon, Simpson, Margalef and Berger-Parker indices were calculated.

Results. The preparation Zaslon 2+ has multifunctional properties that are caused by the adsorption characteristics of diatomite and the biologically active properties of the essential oils of thyme, lemon, garlic, and sage. In addition, its composition includes a modifier − the strain of the bacterium *Bacillus* sp. in the amount of 10^5 CFU/g.

1. Live weight (g) **of males and females, feed intake** (g · bird^{-1} · day^{-1}) in **two lines of poultry of different age in using dietary antibiotics or a complex enterosorbent** (*M*±SEM; Centre for Genetic Selection Zagorsk EPH, Sergiev Posad, Moscow Province, 2017)

Возраст, нед	Line									
	B5					B9				
	♂		♀		CF	♂		♀		CF
	C	T	C	T		C	T	C	T	
1	230±4.8	234±5.3	225±5.0	231±4.2	272	214±2.6	220±2.8	210±4.2	207±3.8	251
2	337±5.6	342±6.2	328±5.5	337±4.7	294	290±4.4	280±5.2	277±6.0	280±4.9	280
3	621±7.5	630±8.6	600±7.0	610±7.5	350	522±6.2	501±5.8	490±7.2	494±6.8	336
4	784±9.6	777±10.3	710±11.3	687±10.8	434	677±10.3	650±9.5	599±8.8	600±9.0	420
5	941±15.3	939±14.6	801±17.9	815±16.5	469	835±14.6	840±13.3	715±12.4	720±11.6	455
6	1107±18.6	1118±20.0	975±17.6	990±16.9	476	990±17.3	989±15.9	845±14.6	849±14.0	462
7	1255±21.3	1269±20.5	1015±18.3	1009±19.2	504	1225±20.4	1218±18.6	1042±17.1	1034±16.8	490
8	1447±24.8	1427±22.6	1165±20.0	1148±18.4	511	1384±22.6	1370±20.4	1130±18.6	1125±16.2	497
9	1599±22.4	1560±20.1	1233±20.2	1240±21.3	518	1445±24.5	1482±22.7	1162±20.8	1217±20.0	504
10	1784±26.6	1782±25.4	1435±23.3	1437±22.7	525	1581±25.0	1590±26.2	1290±24.1	1280±23.6	511
11	1893±28.0	1919±27.2	1544±26.6	1566±25.1	546	1610±25.3	1609±24.4	1381±25.5	1374±22.4	532
12	2011±30.4	2010±28.2	1710±25.4	1710±27.0	553	1803±28.1	1810±26.6	1464±26.8	1459±25.5	539
13	2107±32.2	2118±30.8	1735±28.7	1736±29.0	560	1881±30.4	1884±28.5	1527±28.1	1510±26.8	546
14	2270±34.3	2269±33.7	1864±32.4	1868±31.6	574	1935±32.6	1937±33.3	1590±30.4	1613±28.3	560
15	2490±36.6	2490±36.0	1907±34.5	1910±33.3	602	1981±34.8	1980±35.5	1680±33.6	1671±33.2	588
16	2525±34.2	2537±32.6	1936±28.6	1949±28.0	623	2133±35.3	2141±34.9	1708±30.7	1715±32.2	609
17	2647±36.4	2650±35.8	2001±34.3	2014±35.2	630	2270±36.8	2271±35.6	1761±32.2	1772±31.8	616
18	2688±35.0	2778±34.3	2187±36.8	2190±34.6	658	2361±37.7	2361±37.1	1802±34.6	1804±35.5	644
19	2933±34.3	2945±35.7	2209±38.4	2217±35.8	714	2484±38.5	2482±38.8	1840±35.2	1850±34.8	672
20	3066±36.5	3082±37.8	2230±36.5	2234±36.8	728	2510±40.2	2509±40.0	1890±37.6	1887±38.2	682
21	3171±38.8	3168±40.1	2307±40.6	2317±38.4	770	2574±44.6	2592±42.8	1924±38.6	1930±39.8	730

N o t e. C and T are, control and test, respectively, CF means consumption of feed per week. For the description of the groups, see the Techniques section.

The conducted experiments showed that the live weight of males and females of B5 and B9 lines from the control and experimental groups practically did not differ (Table 1). At 21 weeks of age, the males and females of the B5 line of the experimental group had a live weight of 3172 and 2318 g, the control group − 3169 and 2316 g; for the B9 line, these figures were 2590 and 1917, respectively (experimental), 2589 and 1920 g (control). The preservation of the birds in all groups was high and amounted to 100%. Over the entire period of growth, feed consumption per bird for the B5 line was 11.311 kg, for the B9 line 10.924 kg. The feed conversion in the experimental groups when using the preparation Zaslon 2+ was not significantly different from the control.

The results of the physiological (balance) experiment, conducted at 4 and 7 weeks of age, are consistent with the data on live weight of the young chickens. Significant differences in digestibility of dry matter of feed, fat, and the use of nitrogen between the birds of the control and experimental groups were not found. However, the males and females of the B5 line digested better by 4.14% the dry matter of the feed with a greater (by 3.04%) use of nitrogen. At the same time, fat digestibility was also higher (by 3.12%).

The weight of testicles in males at 21 weeks of age in the control and experimental group had no significant differences and was in the range of 8.3-9.4 g (B5 line) and 7.5-8.8 g (B9 line) in the control group, 8.1-9.3 g (B5 line) and 7.6-9.4 g (B9 line) in the test group. There are no significant differences in the mass of ovaries and ovaries with oviducts as well. The weight of the ovaries was 1.82-1.89 g (B5 line) and 1.71-1.88 g (B9 line) in the control, and 1.82-1.99 g (B5 line) and 1.788- 1.97 g (B9 line) in the test group; the weight of the ovaries with oviduct was 5.44-5.72 g (B5 line) and 5.68-5.67 g (B9 line) in the control, and 5.67-5.80 g (B5 line) and 5.63-5.77 g (B9 line) in the test group.

2. Microbial community in the duodenum of meat chickens of two lines under the use of dietary antibiotics or a complex enterosorbent (*M*±SEM; Centre for Genetic Selection Zagorsk EPH, Sergiev Posad, Moscow Province, 2017)

Микроорганизм, индекс доминирования	Line B5		Line B9	
	control	test	conttrol	test
Number of phylotypes	110.0±5.4	49.0±2.5*	57.0±2.5	18.0±0.8*
Simpson index	0.91±0.36	0.87±0.03	0.82±0.03	0.72±0.03
Shannon index	3.39±0.23	2.62±0.12***	2.53±0.13	1.70±0.07***
Margalef index	22.55±1.10	9.76±0.47*	11.19±0.51	3.33±0.15*
Berger-Parker index	0.21±0.01	0.27±0.01	0.34±0.02	0.39±0.01
Phylum *Firmicutes*				
Family *Lactobacillaceae*, %	89.05±5.30	39.19±2.30*	74.26±3.60	64.53±3.36**
Family. *Veillonellaceae*, %	B.l.r.d.	3.82±0.23*	B.l.r.d.	B.l.r.d.
Family. *Bacillaceae*, %	B.l.r.d.	12.50±0.58*	3.59±0.15	4.32±0.23***
Staphylococcus sp., %	B.l.r.d.	0.83±0.03	0	B.l.r.d.
Family. *Clostridiaceae*, %	0.31±0.02	0.38±0.02	B.l.r.d.	0.97±0.04
Clostridium novyi and/or *Cl. perfringens*, %	0.48±0.04	B.l.r.d.	B.l.r.d.	B.l.r.d.
Phylum *Bacteroidetes*				
Order *Bacteroidales*, %	B.l.r.d.	4.02±0.25	0.48±0.03	1.43±0.07**
Phylum *Actinobacteria*				
Order *Actinomycetales*, %	B.l.r.d.	0.82±0.03	0.69±0.02	B.l.r.d.
Bifidobacterium sp., %	B.l.r.d.	0.56±0.03*	B.l.r.d.	B.l.r.d.
Phylum *Proteobacteria*				
Campylobacter sp., %	1.04±0.06	0.26±0.01*	0.51±0.01	B.l.r.d.
Family *Pseudomonadaceae*, %	B.l.r.d.	1.66±0.07	B.l.r.d.	B.l.r.d.
Phylum *Fusobacteria*				
Fusobacterium sp. , %	0.40±0.02	1.01±0.04*	B.l.r.d.	B.l.r.d.
Phylum *Tenetricutes*				
Mycoplasma sp., %	B.l.r.d.	B.l.r.d.	0.53±0.01	B.l.r.d..
Uncultivated bacteria				
	3.98±0.28	27.94±1.50*	4.06±0.26	9.22±0.36**

Note. For the description of the groups, see the "Techniques" section.
*, ** and *** Differences from control are statistically significant at $p \leq 0.001$, $p \leq 0.01$ and $p \leq 0.05$, respectively; B.l.r.d. means below the limit of reliable T-RFLP determination.

T-RFLP method revealed from 18.0±0.8 to 110.0±5.4 bacterial phylotypes in the duodenum of B5 and B9 poultry (Table 2). Shannon, Simpson and Margalef indices showed a more pronounced taxonomic diversity and complexity of the structure of the microbial communities of the duodenum in the control variants of both lines. This indicates the uncertainty and heterogeneity of the compositions of the microbiocenosis, the accumulation of entropy and some disorganization compared to the test groups.

A significant proportion of microorganisms detected in the blind processes of the intestine of chickens in all the studied groups could not be attributed to any existing taxon. In chickens of both lines, the number of unidentified bacteria in the experimental groups was significantly higher ($p \leq 0.01$) than in the control.

The composition of the intestine microorganisms of meat chickens in both lines was similar. In the majority of the studied samples, the bacteria in the structure of the intestinal microbiota were assigned to 5 phyla: *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, *Proteobacteria*, and *Fusobacteria*. In the control variant, the representatives of the phylum *Tenericutes* (genus *Mycoplasma*) were found in chickens of the B9 line. Bacteria belonging to the *Firmicutes* phylum were dominant in all the examined variants. These results are partially consistent with the data previously obtained by other authors [30, 31, 36]. Thus, of the 13 bacterial phyla, identified in the intestines of chickens and turkeys, *Firmicutes*, *Bacteroidetes* and *Proteobacteria* dominated, with more than 90% of all analyzed sequences [37].

Despite the general similarity in the composition of the intestinal microbiocenosis of meat chickens of two lines, some differences were observed in the structure of the microflora. For example, in the intestine of chickens in the control group of the B5 line, there were bacteria of the family *Clostridiaceae*, genera

Fusobacterium, *Clostridium novyi/Cl. perfringens*, whereas in the B9 line these microorganisms were not identified. It is known that representatives of the *Clostridiaceae* family can play a positive role in the digestion of birds, since they are able to form a number of digestive enzymes, including cellulases, which allow the macroorganism to effectively use the energy of feeds, rich in fiber. In contrast, species of *Clostridium novyi* and *Clostridium perfringens* are often associated with gastroenteritis and claudication in chickens [38, 39], and pathogenic forms are also found among bacteria of the genus *Fusobacterium*.

The administration of complex enterosorbent with phytobiotic properties in the diet of the poultry of both lines contributed to the change in the qualitative composition of the duodenal microbiome. As a result of the use of the preparation Zaslon 2+ in the duodenum of the poultry of the B5 line, there was an increase in the number of bacteria of the genus *Bifidobacterium* and an order *Bacteroidales*, as well as a decrease in the content of bacteria of the genus *Campylobacter*. This indicates a normalization of the microflora balance in the gastrointestinal tract, since bacteria of the genus *Bifidobacterium*, living in the intestinal lumen, have antimicrobial and immunomodulatory activity, synthesize vitamins and some essential amino acids, and microorganisms of the order *Bacteroidales* are capable of synthesizing enzymes that break down complex polysaccharides and starch of feed. Among the representatives of the genus *Campylobacter*, pathogenic forms are often found, such as *Campylobacter jejuni* [40]. Probably, positive changes in the composition of the microflora under the influence of Zaslon 2+ were associated with the antimicrobial activity of essential oils and the probiotic bacterial strain in the composition of the preparation. On the one hand, the mechanism of action of probiotic bacterial strains was to synthesize biologically active substances with antimicrobial activity, on the other, bacterial strains could colonize the gastrointestinal tract through adhesion, forming a protective layer, covering the surface of the mucous epithelium, which simultaneously mechanically and functionally blocked colonization of the intestine by pathogenic microorganisms [41].

The data of the studies are consistent with the results obtained earlier. So, Wang et al. [42] showed that in the microbiome of the blind processes of the intestine of chickens, in the diet of which probiotic strains of bacteria were introduced, bacteria of the genus *Bacteroides,* belonging to the order *Bacteroidales*, dominated. Despite the positive changes in the composition of the microflora under the influence of the preparation in the experimental group of the authors' study, in the duodenum of the poultry of the B5 line, in the diet of which Zaslon 2+ was included, the number of bacteria of the *Lactobacillaceae* family decreased. Lactic acid bacteria of the *Lactobacillaceae* family play an important role in the intestines of birds, because they synthesize lactate as the main product of metabolism, which reduces the pH of chyme, leading to the suppression of pathogenic forms.

With the administration of the preparation Zaslon 2+ of the B9 line, the number of bacteria of the *Bacillaceae* family and cellulolytic bacteria of the *Clostridiaceae* family increased in the duodenum of poultry. The representatives of the *Bacillaceae* family are able to synthesize a wide range of bacteriocins [43] that effectively inhibit the development of pathogenic bacteria [44]. Therefore, an increase in the number of bacteria in these groups may indicate the correction of dysbiotic disorders in the intestine.

Thus, the use of the feed additive Zaslon 2+ in combined feeds for young chickens of the domestic initial lines B5 (Cornish breed) and B9 (Plymouth Rock breed) instead of dietary antibiotics does not significantly change the live weight of poultry. The development of the reproductive organs of males (tes-

ticles) and females (ovaries and oviducts) in both groups remaines within the normal range without statistically significant differences. The digestibility and the use of nutrient substances of feed do not differ significantly between groups of young chickens. The T-RFLP method reveales some differences in the composition of the microbiocenosis of the duodenum in the test groups. The number of unidentified bacteria in the test groups exceeded that in the control. Zaslon 2+ administering results in an increase in the number of *Bacillaceae* family and cellulolytic *Clostridiaceae* bacteria in the duodenum of the B9 birds, and in the B5 line the number of bacteria of the genus *Bifidobacterium* and the order *Bacteroidales* increase, and the counts of the genus *Campylobacter* bacteria decrease. An increase in the bacteria in these groups and a decrease in the portion of pathogenic microorganisms may indicate a correction of dysbiotic disorders in the intestines of poultry.

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