AGRICULTURAL BIOLOGY,
2019, V. 54, № 4, pp. 798-809
(SEL'SKOKHOZYAISTVENNAYA BIOLOGIYA)

ISSN 2412-0324 (English ed. Online)
ISSN 0131-6397 (Russian ed. Print)
ISSN 2313-4836 (Russian ed. Online)

UDC 636.52/.58:636.084.416:579.6

doi: 10.15389/agrobiology.2019.4.798eng
doi: 10.15389/agrobiology.2019.4.798rus

POULTRY DIETS WITHOUT ANTIBIOTICS. II. INTESTINAL MICROBIOTA AND PERFORMANCE OF BROILER (*Gallus gallus* L.) BREEDERS FED DIETS WITH A PHYTOBIOTIC

I.A. EGOROV[1], T.A. EGOROVA[1], T.N. LENKOVA[1], V.G. VERTIPRAKHOV[1], V.A. MANUKYAN[1], I.N. NIKONOV[1], A.A. GROZINA[1], V.A. FILIPPOVA[2], E.A. YILDIRIM[2], L.A. ILYINA[2], A.V. DUBROVIN[2], G.Yu. LAPTEV[2]

[1]*Federal Scientific Center All-Russian Research and Technological Poultry Institute RAS,* 10, ul. Ptitsegradskaya, Sergiev Posad, Moscow Province, 141311 Russia, e-mail Olga@vnitip.ru, eta164@mail.ru, dissovet@vnitip.ru, Vertiprakhov63@mail.ru, manukyan@vnitip.ru, ilnikonov@yandex.ru, alena_fisinina@mail.ru;
[2]*JSC «Biotrof+»,* 19 korp. 1, Zagrebskii bulv., St. Petersburg, 192284 Russia, e-mail filippova@biotrof.ru, deniz@biotrof.ru, ilina@biotrof.ru (✉ corresponding author), dubrowin.a.v@yandex.ru, georg-laptev@rambler.ru

ORCID:
Egorov I.A. orcid.org/0000-0001-9122-9553
Egorova T.A. orcid.org/0000-0002-5102-2248
Lenkova T.N. orcid.org/0000-0001-8026-3983
Vertiprakhov V.G. orcid.org/0000-0002-3240-7636
Manukyan V.A. orcid.org/0000-0003-4564-4427
Nikonov I.N. orcid.org/0000-0001-9495-0178
Grozina A.A. orcid.org/0000-0002-3088-0454
Filippova V.A. orcid.org/0000-0001-8789-9837
Yildirim E.A. orcid.org/0000-0002-5846-4844
Ilina L.A. orcid.org/0000-0003-2490-6942
Dubrovin A.V. orcid.org/0000-0001-8424-4114
Laptev G.Yu. orcid.org/0000-0002-8795-6659

The authors declare no conflict of interests

Acknowledgements:

Supported financially by Russian Science Foundation, grant No. 16-16-04089-P, for the study of the physiological and microbiological characteristics of the embryonic and postembryonic digestion in meat chicken to develop feeding programs ensuring complete use of genotype potential"

Received April 30, 2019

Abstract

The worldwide experience is explicitly evidencing that genetically conditioned productivity potential in poultry can be realized only in healthy birds. Since the implementation of the antibiotic bans in EC countries a constant search for the effective alternatives to in-feed antibiotic growth promoters (AGP) is still in progress. The additives of different types (probiotics, prebiotics, synbiotics, symbiotics, acidifiers, phytobiotics) with growth-stimulating efficiency close to that in antibiotics and inducing no harmful effects become increasingly popular in practical poultry nutrition. The efficiency of phytobiotic Intebio based on the essential oils in diets for growing chicken of preparental lines B5 and B9 (selected by Smena Center for Genetic Selection) was studied. The parameters of growth efficiency, duodenal and circulatory activity of the digestive enzymes in fistulated birds, the results of molecular genetic analysis of the composition of duodenal and cecal microbiota are presented. It was found that live bodyweight in males and females in both lines at 21 weeks of age was similar in control treatments fed diets supplemented with AGP and experimental treatments fed diets supplemented with Intebio (3172 g in males and 2318 in females vs. 3169 and 2316 g, respectively, in control in B5 line; 2590 and 1917 g vs. 2589 and 1920 g in males and females, respectively, in B9 line). Reproductive organs (testicles in males and ovaries and oviducts in females) were normally developed in all lines and treatments. Supplementation of diets with the phytobiotic significantly increased lipase activity in the duodenal digesta in B5 line (by 30.9 %, $p \le 0.05$) and B9 line (by 98.3 %, $p \le 0.01$), and protease activity in B5 line (by 36.4 %, $p \le 0.05$). The activity of lipase in B9 line was significantly ($p \le 0.001$) lower in compare to B5 line in the duodenal digesta (by 59.9 %) and in blood serum (by 48.3 %). Digestibility of dietary dry matter in males and females of B5 line was higher by 3.11 % in compare to B9 line, digestibility of fat higher by 2.95 %, nitrogen retention higher by 2.12 %. The taxonomic composition of duodenal microbiota in both lines is found to be affected by the dietary phytobiotic. In phytobiotic-treated birds the significant increases were found in the duodenal populations of certain polysaccharide-fermenting species (phylum *Bacteroides*, class *Clostridiales*) and species with high antagonistic activity against avian pathogens (*Bifidobacterium spp., Bacillus* spp.).

Keywords: growing chicken, preparental lines, live bodyweight, digestive enzymes, phytobiotic, intestinal microbiota

Meat poultry may achieve high productive and reproductive qualities on-

ly under full valuable feeding which largely influences the effectiveness of geneticists and breeders work. The nutritional value of feeds, their quantity and quality must provide the planned selected indicators according to weeks of life since the 7-day age of poultry. It is necessary to feed compound feeds balanced in terms of available energy, nutritious, mineral, and biologically active substances taking into account their availability at all stages of breeding young stock of the original lines. They must comply with existing veterinary, sanitary, and hygienic requirements and be non-toxic [1].

Currently, most European countries have implemented a ban on the inclusion of feed antibiotics in poultry feeds. The focus is on feed additives that might replace feed antibiotics without significant changes in feed recipes [2-4].

The widespread use of antibiotics and chemical antibacterial agents often leads to the deterioration of poultry health associated with the development of uncontrolled secondary infections: salmonelloses, campylobacterioses, staphylococcoses, clostridioses, as well as polymicrobial diseases [5, 6]. Pathogenic microorganisms cause a violation of the intestinal microbiota composition, lead to changes in the thickness, appearance, muscle tone, strength, and increased paracellular permeability of the intestinal walls for toxic metabolites, which negatively affects the health and productivity of poultry ultimately. Contamination of poultry products by various causative agents of human infectious diseases also remains relevant [7, 8].

The study of the properties of plant extracts and essential oils is considered one of the most promising approaches to the creation of preparations for preventing diseases and increasing poultry productivity. Unlike antibiotics and drugs obtained through chemical synthesis, substances extracted from plants are less toxic, do not accumulate in the body and may become an ideal raw material for the creation of drugs [9]. In addition, plants are an unlimited renewable source of biologically active substances, including up to 12 thousand compounds, among which about 2 thousand are described [10]. Interest in them is due to their antibacterial effect and the possibility of using instead of therapeutic and feed antibiotics, as well as other properties that affect the metabolism and productivity of animals [11-15]. However, data on many aspects of the effect of essential oils are still contradictory, which may be explained by the difference in the nutritional diet, maintenance conditions, and poultry breed characteristics.

The need for breeding stock for broiler farming in the country is mainly met by foreign poultry crosses. However, in recent years, the Smena Center for Genetic Selection in collaboration with scientists of All-Russian Research and Technological Poultry Institute RAS has been working to create a new Russian cross of meat chickens [16, 17]. Its main advantages over foreign analogs include high viability and genetic potential of productivity, as well as adaptation to the local conditions of feeding and maintenance.

The study of the influence of phytobiotics on the microbiome of poultry bowels of different genetic lines is of substantial interest. A decrease in the risk of infectious pathologies is associated with the formation of healthy microbiota of the digestive tract, which is able to provide high resistance to colonization of the bowel by pathogens [18, 19] due to the synthesis of volatile fatty acids (VFAs), bacteriocins and other compounds that inhibit the growth and development of pathogenic species [20, 21]. It is known that microorganisms, interacting with each other, as well as with the host organism, are able to have a profound impact on immunity, nonspecific resistance to infections, and general processes of poultry life [22]. In addition, the active participation of the microbial community of poultry bowels in digestive processes, in particular, in the cleavage of complex polysaccharides and proteins [23, 24], in the use and for-

mation of nutrients, the synthesis of vitamins [25], the development of intestinal villi, increasing the absorbable surface [26, 27], was shown.

The most promising modern approaches in microbiology avoiding microorganisms' culture are based on molecular genetic methods, the NGS-sequencing (next-generation sequencing) and T-RFLP-analysis (terminal restriction fragment length polymorphism) [27-30].

The data on the activity of digestive enzymes in fistulated birds that received the phytobiotic Intebio (developed and produced by OOO BIOTROF, St. Petersburg) which confirm that this preparation may serve as a replacement to fodder antibiotics are given in the presented paper for the first time.

The work objective was to study the effect of phytobiotics based on essential oils on the growth and sexual development of young meat chickens (*Gallus gallus domesticus*), the activity of digestive enzymes and the state of intestinal microbiocenosis as compared to feed antibiotic.

Techniques. Zootechnical and physiological experiments were carried out on the original lines of poultry B5 (paternal line of the paternal parental breed of the Cornish form) and B9 (maternal line of the maternal parental breed of the Plymouth Rock form) in the genetic and selection center Zagorskoe Experimental Breeding Farm (EBF) (Sergiev Posad, Moscow Province) in 2017. From 1-day to 21-week age, birds were kept in cages (50 birds in a group). Humidity, temperature, and light regimes, feeding and watering were consistent with the recommendations of ARRTPI [31]. The viability and live weight of poultry, as well as the weight of reproductive organs (testicles and ovaries with oviducts), were estimated.

In the 1st week, the young stock received feed free, without limitation in the quantity. Then the quantity of feed was fixed weekly, thus normalizing the feeding. The control group received mashes of plant type, balanced in all nutrients according to age periods, with the addition of the Bacitracin-30 feed antibiotic (42 U/mg) in an amount of 100 g/t during the entire experimental period. Poultry of the experimental group received feed additive Intebio (OOO BIOTROF, St. Petersburg) at 1000 g/t feed. Intebio is a phytobiotic (TU 9362-011-50932298-2011) consisting of a carrier (wheat bran, GOST 7169-66) and a mixture of essential oils (garlic, lemon, thyme, and eucalyptus). The poultry of the original lines was fed with crumbled mashes of the following nutritional value: 1st-21st day — 280 kcal/100 g of metabolic energy, 20% of crude protein, 1.0% calcium, 0.7% of phosphorus, 1.15% of total lysine, 0.95% of available lysine, 0.45% of total methionine, 0.39% of available methionine; 22nd-35th day — 275 kcal/100 g and 18%; 1.0%; 0.7%; 0.9%; 0.76%; 0.38%; 0.32%, respectively; 36th-105th day — 265 kcal/100 g and 14%; 1.0%; 0.65%; 0.65%; 0.58%; 0.30%; 0.26%, respectively; 106th-147th day — 270 kcal/100 g and 15%; 1.5%; 0.7%; 0.64%; 0.57%; 0.30%; 0.26%, respectively.

To obtain the duodenum digesta, the young stock was operated at the age of 6 weeks to implant a T-shaped cannula of 1 cm from the confluence of three pancreatic and two bile ducts into the bowel. In 5 days after surgery, when the bird recovered, it was used in experiments. Five birds were selected for experiments from the control and experimental groups; the test period lasted 10 days. In the morning after 14-hour starvation, the birds received 30 g of feed, the duodenal chyme (5.0 ml) was sampled in 1 h after feeding, centrifuged at 5,000 rpm for 3 minutes (DM0412, Dragonlab, PRC), the supernatant was diluted with Ringer's solution 10 times and the activity of digestive enzymes was determined. Amylase activity was evaluated by Smith-Roy-Ugolev [32], with colorimetry (a KFK-3, OAO Zagorsk Optical and Mechanical Plant, Russia) at $\lambda = 670$ nm and expressed as the amount of disorganized starch (mg) per 1 ml

of chyme for 1 min ($mg \cdot ml^{-1} \cdot min^{-1}$). The activity of proteolytic enzymes was determined photometrically by the amount of cleaved casein ($mg \cdot ml^{-1} \cdot min^{-1}$) (KFK-3, ZOMP, $\lambda = 450$ nm) [33], lipase activity was evaluated on a semi-automatic biochemical analyzer BS3000P (SINNOWA Medical Science & Technology Co., Ltd., China) with a kit for lipase determination (DIACON-VET, Russia). Biochemical analysis of blood collected on an empty stomach from the axillary vein were performed on an automatic biochemical analyzer Chem Well 2900 (T) (Awareness Technology, USA) with the appropriate reagent kits (Human GmbH, Germany), and on a semi-automatic biochemical analyzer BS3000P (SINNOWA Medical Science & Technology Co., Ltd., China).

Samples of the duodenum digesta for the analysis of microflora in females of both lines of the experimental and control groups ($n = 3$) were collected at the end of the experiment (three repetitions from each group) with strict observance of sterility [33] and frozen immediately.

The composition of microflora was investigated by the T-RFLP method. Total DNA from the samples was isolated using the DNA Purification Kit (Fermentas, Inc., Lithuania), following the manufacturer's recommendations. PCR was performed with a Verity DNA amplifier (Life Technologies, Inc., USA) using eubacterial primers 63F (5′-CAGGCCTAACACATGCAAGTC-3′) labeled at the 5′-end (fluorophore D4 WellRed) and 1492R (5′-TACGGHTACCTTG-TTACGACTT-3′), which allow amplification of the 16S rRNA gene fragment from the positions 63 to 1492 (enumeration is specified for the 16S rRNA gene of *Escherichia coli*), in the following 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 16S rRNA gene amplicons were purified with standard methods [35]. The concentration of purified DNA fragments of the 16S rRNA gene was determined (a Qubit 2.0 fluorimeter, Invitrogen, Germany). 30-50 ng amplicons of 16S rRNA were treated with HaeIII, HhaI and MspI restrictases (Fermentas, Lithuania). Restriction products were analyzed (a CEQ 8000 sequencer, Beckman Coulter, USA) according to the manufacturer's recommendations.

Bacteria were attributed to a certain taxon with Fragment Sorter software and the database (http://www.oardc.ohiostate.edu/trflpfragsort/index.php).

Statistical processing of the results was performed with Microsoft Excel, by determining the mean values (M) and standard errors of the means (±SEM). The significance of differences was assessed according to Student's t-test. The differences were statistically significant at $p < 0.05$. The Past program calculated the Shannon (H) and Simpson (D) biodiversity indices (http://folk.uio.no/ohammer/past/).

Results. Our findings have shown that both test and control groups had 100% viability. The live weight of the young stock of the lines in the control and test groups was almost identical, which indicates a positive effect of the phytobiotic on the growth of poultry when compared to a feed antibiotic (Table 1). For example, at the end of growing, males of the B5 line in the control group weighted 3,169 g, in the experimental group 3,172 g. Females at the age of 21 weeks showed the same trend. The males' bodyweight in both groups of the B9 line also did not differ, although in absolute values it was lower than in the B5 line, which is due to the breeding direction. Similar results were obtained for females. The feed consumption per 1 bird for the entire period was 11,305 kg in the B5 line and 10,934 kg in the B9 line. Feed conversion in the experimental groups had no significant differences with the control of both studied lines.

The dietary Intebio contributed to an increase in lipase activity compared to the control (by 30.9% at $p \leq 0.05$ in B5 line chickens, by 98.3% at $p \leq 0.001$ in B9 line chickens) and proteases (by 36.4% at $p \leq 0.05$ in B5 line chickens) in the duodenal digesta (Table 2). It can be assumed that essential oils have a stimulating

effect on the production of gastric juice of poultry, which increases the activity of pancreatic enzymes. Lipolytic activity in the intestinal digesta and blood in B9 line was lower than in B5 line by 59.9% and 48.3%, respectively.

1. Age-depended live weight (g) **of young meat chickens (*Gallus gallus domesticus*) of two lines and feed consumption** (g/bird per day) **under the use of dietary antibiotic Bacitracin-30 or essential oils-based phytobiotic Intebio** (*M*±SEM, GSC Zagorskoe EBF, Sergiev Posad, Moscow Region, 2017)

Age, weeks	Line									
	B5					B9				
	♂		♀		FC	♂		♀		FC
	C	T	C	T		C	T	C	T	
1	229±5.4	219±6.2	220±4.8	215±4.3	266	209±3.8	201±5.0	213±2.9	207±4.5	259
2	340±6.3	335±6.5	332±5.9	327±5.8	294	187±6.0	180±6.5	270±5.6	260±4.8	280
3	630±8.6	629±8.8	605±7.8	600±6.9	350	527±7.5	521±6.8	492±6.6	489±7.2	336
4	780±16.3	775±15.8	700±15.2	689±16.0	434	679±12.5	667±13.4	605±10.6	600±11.3	420
5	938±20.3	940±21.8	807±18.8	800±18.1	469	849±18.6	835±20.1	718±17.7	710±16.5	455
6	1110±21.6	1115±20.8	980±17.6	982±18.0	476	992±19.3	990±18.4	850±17.0	842±16.8	462
7	1260±22.3	1262±222.1	1005±118.4	1001±18.6	504	1222±20.3	1215±18.6	1039±16.6	1030±16.8	490
8	1450±23.6	1440±24.0	1170±20.7	1162±20.1	511	1390±21.4	1382±19.9	1127±18.8	1122±19.2	497
9	1595±25.2	1589±24.8	1245±23.3	1240±22.9	518	1450±22.6	1455±23.1	1200±20.4	1207±21.1	504
10	1790±28.3	1794±26.9	1440±27.9	1437±26.8	525	1590±27.6	1587±28.0	1295±25.2	1290±26.4	511
11	1900±30.2	1910±31.0	1550±28.3	1555±27.8	546	1605±28.8	1600±30.3	1375±25.5	1367±24.8	532
12	2020±32.3	2015±30.8	1700±26.6	1692±27.0	553	1810±25.6	1812±26.1	1460±24.4	1462±23.7	539
13	2110±30.6	2117±31.2	1740±27.6	1747±26.5	560	1890±28.0	1884±27.4	1530±22.6	1525±23.1	546
14	2275±31.2	2269±29.9	1875±28.3	1880±27.6	574	1940±25.6	1932±24.8	1600±20.7	1592±21.4	560
15	2495±28.8	2490±28.0	1910±26.4	1905±25.9	602	1995±27.7	1990±26.3	1687±24.4	1680±22.6	588
16	2530±32.4	2537±29.9	1947±27.7	1951±26.8	623	2140±28.5	2147±29.6	1710±26.6	1712±25.7	609
17	2650±31.7	2649±28.8	2005±30.3	2001±30.0	630	2267±30.1	2260±31.2	1775±28.0	1771±27.8	616
18	2795±32.5	2790±28.4	2190±29.6	2185±27.7	658	2368±32.5	2351±33.0	1804±26.5	1801±24.7	644
10	2940±30.8	2947±31.1	2210±29.6	2215±29.1	714	2478±33.4	2480±34.6	1843±30.2	1845±28.8	672
20	3075±32.6	3077±33.2	2235±28.8	2240±29.0	728	2505±36.2	2500±35.8	1885±27.7	1890±27.0	728
21	3169±35.0	3172±35.7	2316±30.9	2318±30.7	770	2589±38.8	2590±39.2	1920±29.8	1917±28.7	756

N o t e. C and T — control and test, respectively, FC — feed consumption for 1 week. For a description of the groups, see the Techniques section.

2. Enzymatic activity of duodenal chyme and activity of blood pancreatic enzymes in meat chickens (*Gallus gallus domesticus*) of two lines under the use of dietary antibiotic Bacitracin-30 or essential oils-based phytobiotic Intebio (*M*±SEM, GSC Zagorskoe EBF, Sergiev Posad, Moscow Region, 2017)

Indicator	Group		To control, %
	control (n = 5)	test (n = 5)	
	Line B5		
	Enzymatic activity of the chyme		
Amylase, mg · ml^{-1} · min^{-1}	219±21.1	231±25.5	105.5
Lipase, U/l	750±54.7	982±76.5*	130.9
Proteases, mg · ml^{-1} · min^{-1}	22±1.8	30±1.7*	136.4
	Enzymatic activity of the blood		
Amylase, mg · ml^{-1} · min^{-1}	395±43.5	322±20.5	81.5
Lipase, U/l	29±2.2	28±2.1	96.5
Trypsin, U/l	35±5.4	34±3.5	97.1
	Line B9		
	Enzymatic activity of the chyme		
Amylase, mg · ml^{-1} · min^{-1}	266±31.0	305±41.0	114.7
Lipase, U/l	301±37.5	597±50.3**	198.3
Proteases, mg · ml^{-1} · min^{-1}	36±0.8	36±1.0	100.0
	Enzymatic activity of the blood		
Amylase, mg · ml^{-1} · min^{-1}	290±25.1	263±6.5	90.7
Lipase, U/l	15±0.9	19±0.6*	126.7
Trypsin, U/l	29±0.5	30±0.9	103.4

N o t e. For a description of the groups, see the Techniques section.
* and ** Differences with control are statistically significant at $p \le 0.05$ and $p \le 0.01$, respectively.

The live weight of poultry under the use of feed antibiotic and phytobiotic indicates almost the same their effects, which was confirmed by the digestibility and use of feed nutrients. Indicators of the digestibility of dry matter, fat, and nitrogen between the test and control males and females had no significant

differences. It was noted only that the males and females of the B5 line digested the dry matter better (by 3.11%), digested fat better (by 2.95%) and used nitrogen better (by 2.12%).

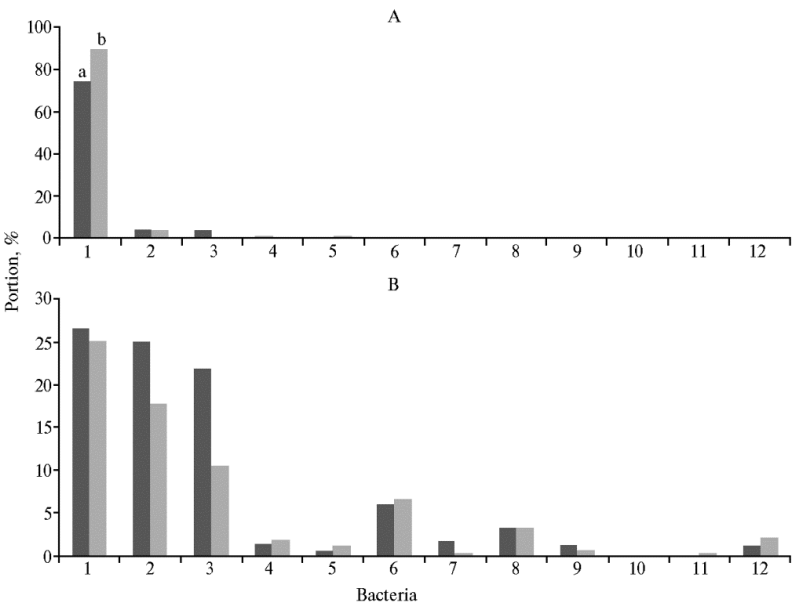
The rearing flocks should have not only the live weight corresponding to standards but also well-developed reproductive organs (testicles, ovaries, and oviducts). The weight of testicles in 21-week-old males of the B5 line in the control group was 7.5-9.1 g, in the experimental group 7.7-9.3 g, i.e., the differences were insignificant. In the B9 line in the control group, the indicators were 7.2-8.7 g, in the experimental group 7.4-9.0 g, which is almost equivalent. A similar trend occurred in reproductive organs of females. Thus, the weight of ovaries and oviducts in the control group of the B5 line was 1.75-1.86 and 5.52-5.61 g, in the test group 1.74-1.91 and 5.64-5.70 g; for the B9 line — 1.73-1.95 and 5.61-5.64 g, respectively (control group), 1.77-1.95 and 5.67-5.72 g (test group).

T-RFLP-analysis of the bacterial community of the bowel revealed a significant number of phylotypes of microorganisms, the total number of which was from 125.65±3.12 to 170.36±6.09 depending on the poultry origin and the use of phytobiotics (Table 3). Part of the phylotypes, ranging from 3.98±0.42% to 24.88±1.61%, depending on the group, could not be identified to phylum, which indicates the presence of absolutely unknown microorganisms in the poultry bowel digesta, whose nucleotide sequences have no analogs with the described taxa. Unidentified sequences have also been identified at lower taxonomic levels.

3. Biodiversity of the bacterial community in the duodenum digesta in meat chickens (*Gallus gallus domesticus*) of two lines under the use of dietary antibiotic Bacitracin-30 or essential oils-based phytobiotic Intebio (*M*±SEM, GSC Zagorskoe EBF, Sergiev Posad, Moscow Region, 2017)

Parameter	Line B5		Line B9	
	control (n = 3)	test (n = 3)	control (n = 3)	test (n = 3)
Shannon biodiversity index (H)	2.53±0.11	2.43±0.09	3.39±0.15	1.67±0.07
Simpson biodiversity index (D)	0.82±0.04	0.82±0.06	0.91±0.02	0.68±0.08
The number of phylotypes	149.05±5.23	130.82±3.68	125.65±3.12	170.36±6.09

N o t e. For a description of the groups, see the Techniques section.



Bacteria of the duodenum digesta in parent lines of broiler chickens (*Gallus gallus domesticus*) B9 (a) **and B5** (b) **in the control** (A) **and test** (B) **groups:** 1 — order *Lactobacillales*, 2 — unidentifiable bacteria, 3 — genus *Bacillus*, 4 — phylum *Actinobacteria*, 5 — femily *Campylobacteriaceae*, 6 — phylum *Bacteroidetes*, 7 — class *Clostridiales*, 8 — order *Selenomonadales*, 9 — genus *Bifidobacterum*, 10 —

phylum *Fusobacteria*, 11 — genus *Staphylococcus*, 12 — order *Pseudomonadales*. For a description of the groups, see the Techniques section.

According to the taxonomic affiliation, the majority of the identified phylotypes were attributed to three phyla, the *Firmicutes*, *Bacteroidetes*, and *Proteobacteria*, comprising in total not less than 77.37±4.29% and reaching a maximum of 95.69±6.15% (Fig.). To a lesser extent, the bacteria of the phylum *Actinobacteria* were represented; members of the phyla *Tenericutes* and *Fusobacteria* were the minority.

A significant number of opportunistic and pathogenic microorganisms were detected in the bacterial community of gut, the dominant among which were members of the family *Campylobacteriaceae*. This fact arouses interest since the presence and distribution of infectious agents in the chyme of the duodenum is not well-studied.

It should be noted that the obtained data, in general, correspond to modern ideas about poultry gut the microbiota [24, 36-39]. Thus, the representatives of 13 bacterial phyla, with more than 90% of *Firmicutes*, *Bacteroidetes*, and *Proteobacteria*, was found in the chicken and turkey gut during the taxonomic analysis of about 5,000 sequences from GenBank (https://www.ncbi.nlm.nih.gov/genbank/), Ribosomal Database Project (https://rdp.cme.msu.edu/), and Silva (https://www.arb-silva.de/) databases [24].

In general, the comparison of ecological indices between poultry revealed lower biodiversity for the Plymouth Rock breed of chickens, which indicates a lower entropy accumulation and a higher organization and uniformity of the bacterial community compared to that of the Cornish breed.

Comparative analysis of the bacterial community of the duodenum digesta allowed us to determine statistically significant differences in the composition of the microbiota associated with the use of dietary phytobiotic. Some differences in the structure of microbiocenosis of the digestive tract depending on the breed of poultry were noted. As per Shannon biodiversity and Simpson dominance indices, in Cornish birds, phytobiotic contributed to a significant ($p \leq 0.05$) decrease in the heterogeneity of the intestinal microbiota. According to the results of the taxonomic assessment, significant changes in response to phytobiotic occurred in the bacterial community of the duodenum of birds in both lines. First, the significant increase in the representation of the members of the *Bacteroidetes* phylum, as well as the *Clostridiales* class, including representatives of the families *Eubacteriaceae*, *Clostridiaceae*, and *Lachnospiraceae* was observed ($p \leq 0.05$), which indicates an increase in potential of fermentation of plant polysaccharides by the microbial community, since these microorganisms have the ability to metabolize starch, fiber, and some other carbohydrates, proteins and deaminate amino acids.

The results we obtained on the intestinal microbiota composition are quite expected and have a clear relationship with the physiological state of birds. For example, the increase in the microbiota having enzymatic activity is consistent with the above data on the increase in the activity of pancreatic enzymes in the duodenal digesta. Our data support reports that some obligate inhabitants of the birds' gut are able to directly impact on birds' productivity. For example, Torok et al. [37] in a series of experiments revealed a significant correlation between the composition of microorganisms in the caecum and the efficiency of feed digestion. The presence of a relationship for bacteria of the *Firmicutes* phylum was noted [40], including representatives of the genera *Eubacterium* (*Eubacteriaceae* family), *Roseburia* (*Lachnospiraceae* family), *Faecalibacterium* (*Ruminococcaceae* family) [41]. Metabolism of these microorganisms is associated with the synthesis of various volatile fatty acids (butyric, acetic, etc.), which are

necessary for poultry as a source of energy. Some acids (e.g., butyric acid) increase the size of the intestinal epithelium [42, 43], thus providing a barrier to toxic agents [44].

It should be noted that the increase in the counts of VFA-synthesizing microorganisms in our experiments with dietary phytobiotics had a positive impact on the representation in the intestine of *Selenomonadales* bacteria, which can transform organic acids to various useful compounds thus participating actively in metabolism.

In addition, interesting changes related to the phytobiotic were observed in respect of the obligate representatives of the poultry gut, the lactictobacteria of *Lactobacillus*, *Enterococcus*, *Bacillus* and bifidobacteria of the genus *Bifidobacterium*, which, due to the synthesis of different organic acids and bacteriocins, are capable of antagonistic displacement of intestinal pathogens such as salmonellae, protea, staphylococci, *E. coli*, pseudomonades, and streptococci [21, 41]. It was found that in response to the use of dietary phytobiotics in the of birds of both breeds, the representation of bacteria of the genera *Bacillus* and B*ifidobacterium* increased significantly ($p \leq 0.05$), along with a decrease in the number of other microorganisms having similar properties.

Among the bacteria that cause infectious diseases, we have found pathogens of campylobacteriosis (family *Campylobacteraceae* – *Arcobacter*, *Campylobacter*), pasteurellosis (family *Pasteurellaceae* – *Pasteurella, Haemophilus*), mycoplasmosis (phylum *Tenericutes* – *Mycoplasma*), necrotic enteritidis (phylum *Fusobacteria*), purulonecrotic infections (genus *Staphylococcus*), *Clostridia* (species *Clostridium novyi* and *C. perfringens*) in birds. Most of these microorganisms were minor is gut community, with the exception of *Campylobacteria*.

The maximum number of opportunistic bacteria was found in Cornish birds, which also indicates some imbalance in their gut microbial community (see Fig.). The presence of *Campylobacter*, Fusobacterium, as well as *C. perfringens* and *Clostridium novyi* species was lower in birds of the maternal line. Any regularity characterizing similar changes in the number of pathogens in the duodenal digesta of the poultry of maternal and paternal lines in response to the use of phytobiotics was not found. This fact probably is connected with the genetic differences of the bird and requires additional elucidation.

The obtained results also indicate that the Cornish birds had a tendency to decrease the number of pathogenic fusobacteria, the causative agents of necrotic enteritidis leading to lesions of internal organs and joints, in the duodenal content.

Thus, our data draw to a conclusion that replacement of a fodder antibiotic by essential oils of plant origin in mashes for young meat chickens (original lines B5 and B9) makes it possible to reach an almost identical live weight of poultry. The development of reproductive organs of males (testicles) and females (ovaries and oviducts) in both groups remained normal without significant differences. The revealed increase in lipase activity in the duodenal chyme by 30.9% in the B5 line and by 98.3% in the B9 line, as well as proteases in the B5 line by 36.4% is consistent with data on the digestibility of fat and nitrogen in these groups. The results of this investigation indicate the noticeable changes in the bacterial community of duodenal digesta in both breeds, associated primarily with an increase in the number of microorganisms with enzymatic activity towards complex polysaccharides (class *Clostridia*, phylum *Bacteroidetes*), as well as bacteria with high antagonistic properties (*Bifidobacterium*, *Bacillus*, etc.).

REFERENCES

1. Fisinin V.I., Egorov I.A., Laptev G.Yu., Lenkova T.N., Nikonov I.N., Il'ina L.A., Manuky-

an V.A., Grozina A.A., Egorova T.A., Novikova N.I., Iyldyrym E.A. *Voprosy pitaniya*, 2017, 86(6): 114-124 (doi: 10.24411/0042-8833-2017-00013) (in Russ.).
2. Konstantinov V. *Kombikorma*, 2010, 6: 115-116 (in Russ.).
3. Smith J.A. The future of poultry production in the USA without antibiotics. *Poultry International*, 2002, 41: 68-69.
4. Fisinin V.I., Okolelova T.M., Prosviryakova O.A., Andrianova E.N., Kryukov O.V., Kuzovnikova A., Narmontiene M., Bevzyuk V.N., Papazyan T.T., Tardat'yan A.G., Shchukina S.A., Kosintsev Yu.V., Timofeva E.N. *Organicheskie kisloty i podkisliteli v kombikormakh dlya ptitsy* [Organic acids and acidifiers in poultry feed]. Sergiev-Posad, 2008 (in Russ.).
5. Dzhavadov E.D., Dmitrieva M.E., Trefilov B.B, Novikova O.B., Titova T.G. *Veterinariya i kormlenie*, 2016, 2: 24-27 (in Russ.).
6. Collier C.T., Hofacre C.L., Payne A.M., Anderson D.B., Kaiser P., Mackie R.I., Gaskins H.R. Coccidia induced mucogenesis promotes the onset of necrotic enteritis by supporting *Clostridium perfringens* growth. *Veterinary Immunology and Immunopathology*, 2008, 122(1-2): 104-115 (doi: 10.1016/j.vetimm.2007.10.014).
7. Hermans D., Pasmans F., Messens W., Martel A., Van Immerseel F., Rasschaert G., Heyndrickx M., Van Deun K., Haesebrouck F. Poultry as a host for the zoonotic pathogen *Campylobacter jejuni*. *Vector-Borne and Zoonotic Disease*, 2012, 12(2): 89-98 (doi: 10.1089/vbz.2011.0676).
8. Seys S.A., Sampedro F., Hedberg C.W. Assessment of meat and poultry product recalls due to *Salmonella* contamination: product recovery and illness prevention. *Journal of Food Protection*, 2017, 80(8): 1288-1292 (doi: 10.4315/0362-028X.JFP-16-424).
9. Hashemi S.R., Zukifli I., Hair Bejo M., Farida A., Somchit M.N. Acute toxicity study and phytochemical screening of selected herbal aqueous extract in broiler chickens. *International Journal of Pharmacology*, 2008, 4(5): 352-360 (doi: 10.3923/ijp.2008.352.360).
10. Kryukov V.S., Glebova I.V. *Problemy biologii produktivnykh zhivotnykh*, 2017, 3: 5-25 (in Russ.).
11. Nazzaro F., Frantianni F., De Marino L., Coppala R., De Feo V. Effect of essential oils on pathogenic bacteria. *Pharmaceuticals*, 2013, 6(12): 1451-1474 (doi: 10.3390/ph6121451).
12. Christaki E., Bonos E., Giannenas I., Florou-Paneri P. Aromatic plants as a source of bioactive comppouds. *Agriculture*, 2012, 2(3): 228-243 (doi: 10.3390/agriculture2030228).
13. Zeng Z., Zhang S., Wang H., Piao X. Essential oil and aromatic plants as feed additives in non-ruminant nutrition: a review. *Journal of Animal Science and Biotechnology*, 2015, 6(1): Article 7 (doi: 10.1186/s40104-015-0004-5).
14. Odoemalam V.U., Etuk I.F., Ndelekwute E.K., Iwuji T.C., Ekwe Ch.C. Herbs and spices: option for sustainable animal production. *Journal of Biology, Agriculture and Healthcare*, 2013, 3(7): 116-124.
15. Costa L.B., Luciano F.B., Miyada V.S., Gois F.D. Herbal extracts and organic acids as natural feed additives in pig diets. *South African Journal of Animal Science*, 2013, 43(2): 181-193 (doi: 10.4314/sajas.v43i2.9).
16. Egorova A.V. *Ptitsevodstvo*, 2012, 12: 8-10 (in Russ.).
17. Egorova A.V., Tuchemskii L.I., Emanuilova Zh.V. *Zootekhniya*, 2015, 6: 2-4 (in Russ.).
18. Yeoman C.J., Chia N., Jeraldo P., Sipos M., Goldenfeld N.D., White B.A. The microbiome of the chicken gastrointestinal tract. *Animal Health Research Reviews*, 2012, 13(1): 89-99 (doi: 10.1017/S1466252312000138).
19. Kerr A.K., Farrar A.M., Waddell L.A., Wilkins W., Wilhelm B.J., Bucher O., Wills R.W., Bailey R.H., Varga C., McEwe S.A., Rajić A. A systematic review-meta-analysis and meta-regression on the effect of selected competitive exclusion products on *Salmonella* spp. prevalence and concentration in broiler chickens. *Preventive Veterinary Medicine*, 2013, 111(1-2): 112-125 (doi: 10.1016/j.prevetmed.2013.04.005).
20. Dobson A., Cotter P.D., Ross R.P., Hill C. Bacteriocin production: a probiotic trait? *Applied and Environmental Microbiology*, 2012, 78(1): 1-6 (doi: 10.1128/AEM.05576-11).
21. Messaoudi S., Kergourlay G., Dalgalarrondo M., Choiset Y., Ferchichi M., Prйvost H., Pilet M.F., Chobert J.M., Manai M., Dousset X. Purification and characterization of a new bacteriocin active against *Campylobacter* produced by *Lactobacillus salivarius* SMXD51. *Food Microbiology*, 2012, 32(1): 129-134 (doi: 10.1016/j.fm.2012.05.002).
22. Sekirov I., Russell S.L., Antunes L.C.M., Finlay B.B. Gut microbiota in health and disease. *Physiol. Rev.*, 2010, 90(3): 859-904 (doi: 10.1152/physrev.00045.2009).
23. Sun H., Tang J.W., Fang C.L., Yao X.H., Wu Y.F., Wang X., Feng J. Molecular analysis of intestinal bacterial microbiota of broiler chickens fed diets containing fermented cottonseed meal. *Poultry Science*, 2013, 92(2): 392-401 (doi: 10.3382/ps.2012-02533).
24. Wei S., Morrison M., Yu Z. Bacterial census of poultry intestinal microbiome. *Poultry Science*, 2013, 92(3): 671-683 (doi: 10.3382/ps.2012-02822).
25. Stanley D., Geier M.S., Chen H., Hughes R.J., Moore R.J. Comparison of fecal and cecal microbiotas reveals qualitative similarities but quantitative differences. *BMC Microbiology*, 2015, 15(1): Article 51 (doi: 10.1186/s12866-015-0388-6).
26. LeBlanc J.G., Milani C., de Giori G.S., Sesma F., van Sinderen D., Ventura M. Bacteria as vitamin suppliers to their host: a gut microbiota perspective. *Curr. Opin. Biotechnol.*, 2013,

24(2): 160-168 (doi: 10.1016/j.copbio.2012.08.005).
27. Fasina Y.O., Hoerr F.J., McKee S.R., Conner D.E. Influence *of Salmonella enterica* serovar Typhimurium infection on intestinal goblet cells and villous morphology in broiler chicks. *Avian Diseases*, 2010, 54(2): 841-847 (doi: 10.1637/9055-090809-Reg.1).
28. Chae B., Ingale S., Kim J., Kim K., Sen S., Lee S., Khong C., Kim E.K., Kwon I.K. Effect of dietary supplementation of probiotics on performance, caecal microbiology and small intestinal morphology of broiler chickens. *Animal Nutrition and Feed Technology*, 2012, 12(1): 1-12.
29. Liao N., Yin Y., Sun G., Xiang C., Liu D., Yu H.D., Wang X. Colonization and distribution of segmented filamentous bacteria (SFB) in chicken gastrointestinal tract and their relationship with host immunity. *FEMS Microbiology Ecology*, 2012, 81(2): 395-406 (doi: 10.1111/j.1574-6941.2012.01362.x).
30. Sekelja M., Rud I., Knutsen S.H., Denstadli V., Westereng B., Naes T., Rudi K. Abrupt temporal fluctuations in the chicken fecal microbiota are explained by its gastrointestinal origin. *Applied and Environmental Microbiology*, 2012, 78(8): 2941-2948 (doi: 10.1128/AEM.05391-11).
31. Merina-Gluzkina V.M. *Laboratornoe delo*, 1965, 3: 142-146 (in Russ.).
32. Batoev Ts.Zh. *Sbornik nauchnikh trudov Buryatskogo SKHI (Ulan-Ude)*, 1971, 25: 122-126 (in Russ.).
33. *Instruktsii po sanitarno-mikrobiologicheskomu kontrolyu tushek, myasa ptitsy, ptitseproduktov, yaits i yaitseproduktov na ptitsevodcheskikh i pererabatyvayushchikh predpriyatiyakh* [Instructions for sanitary and microbiological inspection of poultry carcasses, meat and products, eggs and egg products in poultry farming and during processing]. Moscow, 1990 (in Russ.).
34. Wilson K., Walker J. *Printsipy i metody biokhimii i molekulyarnoi biologii* [Principles and techniques of biochemistry and molecular biology]. Moscow, 2015 (in Russ.).
35. Mikhailova A.G., Khairullin R.F., Demidyuk I.V., Kostrov S.V., Grinberg N.V., Burova T.V., Grinberg V.Y., Rumsh L.D. Cloning, sequencing, expression, and characterization of thermostability of oligopeptidase B from *Serratia proteamaculans*, a novel psychrophilic protease. *Protein Expression and Purification*, 2014, 93: 63-76 (doi: 10.1016/j.pep.2013.10.011).
36. Egorov I.A., Manukyan V.A., Lenkova T.N., Okolelova T.M., Lukashenko V.S., Shevyako A.N., Ignatova G.V., Egorova T.V., Andrianova E.N., Rozanov B.L., Lysenko M.A., Egorova T.A., Grozina A.A., Laptev G.Yu., Nikonov I.N., Aleksandrova I.L., Il'ina L.A., Novikova N.I., Fisinin V.I. *Metodika provedeniya nauchnykh i proizvodstvennykh issledovanii po kormleniyu sel'skokhozyaistvennoi ptitsy. Molekulyarno-geneticheskie metody opredeleniya mikroflory kishechnika* [Methodology of laboratory and farm tests on poultry feeding. Molecular methods of intestinal microflora analysis]. Sergiev Posad, 2013 (in Russ.).
37. Torok V.A., Hughes R.J., Mikkelsen L.L., Perez-Maldonado R., Balding K., MacAlpine R., Percy N.J., Ophel-Keller K. Identification and characterization of potential performance-related gut microbiota in broiler chickens across various feeding trials. *Applied and Environmental Microbiology*, 2011, 77(17): 5868-5878 (doi: 10.1128/AEM.00165-11).
38. Diaz-Sanchez S., Hanning I., Pendleton S., D'Souza D. Next-generation sequencing: the future of molecular genetics in poultry production and food safety. *Poultry Science*, 2013, 92(2): 562-572 (doi: 10.3382/ps.2012-02741).
39. Choi J.H., Kim G.B., Cha C.J. Spatial heterogeneity and stability of bacterial community in the gastrointestinal tracts of broiler chickens. *Poultry Science*, 2014, 93(8): 1942-1950 (doi: 10.3382/ps.2014-03974).
40. Bjerrum L., Engberg R.M., Leser T.D., Jensen B.B., Finster K., Pedersen K. Microbial community composition of the ileum and cecum of broiler chickens as revealed by molecular and cellular-based techniques. *Poultry Science*, 2006, 85(7): 1151-1164 doi: 10.1093/ps/85.7.1151).
41. Louis P., Young P., Holtrop G., Flint H.J. Diversity of human colonic butyrate-producing bacteria revealed by analysis of the butyryl-CoA:acetate CoA-transferase gene. *Environmental Microbiology*, 2010, 12(2): 304-314 (doi: 10.1111/j.1462-2920.2009.02066.x).
42. Le Blay G., Blottiere H.M., Ferrier L., Le Foli E.C., Bonnet J.P., Galmiche C., Cherbut C. Short-chain fatty acids induce cytoskeletal and extracellular protein modifications associated with modulation of proliferation on primary culture of rat intestinal smooth muscle cells. *Dig. Dis. Sci.*, 2000, 45(8): 1623-1630 (doi: 10.1023/a:1005529414765).
43. Fukunaga T., Sasaki M., Araki Y., Okamoto T., Yasuoka T., Tsujikawa T., Fujiyama Y., Bamba T. Effects of the soluble fibre pectin on intestinal cell proliferation, fecal short chain fatty acid production and microbial population. *Digestion*, 2003, 67(1-2): 42-49 (doi: 10.1159/000069705).
44. Niba A.T., Beal J.D., Kudi A.C., Brooks P.H. Bacterial fermentation in the gastrointestinal tract of non-ruminants: influence of fermented feeds and fermentable carbohydrates. *Tropical Animal Health and Production*, 2009, 41(7): 1393-1407 (doi: 10.1007/s11250-009-9327-6).