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THE IMPACT OF VIRGINIAMICIN AND PROBIOTICS ON INTESTINAL MICROBIOME AND GROWTH PERFORMANCE TRAITS OF CHICKEN (*Gallus gallus* L.) BROILERS

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Abstract

Today, there is great interest in the development of environmentally friendly feed additives for poultry farming as a worthy alternative to antibiotics capable of positively modulating the microbiota to control pathogenic microorganisms. However, very few studies have been devoted to comparing the effects of probiotics and antibiotics on the structure of the gut microbiome in broilers. In this study, we compared the composition of the intestinal microbiota and zootechnical parameters in chickens of the Cobb 500 cross during the starter, growth and finishing periods when a probiotic (*Bacillus subtilis* in the composition of Cellobacterin®-T) or an antibiotic (Stafac® 110 based on virginiamycin) was added to the diet and showed that the *B. subtilis* strain accelerates the formation of intestinal microflora. The probiotic also reduces the number of microorganisms of the *Campylobacteriaceae* family which includes many types of gastroenteritis pathogens, and also increases the digestibility of fiber. T-RFLP analysis and qPCR method were used to assess changes in the intestinal microbiota of Cobb 500 broiler chickens fed a *Bacillus subtilis*-based dietary probiotic and virginiamycin-based dietary antibiotic Stafac® 110. On day 14, the total counts of cecal bacteria, as compared to control, were 9.1 times higher ($p \le 0.05$) in broilers fed Stafac® 110, and 54.2 times higher ($p \le 0.001$) when fed *B. subtilis* preparation. This indicates rapid microbial colonization of gastrointestinal tract of the chickens fed Stafac® 110 and *B. subtilis*. T-RFLP analysis revealed two dominant cecal phyla, *Firmicutes* and *Proteobacteria*, while phyla *Actinobacteria*, *Bacteroidetes*, and *Fusobacteria* were less abundant. The taxa are detected which ferment non-starch polysaccharides to produce short-chain fatty acids, inhibit the competing pathogens due to production of bacteriocins, and acidize the chyme as synthesize organic acids. Administration of the dietary antibiotic mostly positively influences the cecal microbiota, e.g., the cellulolytic bacteria and *Clostridia* forms involved in the synthesis of organic acids became more abundant ($p \le 0.05$). Similar beneficial effects, e.g., an increase in *Clostridia* counts ($p \le 0.05$) compared to control, occurred when the probiotic strain was administered. On day 14 of rearing, the dietary antibiotic and probiotic reduced abundance of *Campylobacteriaceae* family comprising gastroenteritis pathogens ($p \le 0.05$) when compared to control. An increase in bodyweight as compared to control (from 1845.8±20.9 to 1936.4±17.9 g, $p = 0.046$) occurred in 36-day-old chickens fed Stafac® 110 but not the probiotic strain but not the probiotic strain, despite recovery of gut microbiota in the chickens fed *B. subtilis*. A 7.1 % increase in fiber digestibility ($p = 0.0027$) occurred in broilers fed dietary probiotic and a 2.3 % increase ($p = 0.047$) in those fed the dietary antibiotic, which may be due to the action of cellulolytic microorganisms. Therefore, a dietary *B. subtilis*-based probiotic which promotes recovery of gut microbiota and increases fiber digestibility in feeds for broiler chickens can be an effective alternative to the virginiamycin-based antibiotic Stafac® 110.

Keywords: broiler chickens, Cobb 500, probiotic, *Bacillus subtilis*, Stafac® 110, T-RFLP analysis, microbiome, *Firmicutes*, *Proteobacteria*, *Clostridia*, *Campylobacteriaceae*

The widespread use of antibiotics in livestock and poultry farming leads to the emergence of pathogenic bacteria resistant to antimicrobial drugs, which seriously threatens the health of animals and humans [1, 2]. In 2016, the UN General Assembly recognized the use of antibiotics in livestock and poultry as one of the main causes of antimicrobial resistance in humans (United Nations meeting on antimicrobial resistance, 2016) [3]. In the European Union, the use of antibiotics was banned in 2006, in the United States, the Center for Veterinary Medicine, Food and Drug Administration (FDA) prepared an FDA Guidance for Industry in 2012, which recommends the use of antibiotics exclusively for therapeutic purposes for limited periods in case of outbreaks of infectious diseases [4]. In recent years, antibiotics have been widely used in the poultry industry in Russia for mass prevention of diseases and poultry growth stimulation, however, since 2020, the state has banned the use of antimicrobial drugs intended for veterinary use for non-medicinal purposes.

The microbiome of the gastrointestinal tract of poultry plays a vital role in the digestion and absorption of feed nutrients, the development of immunity, resistance to diseases, and the breakdown of toxins [5]. Disruption of the microbial community of the gastrointestinal tract can adversely affect the efficiency of feeding, productivity and health of poultry [6]. It has been proven [7, 8] that antibiotic therapy often causes a change in the structure of microbial consortia, provoking dysbacteriosis with subsequent physiological and metabolic disorders in the host's body. Disruption of the microbiota in broiler chickens is often associated with villous atrophy, decreased muscle thickness, and increased infiltration of T-lymphocytes in the intestinal mucosa [9].

In the last decade, interest in the development of environmentally friendly feed additives capable of positively modulating microbiota by controlling pathogenic microorganisms has been constantly growing [10-12]. The positive effects of probiotic strains of microorganisms and prebiotics in the prevention and treatment of gastrointestinal disorders in broiler chickens infected with *Clostridium perfringens* [13], *Campylobacter jejuni* [14], *Salmonella* sp. [15].

Antibiotic therapy and, in particular, uncontrolled intake of antibiotics negatively affect the composition of the human intestinal microbiota [15-17]. Thus, the negative effect of β-lactam therapy on the composition of the human microbiome has been proven [16]. The use of 16S rDNA and 16S rRNA sequencing showed that after 14 days of therapy, the microbial biodiversity collapsed. Similar data were obtained for some farm animals. For example, in cows that received penicillin (4.8 g per animal) and streptomycin (5.0 g per animal) for 14 days, disturbances occurred in the rumen microbiome [18]. In the rumen, after 3 days of antibiotic use the abundance of 45 high-level taxa decreased, after 14 days the abundance of 43 taxa.

For broiler chickens, similar information is limited. It was reported [19] that the abundance of *Lactobacillus* spp. in the ileum of chickens whose feed contained tylosin and a bacteriostatic was significantly lower than that of those who did not receive tylosin. Similar effects have been described in other studies [20-22]. However, very few studies have been devoted to comparing the effects of probiotics and antibiotics on the composition of the gut microbiome in broilers [23].

In the presented study, the authors showed that with the introduction of the *Bacillus subtilis* strain into the gastrointestinal tract of broiler chickens, the formation of the intestinal microflora occurred faster (starting from the 1st day of life) than when the antibiotic Stafac® 110, based on virginiamycin, was added to

the feed. The probiotic reduced the abundance of microorganisms of the *Campylobacteriaceae* family, including the *Campylobacter* genus, which includes many types of gastroenteritis causative agents, and also increased the digestibility of fiber and, therefore, can be an effective alternative to the feed antibiotic Stafac® 110.

The goal of the research was to compare the quantitative composition of the intestinal microbiota and zootechnical parameters in chickens in the starting, growth, and finishing periods when a probiotic or antibiotic was added to the diet.

Methods. Chickens of the Cobb 500 cross were randomly divided into three groups of 70 birds. The control group I received the basal diet, that is, complete mash feed, balanced according to the norms for the cross, including wheat, soybean and sunflower meal, soybean oil, fish meal and meat and bone meal, limestone, monocalcium phosphate, vitamin-mineral complex (fiber content in starter, growth and finishing periods is 4%). For poultry from group II, Stafac® 110 (Phibro Animal Health Corporation, USA) was added to the diet at a dosage of 180 g/t of feed. Stafac® 110 contains the active ingredient virginiamycin (11%) and excipients – carboxymethyl cellulose (4.4%), calcium carbonate (11%), mineral oil (0.2%), purified water-soluble granules (73.4%). In group III, the probiotic Cellobacterin®-T containing *Bacillus subtilis* (BIOTROF LLC, Russia) was added to the feed in the morning (10:00) (1 kg/t of compound feed according to the instructions for the preparation). The birds were kept in cage batteries of the R-15 type (Russia) (35 chickens per cage; the vivarium of All-Russian Research Veterinary Institute of Poultry, St. Petersburg, 2014). Chickens were provided with free access to feed and water. Technological conditions corresponded to the recommendations ("Resource-saving technology for the production of broiler meat: guidelines". Zagorsk, 1990).

The mortality of the livestock was recorded and the increase in live weight was assessed by individual weighing during the experiment (1-36 days of life). Physiological balance experiment to assess the digestibility and use of nutrients and minerals of the feed was carried out from days 28 to 36 ($n = 6$) according to the methodology of scientific and industrial research on feeding poultry of Federal State Budgetary Scientific Institution All-Russian Research and Technological Institute of Poultry (Sergiev Posad, 2013).

In each group, the contents of the ceca (5-10 g each) were taken postmortem from six chicks analogous in live weight to study the microbiota. At the age of 1 day, samples were taken 24 h after feeding, at days 7, 14, 21, and 36 samples were taken from individuals with a filled goiter. The collected samples were immediately placed in sterile centrifugal plastic tubes, frozen at –20 °C and delivered in dry ice to the molecular genetic laboratory of the research and production company BIOTROF LLC for DNA isolation.

Total DNA from the studied samples was isolated using a Genomic DNA Purification Kit (Fermentas, Inc., Lithuania) according to the attached instructions.

T-RFLP (terminal restriction fragment length polymorphism) analysis was performed according to the method developed by the authors earlier [24].

For quantitative polymerase chain reaction (qPCR, thermal cycler DT Lite-4, NPO DNA-Tekhnologiya, Russia), a set of reagents for RT-PCR in the presence of an intercalating dye EVA Green (CJSC Syntol, Russia) was used according to the attached instructions. Universal primers were used to determine the total number of bacteria, the HDA1 5′-ACTCCTACGGGAGGCAGCAG-3′ and HDA2 5′-GTATTACCGCGGCTGCTGGCA-3′ [25]; amplification protocol: 3 min at 95 °C (1 cycle); 1 min at 95 °C, 1 min at 57.6 °C, 1min at 72 °C (40 cycles); 5 min at 72 °C (1 cycle).

The diversity of the bacterial community was assessed graphically using a heat map (the "pheatmap" package Version 1.0.12 for R, https://www.rdocumentation.org/packages/pheatmap/versions/1.0.12/topics/pheat-map) [26]. Hierarchical clustering by groups was carried out using the Ward-linkage clustering method on a matrix constructed from the squared Euclidean distances between objects [27, 28].

The software packages Microsoft Office Excel 2003, R-Studio (Version 1.1.453) (https://rstudio.com), and PAST (https://www.bytesin.com/software/PAST/) were used for mathematical and statistical data processing [29, 30]. Quantitative values were compared using Student's *t*-test. Statistical analysis results were considered significant at $p \leq 0.05$. Numerical data are presented as means (*M*) and their standard errors (±SEM).

Results. In our opinion, the changes caused by the anti- and probiotic in the structure of the microbiota of the cecum contents are of the greatest interest. It is in the cecum that the main processes of fermentation and digestion of complex substrates (cellulose, starches, other polysaccharides) occur, and the retention of feed here is the longest (12-20 h) [5]. For comparison with the antibiotic Stafac® 110, we chose Cellobacterin®-T, a feed additive with probiotic properties (TU 10.91.10-014-50932298-2019, registration number PVR-2-18.11/02763). It contains wheat bran (GOST 7169-2017), on which the microorganisms *Bacillus subtilis* are applied.

The results of determining the number of bacteria in the studied samples of the broiler cecum by qPCR are shown in Figure 1. Depending on the age and the treatment, it ranged from $2.4\times10^9\pm 4.7\times10^8$ to $1.4\times10^{11}\pm7.0\times10^9$ cells/g. This coincides with the known data reporting [31] that the number of bacteria in the ceca in 1-day-old chickens ranged from 10^8 to 10^{10} cells/g, reaching values from 10^9 to 10^{11} cells/g with age.

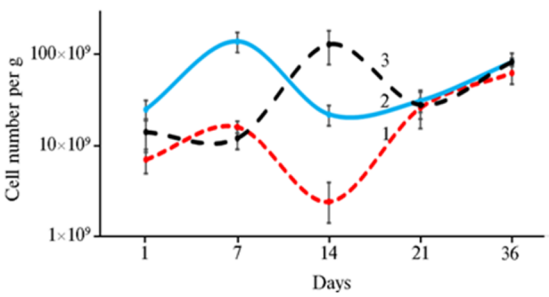

Fig. 1. Age dynamics of the total number of bacteria in the cecum of Cobb 500 cross broiler chickens fed the basal diet (BD, 1, control)**, BD supplemented with antibiotic Stafac® 110** (2) **or BD with the feed additive Cellobacterin®-T containing *Bacillus subtilis* with probiotic properties** (3) (n = 3, *M*±SEM, qPCR analysis; vivarium of the All-Russian Research Veterinary Institute of Poultry, St. Petersburg, 2014).

At the age of 14 days, the total number of bacteria in the cecal chyme of broilers fed Stafac® 110 was 9.1 times higher ($p \leq 0.05$) while in those fed the probiotic *B. subtilis* it was 54.2 times higher ($p \leq 0.001$) compared to the control (Fig. 1). However, under the influence of the antibiotic, a significant increase in the total abundance of bacteria in the blind processes compared to the control was noted already on days 1 and 7 ($p \leq 0.05$ and $p \leq 0.01$, respectively), while we did not find any differences during these periods for *B. subtilis*. The results obtained indicate rapid microbial colonization of the gastrointestinal tract of chickens from the experimental groups (especially when using an antibiotic), which is important during this period of life. Within 2 weeks after hatching, the immune system of the chickens is not yet fully developed, and they are most vulnerable to the negative impact of pathogenic microflora [32]. Thus, it is known [33] that from the first day of life, chicks begin to peck and swallow particles of litter seeded with microorganisms, including pathogenic ones (*Salmonella*, *Clostridium perfringens*, *Campylobacter jejuni*, and *Escherichia coli*).

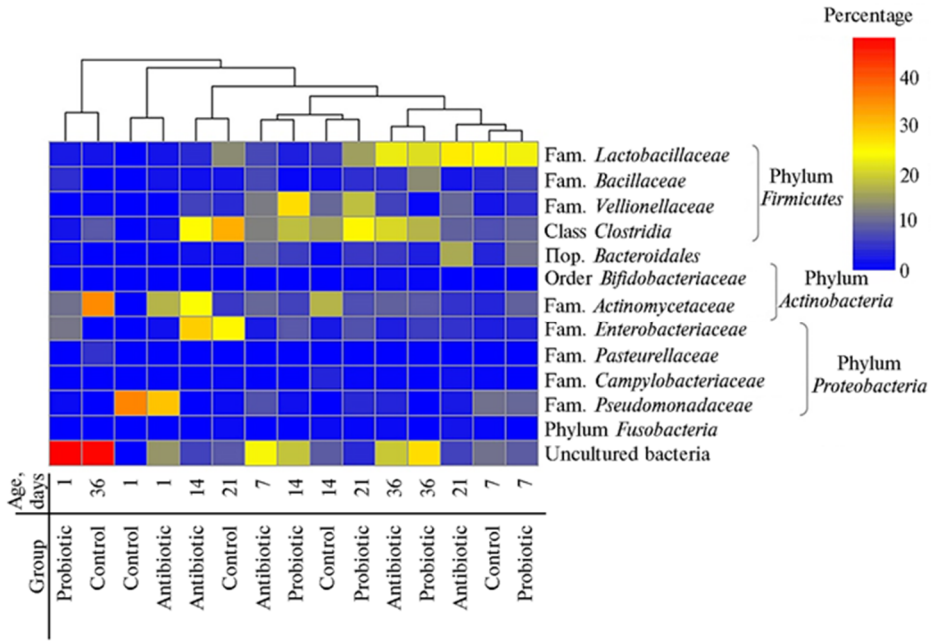

Fig. 2. Cluster analysis and heat map of the cecal bacterial community of Cobb 500 cross broiler chickens fed the basal diet (BD, control)**, BD supplemented with Stafac® 110** (antibiotic)**, or BD with the feed additive Cellobacterin®-T containing *Bacillus subtilis*** (probiotic) **when aged 1, 1, 7, 14, 21, and 36 days** (n = 3, M±SEM, qPCR analysis; vivarium of the All-Russian Research Veterinary Institute of Poultry, St. Petersburg, 2014).

Cluster analysis confirmed the conclusion about the rapid development of the microflora of the gastrointestinal tract of birds (Fig. 2). It can be seen that 1-day-old birds fed the probiotic were allocated to a separate cluster with the control group of adult broilers aged 36 days.

In the microflora of the cecal chyme of chickens, at the phylum level, two taxa dominated, the *Firmicutes* and *Proteobacteria* (see Fig. 2). The phyla *Actinobacteria*, *Bacteroidetes*, and *Fusobacteria* were less abundant. Earlier, other researchers reported [34] that the most common phylotypes of cecal microorganisms belonged to the phyla *Firmicutes* (44-55%), which is consistent with our findings, and *Bacteroidetes* (22-42%), and to the taxa *Actinobacteria*, *Chlorobi*, *Deferribacteres*, *Fusobacteria*, *Verrucomicrobia*, and *Proteobacteria* (the latter dominated in our experiment). The revealed fact of the dominance among the *Firmicutes* phylum of bacteria of the families *Lactobacillaceae*, *Bacillaceae*, *Vellionellaceae* and the class *Clostridia* suggests that the cecal microbiota plays an important role in the digestion of non-starchy polysaccharides associated with the synthesis of short-chain fatty acids, also through the exclusion of in lowering the pH of the chyme due to the synthesis of organic acids [35].

Control and test groups were distinguished into separate clusters on days 7 and 36 of growing. This indicates a more pronounced effect of the age of birds on the composition of microflora vs. the additives used.

Nevertheless, a detailed analysis of changes in the number of taxa showed an increase in the abundance of bacteria of the *Clostridia* class (among them there are forms involved in the breakdown of dietary fiber) when the antibiotic Stafac® 110 ($p \leq 0.05$) and the probiotic *B. subtilis* ($p \leq 0.05$) were used as compared to the control. A similar trend persisted throughout the entire rearing of chickens (excluding day 21). The greatest difference was noted on day 36 when the proportion of bacteria of the *Clostridia* class was 12.7% more ($p \leq 0.01$) in birds fed the antibiotic and 8.8% more ($p \leq 0.05$) in birds fed *B. subtilis* as compared to the

control. This is an important conclusion of great practical importance, since the digestion of cellulose in the intestines of birds is an exclusively microbiological process due to the absence of own cellulases in the macroorganism. In 2013, Stanley et al. [36] using pyrosequencing of the V3 region of the 16S rRNA gene found that an increase in the number of microbial groups in the gastrointestinal tract of birds, including cellulolytic bacteria *Clostridium islandicum* and *Ruminococcus* sp., was associated with an increase in productivity. Our results are consistent with the data of the metagenomic analysis of the cecal microbiota in 42-day-old Ross broilers, in which numerous enzymes that decompose polysaccharides and oligosaccharides have been identified in this intestine region [37].

The tendency of an increase in the number of other important representatives of *Firmicutes* — bacteria of the *Vellionellaceae* family was observed practically throughout the experiment when using Stafac® 110 ($p \leq 0.05$) and *B. subtilis* ($p \leq 0.05$) compared to the group without additives. This conclusion is also important, since it is known that, as a result of the activity of members of the *Vellionellaceae* family, the accumulation of short-chain fatty acids occurs in the cecum, which are further assimilated by the host [38]. It is known that in the cecum of birds, volatile fatty acids (VFAs) are absorbed across the epithelium via passive diffusion and are involved in various metabolic pathways [39]. Up to 95% of VFAs produced during microbial fermentation of carbohydrates [40, 41] are used by the host, providing up to 30% of the total energy requirement. Ruminants receive almost 100% of the required energy as a result of the activity of the rumen microbiome [42]. VFAs are used as a source of energy and carbon. In addition, they affect blood flow, stimulate the growth and proliferation of enterocytes, and regulate mucin production, influencing the intestinal immune response [39]. There is evidence that these compounds activate the immunity of a macroorganism by influencing the expression of Il1β, TNFa, chemokines, and immune barrier genes [43]. Earlier, when analyzing the microbial contents of the cecum of the intestine of birds, genes associated with butyrate production with the participation of 3-hydroxybutyryl-CoA dehydrogenase, phosphate butyryl transferase, and butyrate kinase were found [37]. In addition, the presence of acetate CoA transferase, which is responsible for the synthesis of acetate, and clusters of genes encoding beta, gamma, and delta subunits of methylmalonyl CoA decarboxylase, involved in the formation of propionate, was found [37]. Also, genes of 12 hydrogenases produced mainly by bacteria of the genus *Megamonas*, which belong to the *Vellionellaceae* family, have been identified in the cecum. The authors suggested that these hydrogenases can serve as hydrogen acceptors, promoting the formation of succinate [37].

Among the bacteria of the phylum *Proteobacteria,* the families *Enterobacteriaceae* (up to 28.8±1.8%) and *Pseudomonadaceae* (up to 35.4±2.4%) dominated. *Pseudomonas* sp. were previously also found in the gastrointestinal tract of birds [5]. A large representation of the *Pseudomonadaceae* family in the control and when using the antibiotic Stafac® 110 was noted in chickens on days 1 and 7 (at $p \leq 0.001$ and $p \leq 0.05$, respectively) compared to older birds. Many bacteria of the *Pseudomonadaceae* family are capable of hydrolyzing phytate and degrading starch, but it should be noted that the species *Pseudomonas aeruginosa* causes omphalitis, a dangerous disease that becomes a common cause of death in birds at 1-14 days of age. This species is resistant to sulfisoxazole, ceftiofur, penicillin, lincomycin, bacitracin, oxytetracycline, erythromycin, nalidixic acid, and tetracycline [44].

Among the phylum *Actinobacteria*, bacteria of the *Actinomicetaceae* family dominated (up to 35.3±3.1%). Earlier, a significant amount of metagenomic sequences encoding endoglucanases, usually synthesized by representatives of this

taxonomic group, which degrade polymers, in particular cellulose and xylan, were found in the contents of the gastrointestinal tract of chickens [37]. In our experiment, the representation of bacteria of the *Actinomicetaceae* family was higher than in the control, in chickens of 1-14 days of age when using an antibiotic ($p \leq 0.05$) and 1 and 7 days of age when replacing it with a probiotic ($p \leq 0.05$).

The identification and study of pathogenic bacteria in the microbiota of broiler chickens is important for the health of both poultry and humans. Attention should be paid to the fact that in the authors' experiment, bacteria of the *Campylobacteriaceae* family were detected in the cecum of the intestine in chickens. Gastrointestinal infections in humans caused by a member of this family, *Campylobacter*, are mainly associated with the consumption of poultry products [45]. The use of the antibiotic Stafac® 110 and the *B. subtilis* strain had a significant effect on the decrease in the abundance of these microorganisms in the intestine on day 14 of growing (the differences from the control were statistically significant at $p \leq 0.05$). In chickens, among the previously described taxa that can cause diseases in humans, one can distinguish *Campylobacter* (mainly *Campylobacter jejuni* and *C. coli*), *Salmonella enterica*, *Escherichia coli*, and *Clostridium perfringens* [46].

The results indicating the normalization of the microflora of poultry after the introduction of probiotic strains of bacteria into the diet have been repeatedly confirmed [44, 47, 48]. With regard to the human intestinal microbiota, a stable opinion has been formed that most of the known antibiotics suppress not only pathogenic but also commensal microflora. In our study and in the works of other researchers, the introduction of virginiamycin into the diet of birds had a positive effect on representatives of the intestinal normal flora. Thus, in 2012, an increase in the number of intestinal lactobacilli in broilers was demonstrated under the influence of virginiamycin [49]. Two years later, it was reported [47] that virginiamycin significantly reduced the number of *E. coli* in the intestines of broilers on day 42 of rearing and promoted an increase in the abundance of bacteria of the genus *Lactobacillus* compared to the control group.

1. Age dynamics of zootechnical indicators of Cobb 500 cross broiler chickens fed the basal diet (BD, control)**, BD supplemented with Stafac® 110** (antibiotic), **or BD with the feed additive Cellobacterin®-T containing *Bacillus subtilis*** (probiotic) ($n = 60$, $M \pm$SEM, vivarium of the All-Russian Research Veterinary Institute of Poultry, St. Petersburg, 2014)

Indicator	Group		
	control	Stafac® 110	*B. subtilis*
Mortality, %	2.9	0	0
Live weight, g:			
1 day,	45.1±0.4[a]	45.1±0.3[a]	45.1±0.3[a]
14 days	392.4±7.2[a]	412.7±6.7[a]	410.5±6.9[a]
21 days	786.5±10.4[a]	825.2±9.9[a]	820.54±10.0[a]
36 days			
average for livestock	1989.0	2089.9	2080.3
cockerels	2132.2±38.1[a]	2243.5±31.3[a]	2233.1±32.9[a]
chicken	1845.8±20.9[a]	1936.4±17.9[b]	1927.5±19.4[a]
Daily average live weight gain, g	55.5±2.5[a]	58.4±2.2[a]	58.2±3.4[a]
Feed consumption per 1 head for the entire period, kg	3.5±0.2[a]	3.6±0.2[a]	3.6±0.2[a]
Feed consumption per 1 kg of live weight gain, kg	1.8±0.1[a]	1.7±0.1[a]	1.7±0.2[a]

[a-b] Differences between values marked with different superscript letters are statistically significant at $p \leq 0.05$.

Comparison of zootechnical indicators (Table 1) revealed a significant ($p = 0.046$) increase in live weight in 36-day-old females fed with the antibiotic Stafac® 110. We did not observe such an effect in cockerels during the entire period of rearing. Previously, sex differences in the response to Stafac® 110 in broilers of the Cobb 500 cross at 36 days of age were also described, but a greater increase in body weight was characteristic of males while not observed in females

[50]. It has been shown that the main mechanism of the positive action of antibiotics is associated with the suppression of pathogenic microflora and, as a consequence, a decrease in the amount of toxic metabolites produced by it, especially the decomposition products of ammonia and bile [51], which was probably also observed in our experiment, as followed from the restoration of the composition of microflora. In addition, there is an opinion that the positive effect of antibiotics is associated with an increase in the availability of nutrients for the macroorganism in the intestine and an increase in the digestibility of dietary protein [51]. We also did not find significant differences between the groups in the live weight of hens up to 21 days of rearing.

It was found (see Table 1) that the antibiotic Stafac® 110 and the *B. subtilis* strain did not have a significant effect on feed consumption.

The obtained results agree with the known data. Thus, a group of scientists [52] studied the effectiveness of the probiotic Lacto G based on lactobacilli when introduced into the diet of broiler chickens against the background of artificial infection of birds with the causative agent of coccidiosis *Eimeria tenella*. The results obtained showed that, despite the decrease in pathogen infection with the use of the probiotic, there was no positive effect of the drug on the indicators of live weight gain and feed conversion.

2. Digestibility and nutrient utilization in 28-36-day-old Cobb 500 cross broiler chickens fed the basal diet (BD, control)**, BD supplemented with Stafac® 110** (antibiotic), **or BD with the feed additive Cellobacterin®-T containing *Bacillus subtilis*** (probiotic) (n = 6, M±SEM, vivarium of the All-Russian Research Veterinary Institute of Poultry, St. Petersburg, 2014)

Indicator	Group		
	control	Stafac® 110	*B. subtilis*
	Digestibility, %		
Protein	90.8±4.9[a]	91.9±5.3[a]	91.4±5.2[a]
Fats	80.1±3.8[a]	82.3±5.5[a]	81.7±4.6[a]
Fibre	11.5±0.6[a]	13.8±0.4[b]	18.6±0.5[c]
	Utilization, %		
Nitrogen	53.5±2.6[a]	55.2±3.2[a]	54.6±2.8[a]
Calcium	46.0±2.5[a]	46.9±2.8[a]	46.6±2.1[a]
Phosphorus	38.1±1.7[a]	39.5±2.1[a]	39.1±1.9[a]

[a-c] Differences between values marked with different superscript letters are statistically significant at $p \leq 0.05$.

In our tests (Table 2), the digestibility of fiber in the group with the introduction of the *B. subtilis* strain into the diet was 7.1% higher than in the control ($p = 0.0027$), of the antibiotic 2.3% higher than in the control ($p = 0.047$). This is probably due to the restoration of the intestinal microbiome structure in chickens from the experimental groups and an increase in the number of microorganisms exhibiting cellulolytic activity, for example, bacteria of the genus *Ruminococcus*, as well as cellulolytics of the genus *Clostridium* [53].

As follows from a detailed analysis of the microbial community of the birds' intestines, the cecum was mainly dominated by the microbiota which plays an important role in the digestion of non-starchy polysaccharides and participates in the synthesis of short-chain fatty acids and the displacement of pathogenic microflora using the synthesis of bacteriocins. It is obvious that the bird microbiome, which has such a pronounced effect on the functioning of the macroorganism, needs to be adjusted and maintained. To date, data have been obtained on both the positive and negative effects of antibiotic therapy on the composition of the gut microbiota of birds. Our study revealed a predominantly positive effect of the feed antibiotic Stafac® 110 on the structure of the microbiome due to an increase in the abundance of cellulolytics and bacteria involved in the synthesis of organic acids by the macroorganism in the most important metabolic processes. However, similar data on positive changes in the structure of the microbial

community were obtained during the introduction of the probiotic strain *B. subtilis*. These results may be of great practical interest due to current consumer protests and government restrictions on the use of antibiotics in poultry and livestock. So, by the order of the Government of Russia No. 604-r dated March 30, 2019, within the framework of the state Strategy for Preventing the Spread of Antimicrobial Resistance in the Russian Federation until 2030, from 2020 it is prohibited to use veterinary antimicrobial drugs for non-medicinal purposes. For violation of this prohibition, the introduction of administrative responsibility is expected. In addition, from 2020 the use of antimicrobials in the manufacture of feed should be regulated (with corresponding changes in the existing legislation).

In our opinion, replacing antibiotics in feed with probiotics in conditions of rejection of antibacterial agents is quite real, but it requires additional research to understand the molecular mechanisms of the positive effect of antibiotics and probiotics not only on the microflora of the large intestine but also on other parts of the intestine. It would be interesting in the future to compare the effect of feed and medicinal antibiotics on the structure of the microbiome of chickens, as well as to evaluate changes in the microflora of the gastrointestinal tract of adult birds (parent flocks and layers) under the influence of antibiotics or probiotics.

Thus, the obtained results indicate that the introduction of the *Bacillus subtilis* strain into the gastrointestinal tract of broiler chickens provides a faster formation of the intestinal microflora (already in day 1 of life) in comparison to the basal diet without additives, as well as with the introduction of an antibiotic Stafac® 110 based on virginiamycin. On day 14, both the antibiotic and probiotic strains decreased, as compared to the control, the abundance of microorganisms of the *Campylobacteriaceae* family among which causative agents of gastroenteritis can be found. Dietary antibiotic Stafac® 110 increased body weight in 36-day-old females, but not in males (despite the restoration of their microflora). The digestibility of cellulose during the introduction of the *B. subtilis* strain increased compered to the control and the dietary antibiotic, which may be associated with the activity of cellulolytic microorganisms. The antibiotic Stafac® 110 and the probiotic strain *B. subtilis* had no significant effect on feed consumption. Dietary probiotic *B. subtilis* strain of Cellobacterin®-T restores intestinal microflora in broilers and increase fiber digestibility. Therefore, the Cellobacterin®-T can be an effective alternative to the feed antibiotic Stafac® 110 based on virginiamycin.

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