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Research Article

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Probiotics as an alternative to antibiotics in modulating the intestinal microbiota and performance of broiler chickens

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Abstract

Aims: Gut bacteria play an important role in poultry nutrition and the immune defense system. Changes in the intestinal microbiome affect the physiological state, metabolism, and innate immunity of poultry. The present study aimed to characterize age-related changes in the gastrointestinal tract microflora in broiler chickens, depending on supplementation of the diet with the in-feed antibiotic Stafac® 110 and a *Bacillus subtilis* strain-based probiotic.

Methods and results: In this regard, a comprehensive analysis of the taxonomic structure of the microbial community in the gastrointestinal tract (GIT) of broiler chickens was carried out using a molecular genetic technique of the terminal-restriction fragment length polymorphism analysis and taking into account age dynamics and feeding treatment. A beneficial effect on the microbiological composition and body weight of broilers was observed when using the antibiotic and probiotic in compound feeds. Different bacterial communities were revealed in the duodenum and cecum, and their positive impact on broiler growth was established. The results obtained shed light on the formation of GIT microflora of broiler chickens during the growing period and its changes in response to the use of the antibiotic and the probiotic.

Conclusions: We suggest that the implementation of the tested in-feed antibiotic and probiotic can be beneficial in regulating the intestinal microflora microbiological processes in the GIT and improving the feeding efficiency and productivity of broiler chickens.

Impact Statement

Studying the intestinal microbiome is highly relevant to, and important for, poultry welfare, farming, and industry. Search for feed supplements to modulate bacterial communities in the intestines and performance of broilers can be instrumental in improving nutrition, health, and productivity in poultry.

Keywords: in-feed antibiotic, *Bacillus subtilis* probiotic, broiler chickens, duodenum, cecum, microbiome

1. Introduction

Normally, the intestinal microbiota in poultry contributes to the efficient digestion and absorption of nutrients, the prevention of colonization by pathogens, the improvement of body weight gain, and the biodegradation of feed mycotoxins (Slizewska and Piotrowska 2014, Ye et al. 2021). The gastrointestinal tract (GIT) of chicks can be colonized with microbiota already at the embryonic stage, during the formation of eggs in the oviduct, and during movement along the reproductive tract (Pourabedin and Zhao 2015). However, the chick intestine receives a significant proportion of microorganisms from the environment post hatch (Fathima et al. 2022). After the chick hatch, newly developing bacterial communities experience certain difficulties, since a wide range of environmental impacts (Snel et al. 2002, Aruwa et al. 2021) and genetic factors (Zhou et al. 2022) can affect the GIT microbiome composition. In the growing course of chickens, the nutritional value of feed is changed and some components are replaced by others, e.g. the use of vegetable-type feed is practiced, fish meal is replaced with meat and bone meal, and so on (Fisinin and Surai 2013). All this, to one degree or another, affects the state of the GIT microflora and, as a consequence, the processes of digestion, growth, viability, and feed conversion in birds.

In addition to metabolic functions, the intestinal epithelium constitutes the first line of immune defense against pathogens (Rath and Haller 2022). A number of released factors acting

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in the GIT may protect the bird's body from various negative impacts. The interaction between the immune system of the digestive tract and the intestinal microbiota of chickens begins immediately after hatching and leads to a change in the expression levels of some genes associated with immunity (Bar-Shira and Friedman 2006). The maturation of the body's immune system may be accompanied by a weakening of the immune response to pathogens, which often leads to their persistence in the GIT in adults (Crhanova et al. 2011).

Conventional in-feed antibiotics used in animal husbandry are characterized by a number of well-known significant drawbacks (Ponomarenko et al. 2009), but a positive effect on the body. The latter is exhibited in the suppression and inhibition of pathogenic bacteria of the digestive tract and the creation of a more favorable environment for other types of intestinal bacteria. As a result, resistance of animals to stress and infections ensues (Fisinin and Surai 2013). Among the promising in-feed antibiotics is Virginiamycin, which has a bacteriostatic and, in high concentrations, a bactericidal effect. In a study of this antibiotic, also known under the trademark Stafac® 110 (Phibro Animal Health Corporation, Teaneck, NJ, USA), its introduction into the broiler diet at a dosage of 140 g/t of feed increased the body weight of chickens by 3.1% and the average daily gain by 3.2% (Hoitsman et al. 2012).

To correct GIT dysbacteriosis, probiotics based on certain microbial strains are applicable (Simon et al. 2001, Yu et al. 2022). Particularly, *Lactobacillus* and *Bifidobacterium* strains are most often used in medicine, whereas *Bacillus* and *Enterococcus* bacteria (Plaza-Diaz et al. 2019) and *Saccharomyces* yeasts have been implemented in veterinary medicine (Jin et al. 2000, Kalavathy et al. 2003). One such probiotic, Cellobacterin (All-Russia Research Institute for Agricultural Microbiology, Pushkin, St. Petersburg), was created on the basis of a consortium of microorganisms isolated from the rumen of cattle. The bacteria that make it up produce enzymes that can hydrolyze feed fiber. This allows the use of the probiotic in diets with a higher content of wheat, barley, and sunflower (Laptev et al. 1994, Kislyuk et al. 2004, 2020). Cellobacterin was intended to improve digestion in ruminants; however, experiments have shown that this probiotic is also effective in poultry. Preparations of Stafac® 110 and a *Bacillus subtilis* strain-based probiotic passed a number of extensive production tests in the poultry, pig, and dairy cattle industries (Pervova 2005, Ponomarenko et al. 2009, Bushov and Kurmanaeva 2012, Hoitsman et al. 2012, Grozina 2014). For broiler production, the optimal dosage was established, which was 180 g/t feed for Stafac® 110 and 1 kg/t for the probiotic (Grozina 2014).

Monitoring the state of poultry flocks, including through the implementation of genetic techniques (e.g. Moiseyeva et al. 1993, Feye et al. 2020), contributes to an increase in the efficient production of poultry meat and eggs (e.g. Tereshchenko et al. 2015, Bondarenko and Khvostik 2020). A high-throughput method for studying the intestinal microbiota in chicken ontogenesis (Nikonov et al. 2017a,b, Kochish et al. 2018) is the terminal-restriction fragment length polymorphism (T-RFLP) technique, a molecular genetic method based on the assessment of polymorphism in the lengths of amplified restriction fragments of microbial DNA. This technique is fast and reproducible, allowing qualitative and quantitative comparison of microbial communities by the presence or absence of certain peaks on T-RFLP electropherograms that makes it possible to identify specific taxa of microorganisms (Zhu et al. 2002, Amit-Romach et al. 2004, Grozina 2014). Based on such microbiome composition data, diets can be adjusted to improve the health status and increase the productivity of poultry.

The objective of this study was to explore the composition of the GIT microbiota of broiler chickens and broiler performance in age dynamics, depending on the composition of the feed, i.e. the presence of in-feed antibiotic and probiotic in it. In the course of achieving this goal, we employed the T-RFLP analysis method to identify the effects of the feed additives on the chicken GIT microflora profiles at different ages.

2. Materials and methods

2.1. Experimental birds

Cobb 500 broiler chickens were hatched at a hatchery using commercial hatchers IV-8-M1 (Stimul Ink, OOO Stimul Group, Pushkino, Moscow Oblast, Russia). After hatching, chicks with a body weight of at least 43 g were chosen in the hatchery and transported in a special vehicle manufactured by VEIT Electronics (Moravany, the Czech Republic) and equipped with an automated microclimate control system to the experiment site located in the same region (Moscow Oblast). The total trip duration from the hatchery to the place of the experiment did not exceed 3 h. The chicks were grown in R-15 cage batteries from one day to 36 days of age. The experimental and control groups of chickens with equal body weight were established at 1 day old, 70 birds in each group and 35 birds in one cage. Body weight was determined by weighing all birds individually; however, for statistical analysis, body weight of 35 randomized chickens was taken from each group. Weighing was performed at ages of 14 and 21 days without dividing by sex, and at age of 36 days separately for hens and cockerels, followed by calculating the group mean values and the respective standard errors. The technological parameters of their growing followed the recommendations of the All-Russian Poultry Research and Technological Institute (ARRTPI 1999). Feed and water were offered to birds *ad libitum*. Both the experimental and control groups were fed a complete diet of compound feeds that had a 3% content of ingredients of animal origin and nutritional value according to the commercial Cobb 500 cross recommendations (Cobb 2010), with ingredients of animal origin being totally excluded starting from the 15th day post hatch.

2.2. Experimental diets

In the experiments, the GIT microbiota and growth performance of broilers were studied when administering the in-feed antibiotic Stafac® 110 (experimental Group ANT) and a probiotic based on the *B. subtilis* strain 1–85 we previously developed (Grozina 2014; experimental Group PROB) in the diets daily from Day 1 to Day 36 (i.e. throughout the entire growing period). The antibiotic was added at a dose of 180 g/t feed, and the probiotic at a dose of 1 kg/t. These dosages of antibiotic and probiotic were validated in previous studies (Grozina 2014, Laptev et al. 2020). In the control group (CONT), the same compound feed was used, but with no treatment with the said feed supplements. To examine the intestinal microflora, samples of the duodenum and cecum contents were taken from broiler chickens at 1, 7, 14, 21, and 36 days of age,

with six animals from each group/age. On Day 0, i.e. upon delivery from the hatchery, chicks were immediately given access to water and feed. They were subject to the examination of intestinal microbiome in 24 h after the initial feeding began (i.e. on Day 1). The microbiota data were presented as the actual number of microorganisms, i.e. colony-forming unit per gram (CFU/g). Comparisons were made only within age groups of 1, 7, 14, 21, and 36 days. For the analysis of the growth performance data, weighing was carried out with a 1 g precision on an NP-12KS balance (A&D, Tokyo, Japan) on the 14th, 21st, and 36th days.

2.3. Molecular genetic analyses

Molecular genetic procedures were employed as described elsewhere (Grozina 2014, Laptev et al. 2019, 2021). Bacterial DNA isolation was performed using the Genomic DNA Purification Kit (Fermentas, Vilnius, Lithuania). Polymerase chain reaction (PCR) amplification of the bacterial 16S rRNA gene fragments (e.g. Qiu et al. 2001, Zhu et al. 2002, Romanov et al. 2004) with fluorescently labeled primers was carried out using the eubacterial PCR primers described elsewhere (Laptev et al. 2019, 2021). Determination of the total quantitative content of microorganisms in samples was implemented by real-time PCR (RT-PCR). In addition, the resulting 16S rRNA gene fragments were subjected to T-RFLP analysis to determine the bacterial composition of the samples. For this, the 16S rRNA samples were digested with restriction enzymes HaeIII, HhaI, and MspI and placed in a CEQ8000 automatic sequencer (Beckman Coulter, Brea, CA, USA). The latter was equipped with an in-built program for calculating the length of the restriction fragments of each sample relative to the control size marker embedded in a sample (Size Standard 600, Beckman Coulter). After electrophoresis, results were stored in files containing tabulated gel scanning data and its graphic image, i.e. an electropherogram (Fig. 1). The error of the CEQ 8000 instrument was no more than 5%. Peak sizes and areas were calculated using the Fragment Analysis program (Beckman Coulter), on the basis of which subtypes (phylotypes) were identified with an error of 1.5 nucleotides adopted in the study and their percentage in the microbial community was determined. The taxonomic assignment of microorganisms was determined using the tRFLP Fragment Sorter program (Sciarini 2005).

2.4. Statistical analyses

Mathematical and statistical processing of the data obtained and the generation of the respective bar plots were carried out using the Microsoft Excel 2019 built-in analysis tools and formulae. In particular, all necessary calculations were produced in the Microsoft Excel environment. Student's t-test was used to compare the mean values between the control and experimental groups; differences were considered significant at $P < .05$. The results were also subject to the appropriate treatment using the method of multifactor analysis of variance (ANOVA) in Microsoft Excel and R-Studio (Version 1.1.453; RStudio Team 2018). Significance of differences was determined by Student's t-test, with differences being considered statistically significant at $P < .05$. The Shapiro–Wilk test was used to assess the normality of data distribution by the total content and composition of microflora. To visualize the microbiome beta diversity between samples, a principal component analysis (PCA) plot was composed using the Phantasus webtool (Zenkova et al. 2018).

3. Results

3.1. Total bacterial quantification via RT-PCR

After screening the microbiome profiles in the broiler intestine contents (Fig. 1), the data on the total number and composition of the microbiota had a normal distribution (Shapiro–Wilk test, $P > .05$), i.e. the scatter of data on the microflora in individual birds within groups did not exceed 5%. The range of mean values of total bacterial content for each group was between $9.6 \times 10^7 \pm 6.75 \times 10^6$ and $8.3 \times 10^{10} \pm 2.21 \times 10^9$, with individual variations among the six birds per group shown in the Supplementary Data. With age, an increase in the total number of bacteria was observed Groups ANT and PROB throughout the experiment as compared to Group CONT ($P < .05$; Supplementary Data file). Both in the duodenum and in the caecum, a more pronounced significant effect of the antibiotic (Group ANT) was noted relative to Group CONT ($P < .05$) in the growing period of 1–14 days than in the period of 21–36 days (Supplementary Data file). The respective difference between Groups ANT and CONT was 6.2–58.2 times in the duodenum and 4–9.2 times in the caecum during the growing period of 1–14 days ($P < .05$; Supplementary Data file). On the other hand, there was practically no difference between Groups ANT and CONT in the period of 21–36 days (Supplementary Data file). This was probably due to the fact that younger birds were more responsive to external factors, i.e. feed additives, due to the underdeveloped immune and digestive systems and a lower microbiome composition diversity (Sharma 1991, Ballou et al. 2016). A similar pattern of differences was observed for Group PROB relative to Group CONT ($P < .05$; Supplementary Data file). This was probably due to the fact that the microflora of older birds is more stable than that of chicks on the first days of life, as was also shown previously (e.g. Sun et al. 2022).

The results of quantifying the total bacterial content in the samples showed that in both Groups ANT and PROB, the number of bacteria was higher compared to Group CONT (see the Supplementary Data file). This was indicative of a faster GIT colonization by the microflora in Groups ANT and PROB, being important for intestinal development in the treated chickens. In particular, at 1 day old, the total number of bacteria in the contents of the duodenum in Group PROB was higher as compared to Groups CONT and ANT ($P < .05$; Supplementary Data file). The total number of bacteria in the cecal contents in Groups ANT and PROB was greater than in Group CONT. At the subsequent ages, this indicator in the treated groups elevated relative to Group CONT (Supplementary Data file).

3.2. T-RFLP-based microbiota composition comparison between treatments

T-RFLP analysis showing the profiles of the intestinal microbiome (Fig. 1) resulted in the content data per separate taxonomic groups in the duodenum and cecum of broiler chickens as presented in the Supplementary Data file. Among the representatives of intestinal normocenosis, bacteria with cellulolytic activity play a significant role (Froidurot and Julliand 2022). Since birds practically lack their own diges-

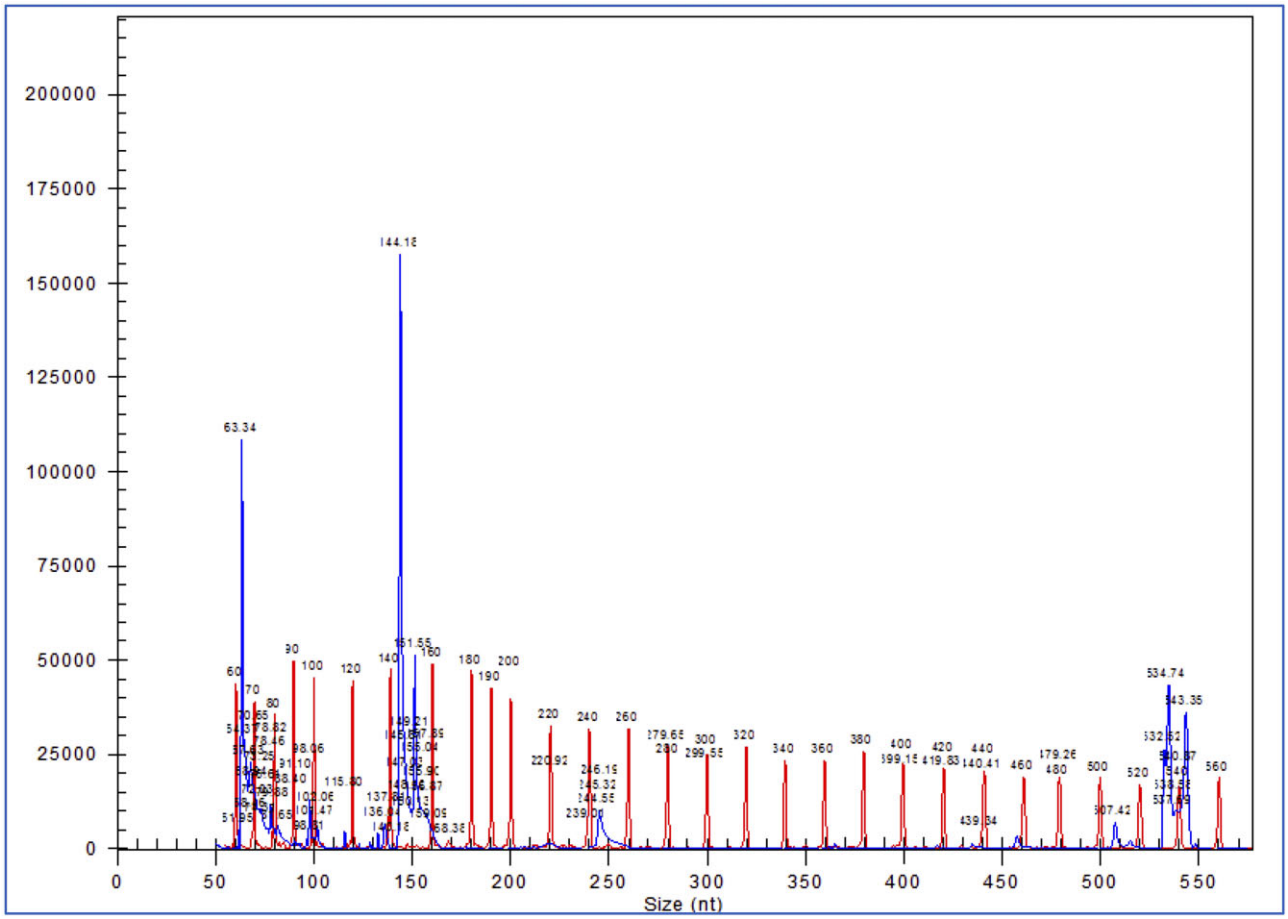

Figure 1. An electropherogram example of the bacterial community profile in the broiler intestine contents. Red peaks represent the size marker, while blue peaks conform to 16S rRNA restriction fragment lengths of a sample.

tive enzymes for the breakdown of cellulose and other non-starch polysaccharides, the role of these microorganisms in the digestion of broiler chickens can hardly be overestimated. The number of cellulolytic bacteria across the studied samples is given in the Supplementary Data file. Already in day-old chicks, the amount of cellulolytic microflora was higher in Groups ANT and PROB as compared to Group CONT, and later on, this preponderance increased ($P < .05$). In particular, the use of the probiotic (Group PROB) resulted in a steady significant increase ($P < .05$; Supplementary Data file) of cellulolytic microorganisms with age in the caecum, i.e. from $6.3 \times 10^7 \pm 3.2 \times 10^6$ cells/g at day-old to $2.3 \times 10^9 \pm 1.8 \times 10^8$ at 36 days of age, although such a pattern was not observed in the duodenum. At the same time, the content of cellulolytics in Group PROB was significantly higher than Groups CONT and ANT ($P < .05$; Supplementary Data file). The fact is that the cecum of birds is characterized by the most active microbiological processes as compared to other parts of the digestive tract (Wilkinson et al. 2017). The internal environment of the caecum appeared to be more favorable for colonization by the probiotic microorganism, which, in turn, contributed to the synthesis of biologically active substances by the *B. subtilis* 1–85 strain and the formation of conditions for the reproduction of cellulolytics. Nevertheless, because the T-RFLP technique does not allow the identification of bacteria to the species level, and even more so to the strain level, we can indirectly assume, based on the results of this study, that the strain of the probiotic colonized the intestinal epithelium. For more accurate validation, more in-depth studies are required, for instance, using a fluorescent labeling approach. Colonization with a strain based on *B. subtilis* seems quite likely, since bacteria of this species often have adhesion genes (Li et al. 2023) and a high colonization potential (Tam et al. 2006). The proposed mechanisms of probiotics action on the composition and function of the intestinal microbiome include not only competition for receptors and binding sites with other intestinal microbes on the intestinal mucosa (Collado et al. 2007), but also the synthesis of antimicrobial agents or metabolites that inhibit the growth of pathogenic microorganisms and stimulate the growth of normal flora (O'Shea et al. 2012). Probably, the conditions for the colonization of the intestinal chyme with normoflora were more suitable in the cecum of older birds than in younger birds.

Among lactic acid microorganisms, lactobacilli and bifidobacteria were identified (Supplementary Data file). The content of lactobacilli was higher in Groups ANT and PROB as compared to Group CONT ($P < .05$). Bifidobacteria in the poultry intestine also have antimicrobial activity against pathogenic microorganisms (Lim and Shin 2020). In chickens, the number of bifidobacteria was higher in Groups ANT and PROB as compared to Group CONT ($P < .05$). In addition, representatives of the families Bacillaceae that have significant antimicrobial activity against pathogens (Zhao et al. 2018) and Veillonellaceae, which have the ability to decompose organic acids and are an important source of energy for a microorganism (Shetty et al. 2013), were found in the GIT

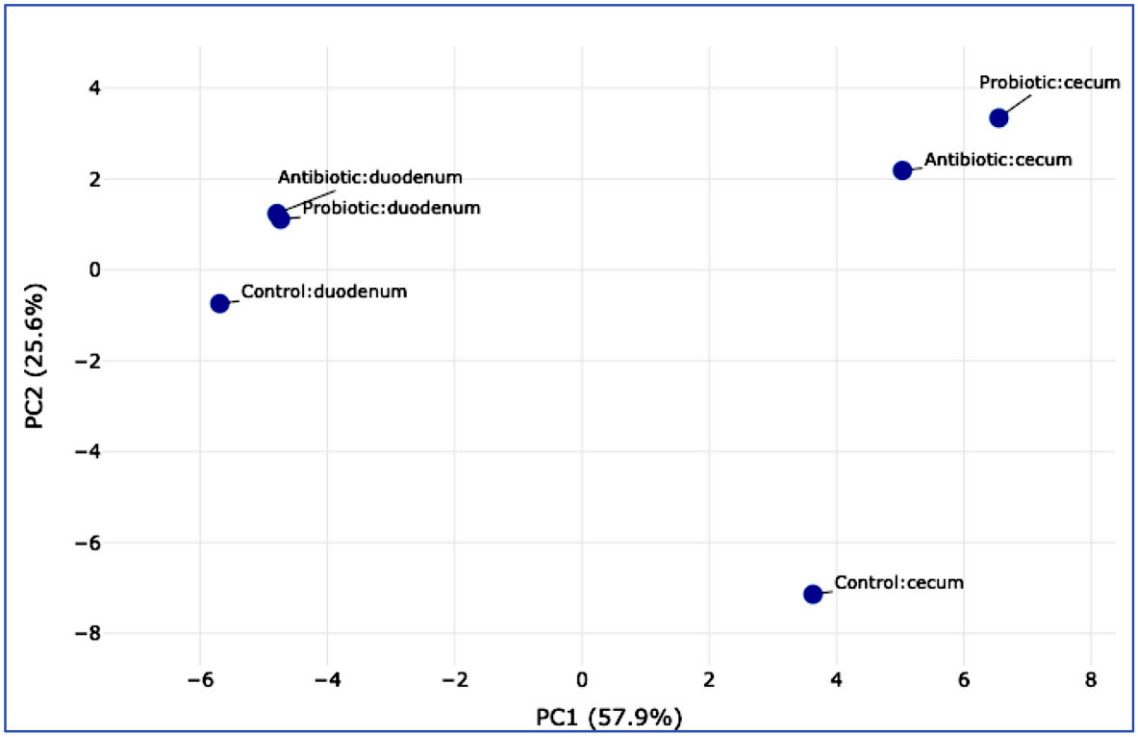

Figure 2. Beta diversity between samples represented as a PCA plot of intestinal bacterial profiles in two experimental (antibiotic and probiotic) and control groups and in two intestine sections of broilers. The graph generated using the Phantasus webtool (Zenkova et al. 2018) is based on the generalization of data on the total bacterial number and content of the main taxonomic groups of bacteria in each chicken group.

of broilers (Supplementary Data file). The respective bacterial content numbers for these two families were greater in Groups ANT and PROB than in Group CONT ($P < .05$). This may be indicative of a positive effect of the antibiotic and probiotic on the microbiome composition.

Enterobacteria (*Salmonella*, *Escherichia coli*, *Proteus*, etc.) belong to the undesirable GIT microbiota in poultry, because they are common causative agents of gastroenteritis (Laham et al. 2015). The examination of the microbial community structure showed that the lowest content of these microorganisms was observed in the intestines of broilers in Groups ANT and PROB as compared to Group CONT ($P < .05$; Supplementary Data file). Actinomycetes causing actinomycosis (Valour et al. 2014) are also undesirable microorganisms in the poultry. In this investigation, the number of actinomycetes was lower in Groups ANT and PROB than in Group CONT ($P < .05$). The number of pathogenic microorganisms such as *Pasteurella* and *Campylobacter* did not exceed the norm, and no pathogenic species were isolated among these bacteria (Supplementary Data file). Additionally, in all the duodenum samples, the numbers of *Pasteurella* and *Campylobacter* were below the limit of available detection, and the data on their content in the cecum differed slightly. The number of *Pasteurella* in the cecum in Groups ANT and PROB was smaller than in Group CONT ($P < .05$). The total number of *Campylobacter* in the cecum of the treated groups was lower than that in Group CONT ($P < .05$; Supplementary Data file). In addition, there were transient microorganisms found in the GIT of broiler chickens, which enter the body with feed and do not play a significant role in the fermentation processes. The number of transient microbiota did not show any trend with age (Supplementary Data file).

3.3. PCA-assisted microbial community structure comparison

To show visually the beta diversity of the chicken GIT microbiome when using the antibiotic and probiotic additives, we presented the results of T-RFLP analysis of the duodenum and cecum contents on a PCA plot (Fig. 2). The treated Groups, ANT and PROB, were located significantly apart from Group CONT on this graph. In the duodenum, these differences were less pronounced, with almost complete similarity of the microbiota profiles in groups ANT and PROB. In the cecum, there were more distinct divergences of all three groups, with some convergence of Groups ANT and PROB.

Collectively, it can be suggested that the introduction of in-feed antibiotic and probiotic preparations into the diet of broiler chickens had a beneficial effect on the GIT microbial communities.

3.4. Broiler performance comparison between treatments

In Table 1, the main performance traits of broiler chickens are compared as a result of their rearing while implementing the antibiotic and probiotic. As was established, the administration of antibiotic and probiotic in the diet of broiler chickens had a significant positive effect on the growth and body weight of birds. The number of chickens (70 per group) in this investigation was comparable to, or exceeded, those in similar published studies (e.g. Qorbanpour et al. 2018, Movahhedkhah et al. 2019, Panaite et al. 2020, Kim et al. 2021, Laptev et al. 2021, An et al. 2022, Shi et al. 2022, Zheng et al. 2022, Zhou et al. 2022) suggesting reliability of the results we obtained. In addition, the veracity of the body weight data in this experiment is supported by their compliance with the

Table 1. Body weight performance in broiler chickens (means ± standard errors).

Body weight	Control group	Antibiotic group	Probiotic group
Mean at 14 days of age, g	410.83 ± 7.41	431.43 ± 7.10*	429.31 ± 7.26
Mean at 21 days of age, g	819.24 ± 10.95	862.71 ± 10.21***	857.94 ± 10.68**
Mean at 36 days of age, g	2012.91 ± 39.73	2117.65 ± 31.18*	2104.71 ± 39.04*
Males	2172.65 ± 41.93	2288.83 ± 39.10***	2273.76 ± 40.16**
Females	1853.18 ± 22.85	1946.47 ± 19.57*	1935.67 ± 20.29*

Differences are significant relative to the control at $^{*}P < .05$; $^{**}P < .02$; $^{***}P < .01$.

requirements of the appropriate broiler raising guidelines (ARRTPI 1999, Cobb 2010).

Thus, based on the data obtained, we suggest that the introduction of these antibiotic and probiotic into the feed containing animal protein in the starter and, partly, grower periods had a beneficial effect on the GIT microflora and, as a consequence, on the performance results. This contributed to an increase in the body weight of broiler chickens by an average of 5.0%–5.2% in Group ANT and by 4.5%–4.7% in Group PROB ($P < .05$; Table 1). Also, the choice of feed supplement for administration should be based on the structure of the diet, the quality of the components, the economic situation at the poultry enterprise, and the epizootic well-being of the area.

4. Discussion

The use of feed additives (in-feed antibiotics, probiotics, phytobiotics, etc.) has become widespread in the poultry industry (Engberg et al. 2000, Chee et al. 2010, Kochish et al. 2019, Laptev et al. 2021). The T-RFLP analysis results reported here confirmed other studies demonstrating that the GIT microbial communities of broiler chickens from the first day of life have a huge biological diversity (Qiu et al. 2001, Hübener et al. 2002, Zhu et al. 2002, Wise and Siragusa 2007, Van den Abbeele et al. 2010, Torok et al. 2011), which may vary depending on the introduction of in-feed antibiotics, probiotics, and other supplements into the diet (Chee et al. 2010, Zhou et al. 2010, Grozina 2014, Laptev et al. 2019). We demonstrated here the new data resulted from the comparative study of the effects of the in-feed antibiotic Stafac® 110 and *B. subtilis* 1–85 strain probiotic on the total content and composition of microbiota in the intestines of the Cobb 500 broilers. The results obtained suggest the prospects of the probiotic supplement instead of administering the in-feed antibiotic as. The tested probiotic can regulate the intestinal microbiome composition of commercial poultry as an effective alternative to antibacterial drugs. Previously, Abudabos et al. (2017) also showed that the introduction of *B. subtilis* at 2×10^7 CFU/g and avilamycin (0.2 g/kg) to *Salmonella*-infected broiler chickens resulted in the same improvement in body weight gain compared to infected control broilers. Those researchers also suggested that a *B. subtilis* probiotic could substitute antibiotics in poultry feeding. Similar findings were reported by Roy et al. (2015) who fed heat-stressed broiler chickens a *B. subtilis* probiotic (0.5 g/kg feed), 2.2% lincomycin (0.15 g/kg feed), and their mixture (0.5 and 0.15 g/kg feed, respectively).

Since there are current legislative restrictions on the use of in-feed antibiotics, the consequences of these restrictions (e.g. economic losses due to reduced feed efficiency, feed consumption, higher morbidity, and mortality in broiler chickens due to the spread of pathogens (Van Immerseel et al. 2009, Ahiwe et al. 2021), an urgent need in developing effective replacements and feeding schemes still exists (Cheng et al. 2014, Ogbuewu et al. 2022). In this study, we introduced and tested a modified feeding scheme that included the probiotic administration, thus resulting in beneficial changes in the intestinal microbiota and performance of broiler chickens.

The GIT microbiome of broiler chickens is a dynamic system. It has previously been established that the change of the starter to the grower diet and the grower to the finisher diet, as well as the administration of in-feed antibiotic and probiotic preparations led to a change in the GIT microbial communities and, as a result, a change in broiler performance, which was also confirmed by other authors (Laptev et al. 1994, Koenen et al. 2004, Risøen et al. 2004, Chee et al. 2010, Zhou et al. 2010, Grozina 2014).

Here, we noted that the in-feed antibiotic and probiotic had a positive effect on the microflora, increasing the number of beneficial bacteria in the GIT, such as lactobacilli, bifidobacteria, bacilli, Veillonellaceae, and cellulolytic bacteria. In a previous study conducted on broiler chickens, Mountzouris et al. (2010) demonstrated a significant increase in the concentration of lactobacilli and bifidobacteria in the caecum of birds fed probiotics. Remarkably, we observed the declined number of conditionally pathogenic bacteria, such as enterobacteria, actinomycetes, *Pasteurella* and *Campylobacter*, in chickens from the treated groups as compared to the control. Possible mechanisms by which *Bacillus* spp. may restrict pathogen reproduction include competition for adhesion sites, production of organic acids, lowering gut pH, maintenance of normal gut microbiota through competitive exclusion and antagonism, production of antimicrobial peptides, improved oxidative stability, modulation of the immune system, increased activity of digestive enzymes, and competition for nutrients (Ogbuewu et al. 2022). The effect of antibiotics leads to destroying the cell membranes of bacteria (Niewold 2007). Previously, in a study evaluating the effectiveness of probiotics isolated from the digestive system of poultry, Garriga et al. (1998) demonstrated that 77 strains out of 296 selected suppressed the proliferation of *E. coli* and *Salmonella* Enteritidis. In our investigation, there was no negative effect from the use of in-feed antibiotic on the beneficial GIT microbiome of chickens. Taking into account the fact that during the growth period there was a deficiency of favorable microbiota, it could be preferable to use a probiotic preparation in this situation. However, a sharp increase in undesirable microflora in the final rearing period of broiler chickens requires continued use of the probiotic in the diet, or replacing it with a stronger antibacterial drug, such as an in-feed antibiotic. The results of our experiments also suggested the suitability of using the T-RFLP analysis method to establish and understand a relatively complete pattern of the GIT microbial communities in poultry, which is in line with previously published studies (Grozina

2014, Witzig et al. 2015, Lindström et al. 2018, Laptev et al. 2019).

5. Conclusions

In this investigation, the GIT microbiota and performance of broiler chickens were analyzed using an in-feed antibiotic and probiotic supplemented in the diet that contained 3% of animal origin ingredients, fed up to 15 days of age, with their subsequent complete exclusion until the end of rearing. Our findings suggested that the in-feed antibiotic and probiotic had a similar regulatory effect on the composition of the microbiome, the amount of normoflora (lactobacteria, bifidobacteria, bacilli, Veillonellaceae, and cellulolytic bacteria) being increased, while the amount of opportunistic (enterobacteria, actinomycetes, *Pasteur ella* and *Campylobacter*) being decreased. This contributed to an increased body weight gain of broilers. The microbiota composition analysis using the molecular genetic technique enabled to trace factors of changes in the poultry productivity and adjust the microbiocenosis balance through the administration of feed additives, which can facilitate the economic efficacy of poultry industry as a whole. The results of this study allow us to propose a way to regulate microbiological processes in the GIT using the tested in-feed antibiotic and probiotic, while contributing to the efficiency of feed use and the productivity improvement in broiler chickens. We suggest that the impact of the probiotic on the microflora composition and broiler performance was not inferior to the effect of the in-feed antibiotic. An alternative application of probiotics is very promising since the use of antibiotics is not in harmony with the concept of global ecologization of food production.

Animal ethics

The study design and procedures used to examine the impact of in-feed additive treatments on broiler microbiota and performance were conducted according to the ethics guidelines of the Declaration of Helsinki and approved by the Institutional Committee for the Care and Use of Animals (Protocol No. 15-065-A, dated 31 October 2015).

Supplementary data

Supplementary data is available at *JAMBIO Journal* online.

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Author contributions

Alena A. Grozina (Investigation, Methodology, Visualization), Larisa A. Ilina (Data curation, Software, Validation, Writing – original draft), Georgiy Yu. Laptev (Methodology, Project administration, Resources, Supervision), Elena A. Yildirim (Software, Validation, Writing – original draft), Ekaterina S. Ponomareva (Data curation, Formal analysis, Investigation), Valentina A. Filippova (Formal analysis), Darya G. Tyurina (Formal analysis), Vladimir I. Fisinin (Conceptualization, Funding acquisition, Project administration, Supervision), Ivan I. Kochish (Writing – original draft), Darren K. Griffin (Writing – review & editing), Peter F. Surai (Writing – review & editing), and Michael N. Romanov (Formal analysis, Software, Visualization, Writing – original draft, Writing – review & editing)

Data availability

The data presented in this study are available in this article and supplementary material.

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