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Effect of a probiotic preparation on the composition of the intestinal microbiome of rabbits

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ABSTRACT

In this work, we studied the effect of a probiotic supplement containing bacteria of the *Bacillus amyloliquefaciens* strain (Vetom 2) on the intestinal microbiome composition of rabbits. Rabbits of the experimental group, in addition to compound feed together with water, received the Vetom 2.0 probiotic feed additive in the amount of 50 mg / kg of live weight of 10 days every 30 days for four months. Genomic DNA was isolated from the intestines of rabbits using a set of ExtractDNABlood&Cells Amplification of variable regions V3–V4 of the 16S rRNA gene using universal primers and subsequent sequencing on the IlluminaMiSeq platform. Bioinformatic analysis was carried out in the QIIME2 environment. The control and experimental groups of crossbred rabbits were dominated by five phyla of intestinal bacteria: *Bacillota, Pseudomonadota, Bacteroidota, Actinomycetota*, and *Verrucomicrobiota*. The following taxa prevailed at the level of the orders: *Eubacteriales, Pseudomonadales, Bacteroidales, Caryophanales, Enterobacteriales*. At the family level, the most important taxa showing significant differences between the groups were *Oscillospiraceae, Lachnospiraceae,* and *Campylobacteraceae*. The probiotic supplement contributed to an increase in the proportion of positive microorganisms, a decrease in the proportion of conditionally pathogenic, and an increase in the biological diversity of the intestinal microbiocenosis.

Keywords: Biodiversity of microbiocenosis intestinal, Metabarcoding, Microbiome, Probiotic supplement, Rabbits.

INTRODUCTION

One of the main problems in rabbit breeding, which hinders the development of this livestock industry in several countries, is the high mortality rate of animals, including as a result of disruption of the normal functioning of their gastrointestinal tract (Kylie *et al.*, 2018; Dabbou *et al.*, 2020; Cremonesi *et al.*, 2022; Curone *et al.*, 2022; Setiaji *et al.,* 2024; Metleva *et al.*, 2024). Young animals are especially susceptible to these processes during weaning from the mother rabbit due to changes in their diet, which can lead to dysbiosis (Mancini and Paci, 2021; Curone *et al.*, 2022; Metleva *et al.*, 2024). In this state, the normal balance of the intestinal microbiota in rabbits is disrupted, which contributes to the maintenance of animal health and affects various vital body processes (Chen *et al.*, 2019; Cotozzolo *et al.*, 2022; Cheng *et al.*, 2024). This can contribute to the development of pathogenic microorganisms that inhibit metabolism and reduce the body's resistance to various diseases, thus having serious health consequences and leading to fatal outcomes (Metleva *et al.*, 2024; Wlazłoł *et al.*, 2024).

Until recently, antibiotics have been used to address the above problems. However, their excessive use may contribute to the emergence of resistance of pathogenic bacteria resistant to drugs (Ikeda-Ohtsubo *et al.*, 2018; Dabbou *et al.*, 2020; Liu *et al.*, 2022; Narkevich *et al.*, 2022; Metleva *et al.*, 2024). Therefore, various biologically active additives and probiotic agents are increasingly being used in rabbit breeding to prevent digestive disorders (Guo *et al.*, 2017; Cotozzolo *et al.*, 2022; Logvinova, 2023). Probiotics are preparations of living bacteria that, upon colonizing the gastrointestinal tract, stimulate the development of beneficial microbiota, and can also synthesize various nutrients. Some research results indicated, probiotic supplements can help reduce the number of opportunistic microorganisms, promote better absorption of nutrients in the diet, and have a positive effect on strengthening the nonspecific immunity of animals (Guo *et al.*, 2017; Ovcharova *et al.*, 2019; Logvinova, 2023).

Curone *et al.* (2022) stated, the most numerous types were *Firmicutes* (correct name *Bacillota*) and *Bacteroidetes*. The dominant families were representatives of the dominant orders and classes, with the main families being the *Ruminococcaceae* and *Lachnospiraceae*. (Curone *et al.*, 2022). In this article, we aimed to study the effect of a probiotic supplement containing bacteria of the *Bacillus amyloliquefaciens* strain (Vetom 2) on the composition of the intestinal microbiome of rabbits by molecular methods.

MATERIALS AND METHODS

Research Object

Crossbred rabbits obtained from crossing the breeds gray giant ´ white giant. Rabbit care and experiments were carried out in accordance with the instructions and recommendations of regulatory acts: Model Law of the Interparliamentary Assembly of the Commonwealth of Independent States "On the Treatment of Animals", Article 20 (Resolution of the MA of the CIS Member States No. 29-17 of 10/31/2007), Guidelines for working with Laboratory Animals (http://fncbst.ru/?page_id=3553). During the research, measures were taken to ensure a minimum suffering for the experimental rabbits and to reduce the number of samples under study.

Experimental Scheme

The research was carried out on crossbred rabbits of unknown origin, which were kept in wooden cages with a mesh metal bottom in the open air. The groups were formed using the pair-analog method. The age of the rabbits at the beginning of the experiment was 2 months. The control and experimental groups each consisted of 8 rabbits: 4 males and 4 females. The live-weight of 1 head in the control was 1565.88±11.46 g, in the experiment – 1565.38±12.07 g. Rabbits in both groups were kept under the same conditions.. The rabbits were fed twice a day (in the morning and in the evening at the same time) with complete compound feed KK-90/2 in the amount of 150 g/head. The feed was fed using automatic feeders. The nutritional value of the diet was 1.59 MJ. Each animal in the experimental group, in addition to the combined feed, was given water containing 50 ml/kg live weight of the probiotic feed supplement Vetom 2.0, containing bacteria of the *Bacillus amyloliquefaciens* strain. The feeding course was 10 days every 30 days for 4 months. At the end of the experiment, intestines were extracted from animals of both groups, to study the composition of complex microbial communities.

Experimental Equipment

DNA was isolated from the mucous membrane and intestinal contents of rabbits using the standard ExtractDNABlood&Cells kit (ZAO EuroGen, Moscow) at the Department of Animal Science of the Yaroslavl State Agrarian University. The results of high-throughput sequencing of amplicon samples obtained using primers specific to the V3–V4 region of the 16S rRNA gene were used to identify intestinal microorganisms: for: 5’-CCTACGGGNGGCWGCAG; rev: 5’-GACTACHVGGGTATCTAATCC.

Statistical Analysis

The samples were analyzed within four groups. The length of the analyzed DNA fragments is 250 nucleotide pairs. Identification of rabbit datasets was carried out on the QIIME 2 data science platform (Bolyen *et al.*, 2019) on the basis of Sequencio LLC. Error correction was performed using DADA2 (Callahan *et al.*, 2016) and an OTE table was obtained. Interactive visualization is available in the vis/stats-dada2.qzv file when viewed using the QIIME2 View tool.

Taxonomic classification was carried out using a naive Bayesian classifier trained on the greengenes database release 2022.10. The following substitutions were made in the names of taxa: *Fusobacteria®Fusobacteriota*; *Firmicutes®Bacillota*; *Proteobacteria®Pseudomonadota*; *Actinobacteria®Actinomycetota*; *Bacteroidetes ®Bacteroidota*; *Cyanobacteria®Cyanobacteriota*; *Verrucomicrobia®Verrucomicrobiota*; *Planctomycetes®Planctomycetota*; *Spirochaetes®Spirochaetota*; *Chlorobi®Chlorobiota*; *Synergistetes®Synergistota*; *Gemmatimonadetes®Gemmatimonadota*.

The analysis of alpha diversity (Shannon index) between groups was carried out at a cut-off threshold of 5,000 readings Interactive visualization and data on the significance of comparisons between groups (Kraskel-Wallis test) are available in the vis/shannon-group-significance.qzv file when viewed using the QIIME2 View tool.

RESULTS

In the control and experimental groups of crossbred rabbits, five phyla of intestinal bacteria dominated: *Bacillota*, *Pseudomonadota*, *Bacteroidota*, *Actinomycetota* and *Verrucomicrobiota* (Table 1). Of the minor types, *Patescibacteria* should also be noted. It was found that the largest relative amount in both chyme and intestinal mucosa in animals of both groups was accounted for by bacteria belonging to the *Bacillota* type. Their proportion ranged from 35.9 to 55.4%, while it was found that in rabbits of the experimental group, the relative number of bacteria of this type was greater both in the intestinal chyme (by 14.0 percentage points) and in the mucous membrane (by 14.6 percentage points). The *Pseudomonadota* type was in second place in terms of representation, the proportion of bacteria of this type varied in the range from 13.6 to 19.9%, however, it should be noted that the relative number of bacteria of this type, in contrats, was higher both in the chyme (by 3.6 percentage points) and in the intestinal mucosa (by 5.2 percentage points) of rabbits of the control group. There are significantly fewer representatives of other types, and such clear patterns are not observed.

Among the dominant orders represented in the chyme and intestines of the rabbit, the following can be noted: *Eubacteriales, Pseudomonadales, Bacteroidales, Caryophanales, Enterobacteriales*, of the minor ones – *Coriobacteriales, Actinomycetales, Lactobacillales* (Table 2).

A similar pattern was observed as for the previously described phyla. It was revealed that the largest relative amount in both chyme and intestinal mucosa in animals of both groups was accounted for by bacteria belonging to the order *Eubacteriales*. Their proportion varied from 26.0 to 44.1%, in rabbits of the experimental group of

Table 1. Representation of some phyla of bacteria in the intestinal microbiome of rabbits, %

Top phylum of bacteria	*Bacillota*	*Pseudomonadota*	*Bacteroidota*	*Actinomycetota*	*Verrucomicrobiota*	*Patescibacteria*
Intestinal chyme						
Proportion in rabbits of the control group, %	35.9±11.3	17.2±8.7	3.2±1.2	1.5±0.4	1.4±0.9	0.1±0.1
Proportion of rabbits in the experimental group, %	49.9±12.3	13.6±9.4	3.1±0.9	2.8±1.1	1.9±0.8	0.2±0.1
Intestinal mucosa						
Proportion in rabbits of the control group, %	40.8±9.8	19.9±9.6	3.3±0.9	1.7±0.5	1.8±0.8	0.1±0.1
Proportion of rabbits in the experimental group, %	55.4±9.5	14.7±6.8	3.8±0.8	1.9±0.6	1.1±0.3	0.5±0.2

Table 2. Representation of some bacterial orders in the intestinal microbiome of rabbits, %

Orders in the intestinal microbiome	*Eubacteriales*	*Pseudomonadales*	*Bacteroidales*	*Caryophanales*	*Enterobacteriales*	*Coriobacteriales*	*Actinomycetales*	*Lactobacillales*
Intestinal chyme								
Proportion in rabbits of the control group, %	26.0±9.8	5.9±2.9	3.1±1.2	1.9±1.5	1.0±0.3	0.5±0.2	0.4±0.2	0.1±0.1
Proportion of rabbits in the experimental group, %	38.0±10.7	4.4±2.5	2.9±0.9	1.5±1.2	1.5±0.9	0.3±0.1	1.9±1.0	0.2±0.1
Intestinal mucosa								
Proportion in rabbits of the control group, %	29.6±9.6	6.8±9.9	3.1±1.1	3.4±1.8	0.9±0.3	0.3±0.1	0.8±0.3	0.1±0.1
Proportion of rabbits in the experimental group, %	44.1±9.5	4.8±1.7	3.8±1.1	2.9±2.2	3.4±2.2	0.4±0.1	1.1±0.5	0.12±0.03

bacteria of this order there were more both in the intestinal chyme (by 12.0 pp.) and in the mucous membrane (by 14.5 pp.). The *Pseudomonadales* order was in second place in terms of representation, the proportion of bacteria of this taxonomic group ranged from 4.4 up to 6.8%. There were more bacteria of this order both in the chyme (by 1.5 percentage points) and in the intestinal mucosa (by 2.0 percentage points) of rabbits of the control group. The proportions of the other groups differed only slightly between the studied groups .To analyze the representation of various types of microorganisms in the intestines of rabbits, we created a composite bar chart (Figure 1, Figure 2) and Table 3.

The dominant species is *Campylobacter_D cuniculorum* (family *Campylobacteraceae*, type *Pseudomonadota*), and its proportion is higher in the microbial community of the intestines of rabbits of the control group by 1.5-4.5 percentage points than in animals of the experimental group. Of the minor genera, which are also more numerous in the control group (the difference in representation is 0.2-1.1 percentage points), *Acinetobacter* (family *Moraxellaceae*, type *Pseudomonadota*) should be noted. Two taxonomic units are contained in approximately the same amount regardless of taking a probiotic drug, their proportion ranges from 1.1 to 2.5%, these are *Psychrobacter faecalis* (family *Moraxellaceae*, type *Pseudomonadota*) and *Akkermansia* (family *Akkermansiaceae*, type *Verrucomicrobiota*). The proportion of three taxonomic units from among the dominant and six from among the minor ones increases in the microbial communities of the intestines of rabbits of the experimental group, this is

Faecousia (family *Oscillospiraceae_88309*, type *Bacillota*)
Lachnospiraceae (type *Bacillota*)
Oscillospiraceae_88309 (*Bacillota* type)
Cryptobacteroides (family *UBA932*, type *Bacteroidota*)
Oscillospiraceae (synonym of *Acutalibacteraceae*) (type *Bacillota*)
Escherichia_710834 (family *Enterobacteriaceae*, type *Pseudomonadota*)
Oscillospiraceae (synonym of *Ruminococcaceae*) (type *Bacillota*)
Gemmiger_A_73129 (family *Oscillospiraceae*, type *Bacillota*)

The Shannon index was calculated for microbial communities of the intestinal mucosa and chyme of control and experimental rabbits (Figure 3).

Biodiversity was higher in the experimental group (average values of the Shannon index 4.0 and 4.5) than in the control group (3.5 and 4.0), and in the mucous membrane higher than in the chyme by about 11% although these differences were not statistically significant. The minimum value of the Shannon index was observed in one

Table 3. Average values of the proportion of the main taxonomic units of intestinal microorganisms of rabbits by groups, %

Phylum	Order	Family	Genus	Species	Share in chyme, %		Share in the intestinal mucosa, %	
					Control	Experience	Control	Experience
Pseudomonadota (synonym *Campylobacteriota*)	*Campylobacterales*	*Campylobacteraceae*	*Campylobacter_D*	*Campylobacter_D cuniculorum*	8.9±8.9	7.4±7.7	9.5±9.5	5.0±5.0
Bacillota_A	*Eubacteriales* (synonym *Oscillospirales*)	*Oscillospiraceae_88309*	*Faecousia*	*NA*	4.2±2.2	7.2±2.6	5.7±2.9	7.0±2.6
Bacillota_A	*Eubacteriales* (synonym *Lachnospirales*)	*Lachnospiraceae*	*NA*	*NA*	3.2±1.9	5.4±2.1	3.9±1.6	7.1±2.4
Pseudomonadota	*Pseudomonadales_660879*	*Moraxellaceae*	*Psychrobacter*	*Psychrobacter faecalis*	2.1±1.2	2.4±1.5	2.4±1.2	2.5±1.1
Bacillota_A	*Eubacteriales* (synonym *Oscillospirales*)	*Oscillospiraceae_88309*	*NA*	*NA*	2.0±0.7	3.3±1.0	1.6±0.6	3.1±0.9
Verrucomicrobiota	*Verrucomicrobiales*	*Akkermansiaceae*	*Akkermansia*	*NA*	1.4±0,9	1.8±0.9	1.3±0.7	1.1±0.4
Bacillota_A	*Eubacteriales* (synonym *Oscillospirales*)	*Oscillospiraceae_88309*	*Faecousia*	*Faecousia sp000434635*	1.3±0.7	1.6±0.7	1.2±0.6	1.8±0.6
Bacillota_D	*Caryophanales* (synonym *Bacillales_A*)	*Caryophanaceae* (synonym *Planococcaceae*)	*Lysinibacillus_304693*	*NA*	1.2±1.1	0.2±0.2	1.6±1.3	0.4±0.3
Bacillota_A	*Eubacteriales* (synonym *Oscillospirales*)	*Oscillospiraceae* (synonym *Ruminococcaceae*)	*Ruminococcus_D*	*NA*	1.1±0.6	1.7±0.5	1.3±0.5	1.8±0.5
Bacillota_A	*TANB77*	*CAG-508*	*CAG-269*	*CAG-269 sp000431335*	1.1±0.5	1.2±0.5	1.0±0.3	1.3±0.4
Bacillota_A	*Eubacteriales* (synonym *Christensenellales*)	*Borkfalkiaceae*	*Borkfalkia*	*NA*	0.9±0.4	0.8±0.3	0.8±0.3	1.4±0.4
Bacteroidota	*Bacteroidales*	*UBA932*	*Cryptobacteroides*	*NA*	0.8±0.6	1.4±0.5	0.8±0.5	1.8±0.6
Bacillota_D	*Erysipelotrichales*	*Erysipelotrichaceae*	*Faecalibaculum*	*NA*	0.8±0.4	0.4±0.1	1.2±0.8	0.5±0.2
Bacillota_A	*TANB77*	*CAG-508*	*NA*	*NA*	0.8±0.3	0.8±0.5	0.8±0.3	0.6±0.3
Bacillota_A	*TANB77*	*CAG-508*	*UMGS1994*	*UMGS1994 sp900553945*	0.7±0.3	1.1±0.5	0.7±0.2	0.8±0.3

Table 3. Average values of the proportion of the main taxonomic units of intestinal microorganisms of rabbits by groups, % (continued)

Phylum	Order	Family	Genus	Species	Share in chyme, %		Share in the intestinal mucosa, %	
					Control	Experience	Control	Experience
Bacillota_A	*Eubacteriales* (synonym *Oscillospirales*)	*Oscillospiraceae* (synonym *Ruminococcaceae*)	*Ruminiclostridium_E*	*Ruminiclostridium_E siraeum*	0.7±0.3	1.1±0.4	0.7±0.3	1.2±0.4
Bacillota_A	*Eubacteriales* (synonym *Oscillospirales*)	*Oscillospiraceae* (synonym *Acutalibacteraceae*)	*NA*	*NA*	0.6±0.5	2.3±1.2	0.8±0.5	3.4±1.4
Pseudomonadota	*Pseudomonadales_660879*	*Moraxellaceae*	*Psychrobacter*	*Psychrobacter sanguinis_A*	0.6±0.5	0.3±0.3	0.7±0.5	0.2±0.1
Pseudomonadota	*Pseudomonadales_650611*	*Pseudomonadaceae*	*Pseudomonas_E_647464*	*NA*	0.6±0.3	0.7±0.6	0.5±0.4	0.9±0.7
Bacillota_A	*Pseudomonadales*	*Oscillospiraceae_88309*	*Limivicinus*	*Limivicinus sp002320035*	0.5±0.2	0.4±0.3	0.5±0.2	0.4±0.2
Pseudomonadota	*Enterobacterales_A_737866*	*Enterobacteriaceae_A*	*Escherichia_710834*	*NA*	0.5±0.2	1.2±1.0	0.5±0.2	2.8±2.3
Bacillota_A	*NA*	*NA*	*NA*	*NA*	0.5±0.3	0.6±0.2	0.3±0.1	0.4±0.1
Bacillota_A	*Eubacteriales* (synonym *Christensenellales*)	*CAG-74*	*NA*	*NA*	0.5±0.2	0.6±0.2	0.7±0.2	0.7±0.2
Bacillota_A	*Eubacteriales* (synonym *Oscillospirales*)	*Oscillospiraceae* (synonym *Ruminococcaceae*)	*HUN007*	*NA*	0.5±0.3	0.5±0.2	0.3±0.3	0.7±0.3
Bacillota_D	*Acholeplasmatales*	*Anaeroplasmataceae*	*Pelethenecus*	*Pelethenecus faecipullorum*	0.5±0.2	0.5±0.4	0.3±0.1	0.4±0.1
Bacillota_A	*Eubacteriales* (synonym *Oscillospirales*)	*Oscillospiraceae* (synonym *Ruminococcaceae*)	*NA*	*NA*	0.5±0.2	1.0±0.4	0.7±0.2	0.9±0.2
Bacteroidota	*Bacteroidales*	*Bacteroidaceae*	*Bacteroides_H*	*Bacteroides_H massiliensis*	0.4±0.3	0.3±0.3	0.3±0.2	0.2±0.2
Bacillota_A	*Eubacteriales* (synonym *Oscillospirales*)	*Oscillospiraceae* (synonym *Ruminococcaceae*)	*Gemmiger_A_73129*	*NA*	0.4±0.2	1.1±0.4	0.8±0.4	1.3±0.4
Bacillota_A	*TANB77*	*CAG-508*	*CAG-793*	*NA*	0.4±0.3	2.0±1.0	0.4±0.2	2.1±1.0
Pseudomonadota	*Pseudomonadales_660879*	*Moraxellaceae*	*Acinetobacter*	*NA*	0.4±0.3	0.2±0.2	1.4±1.4	0.3±0.1

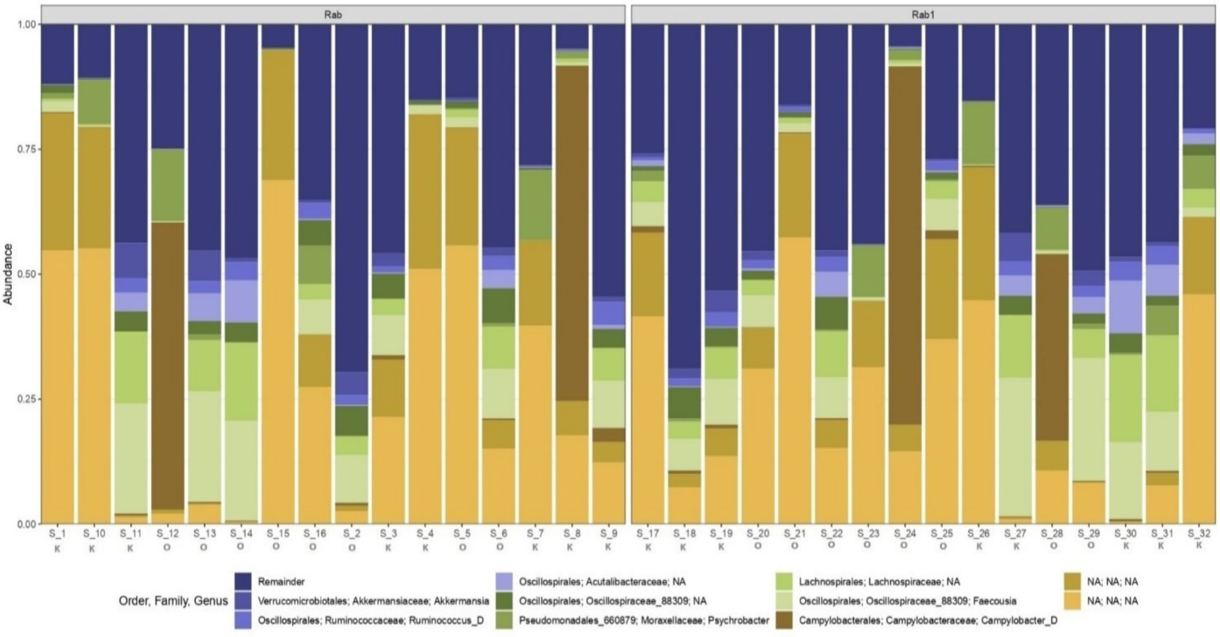

Figure 1. Representation of various taxonomic groups of microorganisms in the intestines of rabbits (samples S_1, S_3, S_4, S_7, S_8, S_9, S_10, S_11 – control chyme; samples S_2, S_5, S_6, S_12, S_13, S_14, S_15, S_16 – experiment chyme; samples S_17, S_19, S_20, S_23, S_24, S_25, S_26, S_27 – control mucosa; samples S_18, S_21, S_22, S_28, S_29, S_30, S_31, S_32 – experiment mucosa)

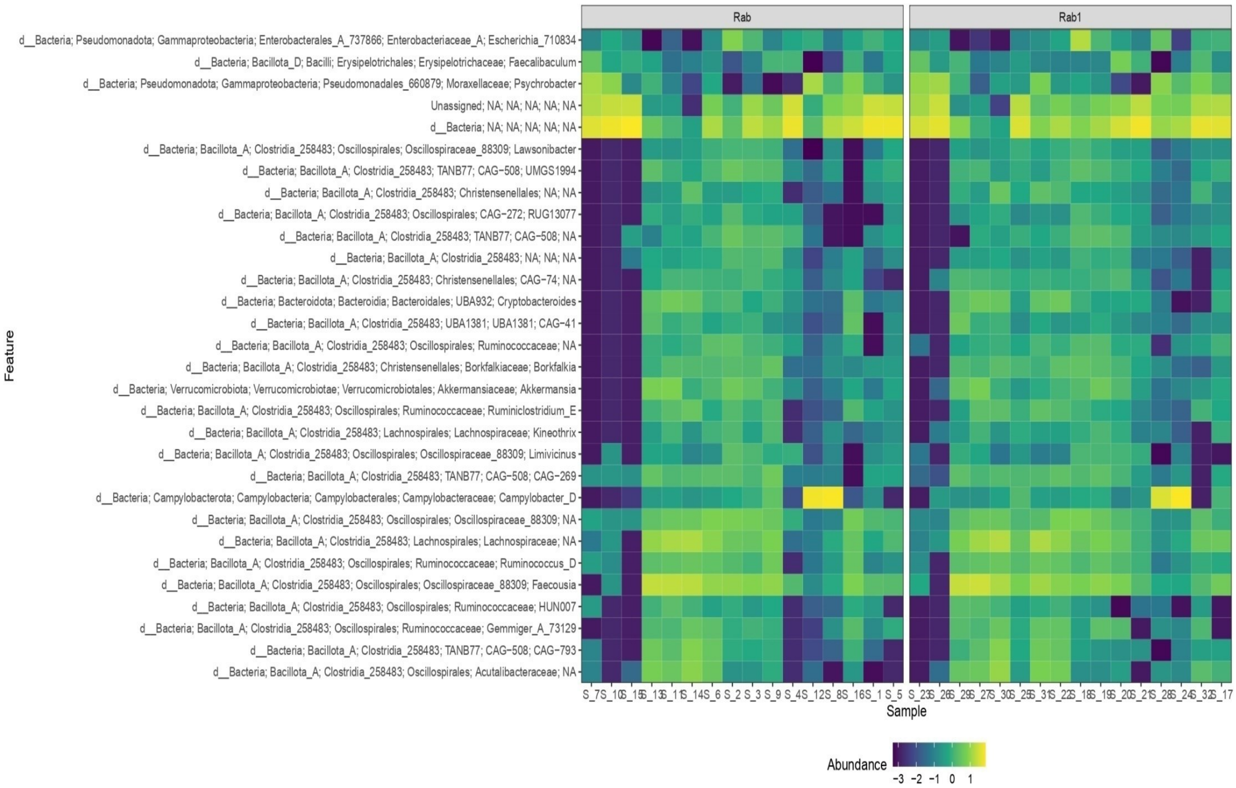

Figure 2. Heat map (samples S_1, S_3, S_4, S_7, S_8, S_9, S_10, S_11 – control chyme; samples S_2, S_5, S_6, S_12, S_13, S_14, S_15, S_16 – experiment chyme; samples S_17, S_19, S_20, S_23, S_24, S_25, S_26, S_27 – control mucous; samples S_18, S_21, S_22, S_28, S_29, S_30, S_31, S_32 – experiment mucous

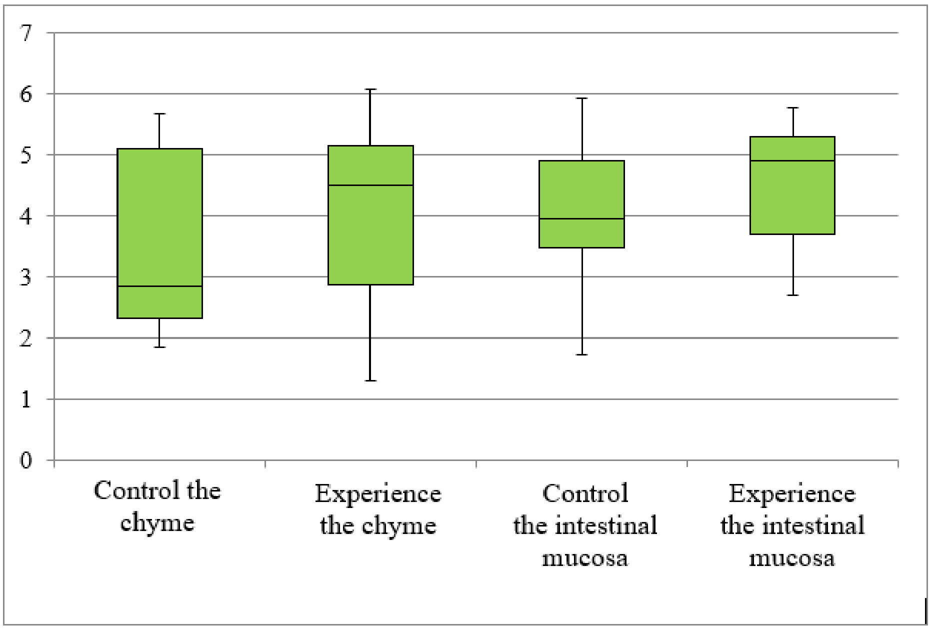

Figure 3. Alpha diversity (Shannon index) of microbial communities of the intestinal mucosa and chyme of control and experimental rabbits.

of the rabbit chyme samples of the experimental group (1.31), in the other groups the minimum value was in the range from 1.72 to 2.70. The maximum values of the Shannon index in all groups were close to 6.0, fluctuations ranged from 5.67 to 6.07. According to the results of statistical data processing using the Kruskal-Wallis criterion, the reliability of differences between the parameters of the experimental and control groups was established (P>0.999). According to the results of the dispersion analysis, the individual characteristics of individuals have the greatest influence on the share of dominant OTUs. The greatest share of the influence of the "use of probiotic" factor was found for the *Lachnospiraceae* group (6.6%).

DISCUSSION

A number of works report studies into the composition of intestinal microbiomes of rabbits, which share one common finding, namely, the predominance of the phylum *Bacillota* (old name *Firmicutes*) in all the studied samples. This type is classified as the most effective cellulose degrader, playing a fundamental role in rabbit digestion (Cotozzolo *et al.*, 2022; Cremonesi *et al.*, 2022). Below we present the results of studies, which, similarly to our findings, distinguish *Bacillota* (*Firmicutes*) as the predominant type.

Narkevich *et al.* (2022) studied the composition of microorganisms of blind processes of the intestine of rabbits under the influence of a complex feed additive and identified 12 phylum of the Bacteria kingdom, among which representatives of the phylum *Firmicutes* (correct name *Bacillota*) dominated in number (80.2±6.2% in group I, 78.2±7.4% in group II), phylum *Bacteroidetes* (corresponding name *Bacteroidota*) turned out to be the second most common in the intestines of rabbits (13.3±1.2% in group I, 12.3±1.8% in group II). In our studies, this phylum ranks third in significance. In the experimental group, compared with the control group, an increase in the number of phylum *Verrucomicrobiota, Actinobacteriota* (now *Actinomycetota*), *Patescibacteria, Proteobacteria* (now *Pseudomonadota*), *Desulfobacterota* (corresponding name *Pseudomonadota*) by 1.3-2.6 times was observed, along with a decrease in the representation of the phylum *Campilobacterota* (corresponding name *Pseudomonadota*) by 4.8 times ($p \leq 0.05$) (Narkevich *et al.*, 2022). In our study, the proportion of *Pseudomonadota* type, on the contrary, was higher in rabbits of the control group.

Animals fed on a high-fiber diet showed a different pattern of bacteria. Within the *Firmicu-*

tes (*Bacillota*) phylum, several taxa, including the genera *Ruminococcus*, *Clostridium* and *Butyrivibrio*, as well as the family *Erysipelotrichaceae*, were positively associated with a higher fiber content (Arrazuria *et al.*, 2016). In our study, from the listed bacteria, only the *Ruminococcus* genus is represented, and their proportion is higher in the experimental group. Similarly, Arrazuria *et al.* (2016) noted that representatives of the *Proteobacteria* type were quite numerous. Examples include the family *Enterobacteriaceae* (class *Gammaproteobacteria*); the family *Comamonadaceae*; the order *Burkholderiales* (class *Betaproteobacteria*); and the genus *Bilophila* (class *Deltaproteobacteria*). *Bacterial* lines belonging to the *Actinobacteria* type (genus *Adlercreutzia*) and the *Tenericutes* type (genus *Anaeroplasma* and family ML615J-28) were also more numerous in the high-fiber group. In our study, only the *Enterobacteriaceae* family is found among the listed ones, and its representatives are more numerous in the experimental group.

An analysis of the composition of the microbial community of rabbit intestines, conducted by Ye *et al.* (2023), he showed that the dominant types of intestinal microbiota in all samples were also *Firmicutes* (*Bacillota*) (53.66%), *Bacteroidetes* (*Bacteroidota*) (27.05%), *Verrucomicrobiota* (10.11%), *Proteobacteria* (*Pseudomonadota*) (1.56%), *Actinobacteria* (*Actinomycetota*) (1.24%) and *Desulfobacterota* (1.07%). In our study, four of the listed five phylum were represented, only *Desulfobacterota* was missing. The authors also note that the relative abundance of these dominant types varied in different seasons. The relative abundance of *Firmicutes* (*Bacillota*) increased significantly from summer to winter. *Verrucomicrobiota* accounted for significantly higher proportions in winter than in summer. Changes in the relative abundance of *Proteobacteria, Actinobacteriota* and *Desulfobacterota* were consistent and had significantly higher values in summer than in winter. At the genus level, *Akkermansia, Oscillospiraceae NK4A214* group, *Christensenellaceae R-7* group, *Ruminococcus, Alistipes, Oscillospiraceae V9D2013* group, *Monoglobus*, *Bacteroides* and *Eubacterium siraeum* group were the nine dominant genera (Ye *et al.*, 2023).

Chen *et al.* (2019) reported, the types *Firmicutes* (*Bacillota*) and *Bacteroidetes* (*Bacteroidota*) with observed frequencies of 69.8% and 17.9%, respectively, prevailed among taxonomically annotated OTE. *Actinobacteria* (*Actinomycetota*) (3.4%), *Desulfovibrionales* (1.2%), *Verrucomicrobia* (*Verrucomicrobiales*) (Chen *et al.*, 2019) were also present, i.e., again, a coincidence was observed for four out of five phyla.

The results of Liu *et al.* (2022) confirmed the dominance of the three most common phylum in studies, but noted their different ratios when changing the diet, namely when adding alfalfa flour *Firmicutes* (*Bacillota*) (73.22%), *Bacteroidetes* (14.54%) and *Proteobacteria* (5.40%); peanut vine – *Firmicutes* (75.29%), *Bacteroidetes* (10.73%) and *Proteobacteria* (5.19%); beet pulp *Firmicutes* (36.14%), *Bacteroidetes* (23.50%) and *Proteobacteria* (34.46%). The authors note that representatives of the *Ruminococcaceae* can break down cellulose and hemicellulose in feed, produce butyrate by fermentation of complex indigestible polysaccharides and play a key role in maintaining intestinal health (Liu *et al.*, 2022). In our study, there were more representatives of this family in rabbits of the experimental group.

The results of the Cremonesi *et al.* (2022) confirmed the predominance of *Firmicutes* (*Bacillota*) in all anatomical tracts in both experimental groups of rabbits. Bacteroidetes was the second most common type, especially in the large intestine (caecum and colon). This type, which does not differ significantly between the two groups and along the digestive tract, is known for its role in stimulating lymphoid tissue associated with the intestine. The families *Ruminococcaceae* and *Lachnospiraceae* were present in all anatomical parts. *Ruminococcaceae* are usually predominant in healthy rabbits, while *Lachnospiraceae* is known to be associated with reduced mortality. These two families seem to play an important role in the digestion of fiber, in particular peptose and cellulose, and are significant producers of short-chain fatty acids (Cremonesi *et al.*, 2022).

Taxonomic identification at the phylum level has shown that the dominant phylum in studies is Wlazło. *et al.* (2021) were also *Firmicutes* (*Bacillota*). Their share was more than 67% of all identified microorganisms. Actinobacteriota was the second most common bacterial phylum in samples of the contents of the caecum. Microorganisms of the *Bacteroidota* phylum, consisting of three large classes of Gram-negative bacteria, were present in similar proportions in the control group and experimental groups. Bacteria of the *Lachnospiraceae* family were present in all animal groups in similar proportions. The occurrence of microorganisms of the *Akkermansiaceae* family was noted only in the material collected from the control group (Wlazło *et al.*, 2021).

Thus, in all the studies presented in the discussion, similar to our findings, *Firmicutes* (*Bacillota*) rank first among the dominant types of bacteria in the intestines of rabbits. The phylum *Bacteroidetes* (*Bacteroidota*) and *Proteobacteria* (*Pseudomonadota*) rank second and third in most studies, with some exceptions. In recent years, researchers of the normal intestinal microbiota tend to believe that it is difficult to isolate the phylogenetic core of the microbiota (core microbiota) – the dominant microorganisms found in at least 50% of individuals. Instead, the concept of a "philometabolic microbiota core" is proposed, which distinguishes groups of microorganisms with the same enzymatic functions (Sitkin *et al.*, 2015; Voroshilina *et al.*, 2023;).

In addition, investigations into the intestinal microbiome of rabbits as model organisms in the study of diseases occurring in humans are extremely interesting (Arrazuria *et al.*, 2016; Chen *et al.*, 2019; Curone *et al.*, 2022). Thus, Cheng *et al.* (2024) noted that there are four main types (*Firmicutes* (corresponding name *Bacillota*), *Proteobacteria* (corresponding name *Pseudomonadota*), *Bacteroidetes* (corresponding name *Bacteroidota*) and *Actinobacteria* (corresponding name *Actinomycetota*)), which compose 80% of the total intestinal microbiome of rabbits. The number of *Bacteroidetes* (corr. the name *Bacteroidota*) was lower in the group with intervertebral disc degeneration than in the control group, while the number of *Firmicutes*, *Proteobacteria* and *Actinobacteria* was higher (Cheng *et al.*, 2024).

In particular, Arrazuria *et al.* (2016) studied the intestinal microbiome of rabbits whose gastrointestinal tract was infected with *Mycobacterium avium* (the causative agent of paratuberculosis). With an increase in fiber in the diet of these rabbits, an increase in the number of *Bacteroidota* (synonym *Bacteroidetes*) was noted, which prevented the development of infection (Arrazuria *et al.*, 2016). Cotozzolo *et al.* (2022) indicated, *Firmicutes* were the dominant phylum in the departments of the gastrointestinal tract of rabbits (corr. the name *Bacillota*). Bacteria belonging to this type play a great role in digestive processes, as they effectively dicompose cellulose. *Firmicutes* (*Bacillota*) is the predominant phylum in animals with a single-chamber stomach, which is also typical of humans (Cotozzolo *et al.*, 2022). There are also studies showing that mycotoxin zearalenone has no effect on the number of *Bacteroidetes* and *Firmicutes*, while significantly increasing the proportion of the type of *Proteobacteria* (corresponding name *Pseudomonadota*) of the caecum. Although *Proteobacteria*, a minor component in the microbial community of the hindgut in rabbits, accounted for only 1.8% in the caecum of rabbits, it included many pathogenic bacteria such as *Escherichia coli*, *Salmonella*, and *Vibrio cholera*; *Thus*, an increased content of *Proteobacteria* was associated with severe intestinal inflammation such as intestinal disease and necrotizing enterocolitis, which was a potential diagnostic microbial signature of epithelial dysfunction (Li *et al.*, 2018).

CONCLUSION

When studying the composition of microorganisms in the intestines of rabbits, five dominant phylum of the *Bacteria* were identified, among which representatives of the *Bacillota* prevailed in number. The predominance of this type contributes to effective digestion in rabbits. In the control group, the superiority of the *Pseudomonadota* phylum was observed, the supplement used reduces the proportion of conditionally pathogenic microorganisms. Rabbits of the experimental group, tended to have a higher content of bacteria of the families *Oscillospiraceae* and *Lachnospiraceae*, which is typical of healthy animals. The level of biological diversity of intestinal microbes was higher in the experimental group.

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