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HABITAT AS A DETERMINING FACTOR FOR THE REINDEER RUMEN MICROBIOME FORMATION IN RUSSIAN ARCTIC

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Abstract

Reindeer (*Rangifer tarandus*) is geographically isolated from other subspecies of the ruminant family *Cervidae*. It is known that belonging to certain environmental conditions can have a significant impact on the composition of the ruminant rumen microbiome. With the use of molecular-biological analysis, we studied for the first time the patterns of formation of the reindeer's rumen microbial communities for the *Rangifer tarandus* living in different natural and climatic zones of the Russian Federation. The purpose of the study is to assess the regional features of the reindeer's rumen microbiome into the different Arctic regions of Russia using T-RFLP analysis and quantitative PCR. It was made a comparative analysis of the influence of a number of factors on the composition of the reindeer rumen microbiome, incl. gender and age peculiarities, regional habitat conditions and feeding ration features. Samples of the rumen content were collected in the summer-autumn period of 2017 from 58 individuals ($n \geq 3$ from each age group) in the Yamalo-Nenets Autonomous District (AO), Nenets Autonomous District and the Murmansk region. The total number of bacteria, archaea, and fungi of the *Neocallimastigales* was analyzed by quantitative PCR, and the composition of the bacterial community by T-RFLP (terminal restriction fragment polymorphism) method. The main determinant of all components of the microbial community of the reindeer's rumen is regional habitat conditions, which, apparently, is due to differences in the composition of the pasture diet and the epizootic situation in the herd. The smallest similarity with other regions was found for samples from the Murmansk region, which is probably due to differences in reindeer pasture ration in this region, i.e. the differences in the composition of vegetation and lower nutritional values. Gender and age differences of animals were less significant though made a certain contribution to the ratio of microorganisms in the rumen. The clearest differences in the rumen microbiota were detected between groups of animals under 2 years of age and older than 2 years. In general, significant changes in the representation of a taxa number were noted in connection with the nutritional value of pasture ration. A statistically significant relationship was established between the level of fiber in grazing feed and members of the families *Veillonellaceae* ($r = -0.75$), *Nostocaceae* ($r = 0.52$), *Rivulariaceae* ($r = -0.88$), etc. in addition to traditionally associated with the processes of cellulose degradation bacteria. There is no significant correlation between the content of conditionally pathogenic microorganisms from the *Fusobacteria*, *Tenericutes* (*Mycoplasmataceae*), *Proteobacteria* (*Enterobacteri-*

aceae, *Campylobacteraceae*) and the nutritional value of feeds and other groups of microorganisms, which indicates the need for more research in this direction. The obtained data clarify aspects of the interaction and cohabitation of symbionts in the complex-component system of the reindeer rumen, which is characterized by the diversity of sources of plant polysaccharides and the variety of enzymes produced by microorganisms.

Keywords: *Rangifer tarandus*, T-RFLP analysis, quantitative PCR, rumen microbiome, reindeer, Russian Arctic

Reindeer (*Rangifer tarandus*) occupies a special place among herbivorous ruminants. The geographical isolation of this species from other subspecies of the *Cervidae* family [1] implies not only anatomical and morphological features in the structure of the digestive system but also the formation of specific rumen microbial communities [2-4]. Interest in the study of complex symbiotic communities of the reindeer rumen is also associated with the study of the adaptative features of the organism of these animals to adverse factors, in particular, the ability to effectively use the scarce plant resources of the tundra, forest-tundra and northern taiga areas [5]. The formation of microbial communities in the rumen of ruminant animals is influenced by a complex of interdependent factors: a variety of sources of plant fiber, interactions between microorganisms of various groups, taxonomic diversity of enzyme systems, the molecular structure of enzymes, physiology of microorganisms, as well as environmental aspects [6-8]. In this regard, the adaptive facilities of reindeer should be considered taking into account the conditions of their habitat, the availability of nutritional resources, and other factors [9].

Relation to certain environmental conditions affects the composition of the rumen microbiota in ruminants [10]. However, such studies focus mainly on the analysis of the representation of individual groups of microorganisms, for example, cellulolytic ones. Thus, a significant difference in the rumen microbial communities is shown for two geographically separated subspecies of the reindeer of Norway — *Rangifer tarandus* (Eurasian tundra reindeer, *R. tarandus tarandus*) of the continent part of the country and *R. tarandus platyrhynchus* (Svalbard reindeer, *R. tarandus platyrhynchus*) of the high Arctic Spitsbergen archipelago, located between Norway and the North Pole. At the same time, the representatives of *R. tarandus platyrhynchus*, which during the 8-10-month winter period are forced to eat food with a high lignin content, have a 6-14 times higher content of cellulolytic bacteria than *R. tarandus tarandus* [1]. However, the bacteria associated with plant feed fermentation processes were similar in species composition: *Peptostreptococcus anaerobius*, *Lachnospira multiparus*, *Butyrivibrio fibrisolvens*, *Eubacterium ruminantium*, *Selenomonas ruminantium*, *Fibrobacter succinogenes*, *Eubacterium pyruvovorans* and *Fusocillus* sp. Moreover, according to Aagnes et al. [11], the subspecies of *R. tarandus tarandus* has serious limitations in the digestion of fiber.

Molecular genetic technologies, such as T-RFLP analysis (terminal restriction fragment length polymorphism) and NGS sequencing (next-generation sequencing), provide new possibilities in studying the structure of microbial communities, which allows giving a deep characterization of their biodiversity, revealing not only dominant taxa but also other components, including uncultured microorganisms [12-15]. However, there are few reports to date of the reindeer rumen microbiome [16, 17].

In the work, using molecular analysis, it was for the first time shown that one of the key factors affecting the formation of the reindeer (*Rangifer tarandus*) rumen microbiome was the habitat, which is probably related to the characteristics of the feed base of animals.

The purpose of the work was to assess the regional and age-gender features of the rumen microbiome of reindeer living in various climatic zones of the Russian Federation using T-RFLP analysis and quantitative PCR.

Techniques. The object of the study was 58 reindeer animals (*Rangifer tarandus*) of the Nenets breed, calves (4-8-month old) and adult animals (males and females). The content of the reindeer rumen was samples in the summer-autumn period of 2017 ($n \geq 3$ from each age group) in the Yamalo-Nenets Autonomous District (Kharp village, forest-tundra natural and climatic zone), the Nenets Autonomous District (Nelmin-Nos village, tundra natural and climatic zone) and the Murmansk Province (station Loparskaya, tundra natural and climatic zone).

The total number of bacteria, archaea, and fungi of the *Neocallimastigales* class in the rumen content was analyzed by quantitative (real-time) polymerase chain reaction (qRT-PCR) using a kit for real-time PCR (RT-PCR) with intercalating colorant EVA Green (ZAO Syntol, Russia) and primers F — 5′-ACTCCTACGGGAGGCA-GCAG-3′, R — 5′-ATTACCGCGGCTGCTGG-3′ (bacteria), F — 5′-AG-GAATTGGCGGGGGAGCAC-3′, R − 5′-GCCATGC-ACCWCCTCT-3′ (archaea), F — 5′-GCACTTCATTGTGTGTACTG-3′, R — 5′-GGATGAAACTCGTTGACTTC-3′ (fungi) on the DT Lite-4 detection amplifier (NPO DNA-Technology, Russia) in the following mode: the first cycle − 3 min at 95 °C (1 repetition); second cycle − 13 sec at 95 °C, 13 sec at 57 °C, 30 sec at 72 °C (40 repetitions).

The rumen bacterial community was studied using the T-RFLP method [18]. DNA was extracted from the samples with the Genomic DNA Purification Kit (Fermentas, Lithuania) in accordance with the manufacturer's instructions. PCR (a Verity amplifier, Life Technologies, USA) was conducted with 63F bacterial primers (5′-CAGGCCTAACACATGCAAGTC-3′) with a fluorophore label at the 5′-end (WellRed D4 fluorophore, Beckman Coulter, USA) and 1492R (5′-TACGGHTACCTTGTTACGACTT-3′), amplifying the 16S pRNA gene fragment from the 63rd to the 1492th position, the first cycle — 3 min at 95 °C (1 repetition); the second cycle — 30 s at 95 °C, 40 s at 55 °C, 60 s at 72 °C (35 repetitions); the third cycle — 5 min at 72 °C. The final concentration of the total obtained DNA was determined by a Qubit 2 fluorimeter (Invitrogen, USA) using the Qubit dsDNA BR Assay Kits (Invitrogen, USA) according to the manufacturer's recommendations.

Amplicons of the 16S rRNA gene fragment, marked by a fluorescent label, were purified in accordance with the standard procedure [19]. Then, 30-50 ng of DNA were treated with restriction enzymes MspI, HaeIII, HhaI (Fermentas, Lithuania) for 2 h at 37 °C. Restriction products were precipitated with ethanol, then 0.2 μl of Size Standart-600 molecular weight marker (Beckman Coulter, USA) and 10 μl of Sample Loading Solution formamide (Beckman Coulter, USA) were added. The analysis was performed using a CEQ 8000 sequencer (Beckman Coulter, USA); the error of CEQ 8000 is not more than 5 %. The height of the peaks and their area were measured with the Fragment Analysis software (Beckman Coulter, USA), as a result of which subtypes (phylotypes) were identified and their relative amount in the microbial community was calculated. The taxonomic affiliation of bacteria was determined using the database (http://mica.ibest.uidaho.edu/trflp.php).

The samples of pasture vegetation, which formed the basis of the diet of reindeer, were selected in each region and their botanical description was conducted. In addition, the ratio of various plant species in the diet and their nutritional value were determined.

The results were statistically processed by the method of variance analysis using the Microsoft Excel 2010 software. The mean values (*M*) and standard errors of the mean (±SEM) were determined, and the significance of the differences was evaluated by Student's *t*-test. The calculation of the Pearson correlation coefficients and the assessment of the similarity of bacterial communities by the Principal Component Analysis (PCA) method based on the Bray-Curtis coefficient, which accounts the number and relative abundance of certain taxa, were conducted using the Past program (http://folk.uio.no/ohammer/past/).

Results. Figure 1 shows the regions where the sampling was conducted. The average composition and nutritional value of the summer-autumn pasture diet of reindeer are presented in Table 1.

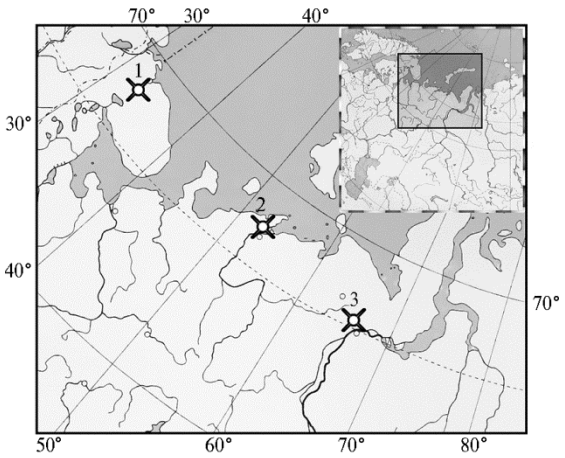

Fig. 1. Regions for selecting the rumen contents of reindeer (*Rangifer tarandus*) of the Nenets breed: 1 — Yamalo-Nenets Autonomous District, 2 — Murmansk Province, 3 — Nenets Autonomous District (2017).

The physiology of reindeer, including the state of rumen microflora, experiences a much lower anthropogenic load than domesticated ruminants have, in particular, bovine cattle. At the same time, the role of the microbial community of the rumen in ruminants in providing nutrition is especially significant, since their diet consists of extremely poor nutritionally valuable plant feeds [20]. It is known that the digestive system of reindeer has unique adaptations for microbiological fermentation of scarce plant resources in natural habitats, which can exert significant selection pressure on the structural and functional organization of the rumen microbiome.

1. Averaged composition and nutritional value of the summer pasture diet of Nenets reindeer (*Rangifer tarandus*) in three zones of Russian Arctic in the summer-autumn period (*M*±SEM, 2017)

Indicator	Yamalo-Nenets Autonomous District	Murmansk Province	Nenets Autonomous District
Nutritional value			
Soluble carbohydrates (sugars), g/kg	20.80±0.80	13.85±0.52	66.86±3.50
Mass fraction of dry matter, %	76.74±1.60	73.28±1.73	82.04±1.46
Crude fat, g/kg	16.90±0.61	12.39±0.84	15.46±0.54
Crude protein, g/kg	95.90±4.70	54.96±3.10	64.03±3.50
Crude ash, g/kg	36.40±1.10	24.99±2.20	23.95±1.80
Crude fiber, g/kg	142.80±7.30	134.62±6.50	160.55±8.60
Dietary components Компоненты рациона, %			
Cladonia	5	10	10
Nephroma	5	–	–
Betula pendula	5	20	20
Salix borealis	5	20	15
Salix polaris	15	–	–
Vaccinium uliginosum	10	–	5
Betula nana	25	20	20
Perennial grasses	30	30	30

Note. Dashes indicate the absence of a component in the diet.

The rumen of ruminants is inhabited by various groups of symbiotic microorganisms [4-7]. In our studies, in animals from the Yamalo-Nenets Autonomous District, a significantly larger ($p < 0.05$) number of bacteria and fungi

(Chytridiomycetes) was noted compared to animals from other regions (Table 2). In the rumen of reindeer from the Murmansk Region and the Nenets Autonomous District, a significantly larger ($p < 0.05$) counts of archaea was noted compared to animals from the Yamalo-Nenets Autonomous District.

The age and gender differences in animals in terms of the abundance of the main groups of microorganisms appeared to be less significant in comparison to regional features. However, the detected microbiota changes were not the same for animal units from different regions. Thus, in reindeer older than 2 years from the Murmansk Region and the Yamalo-Nenets Autonomous District, a significant increase in the number of bacteria ($p < 0.05$) was observed compared with animal units from the Nenets Autonomous District. Significant changes in the number of Chytridiomycetes in animals from the Murmansk Region and the Nenets Autonomous District were not observed with age, while they were noted in animals from the Yamalo-Nenets Autonomous District.

It can be assumed that the regularities that we found in the change in the number of microorganisms in the reindeer rumen community are interrelated with the structure of the summer pasture diet and its nutritional value, which is consistent with reports of foreign researchers. Thus, Olsen et al. [21] showed a decrease in the number of viable zoospores of *Chytridiomycetes* in winter, which have a wide range of multifunctional polysaccharide enzymes [22-23] in the rumen content of Norwegian reindeer living in natural pastures. Similar results were obtained for methanogenic archaea, the abundance of which decreased in the rumen of *R. tarandus* during the spring season compared to the autumn period [24]. At the same time, the reindeer *R. tarandus platyrhynchus* of the Spitsbergen archipelago showed the absence of significant changes in the rumen of the number of methanogens, bacteria and protozoa due to a change in the composition of the vegetation of natural pastures in the autumn and spring periods [25].

2. Abundance of microorganisms in the rumen community of Nenets reindeer (*Rangifer tarandus*) younger (I) **and older** (II) **than 2 years of age from Russian Arctic regions** (M±SEM, 2017)

Region, age	Bacteria	Archaea	Fungi *Neocallimastigales*
Yamalo-Nenets Autonomous District:			
I	$1.56\times10^{9}\pm5.38\times10^{7}$ [d]	$1.20\times10^{8}\pm3.94\times10^{6}$ [d]	$2.81\times10^{6}\pm8.24\times10^{4}$ [b]
II	$2.58\times10^{9}\pm1.84\times10^{6}$ [c]	$1.42\times10^{8}\pm6.72\times10^{6}$ [d]	$4.53\times10^{6}\pm1.95\times10^{5}$ [a]
Murmansk Province:			
I	$8.10\times10^{8}\pm4.01\times10^{3}$ [e]	$1.03\times10^{9}\pm5.25\times10^{4}$ [b]	$2.07\times10^{5}\pm8.09\times10^{2}$ [c]
II	$1.75\times10^{9}\pm2.87\times10^{8}$ [c. d]	$7.79\times10^{8}\pm2.87\times10^{6}$ [c]	$2.07\times10^{5}\pm8.09\times10^{2}$ [c]
Nenets Autonomous District:			
I	$6.37\times10^{9}\pm4.72\times10^{8}$ [a]	$8.74\times10^{7}\pm6.02\times10^{6}$ [e]	$3.24\times10^{5}\pm9.50\times10^{3}$ [c]
II	$4.41\times10^{9}\pm2.12\times10^{7}$ [b]	$2.15\times10^{9}\pm1.56\times10^{8}$ [a]	$3.39\times10^{5}\pm5.10\times10^{4}$ [c]

[a-e] Differences in indicators without a common upper index are statistically significant at $p < 0.05$.

The analysis of the similarity of the bacterial rumen communities in the examined animals based on the principal component method (Fig. 2) showed that all samples were divided into three main groups corresponding to individual regions, the Murmansk Region, the Yamalo-Nenets and the Nenets Autonomous Districts. The revealed patterns were presumably caused by differences in the composition of the pasture diet and the epizootic situation in the herd, since the meteorological parameters in the regions were similar for the summer-autumn period in which the study was conducted. Interestingly, the age and gender characteristics of animals, determined by the method of principal components, showed a less significant division of samples into subgroups. In general, the most significant differences were detected in the composition of the bacterial rumen community between groups of young animals (up to 2 years old) and adult animal units (over 2 years old) (see Fig. 2). It is worth noting that the au-

thors did not find any significant changes in the composition of the microbial rumen community in females and males.

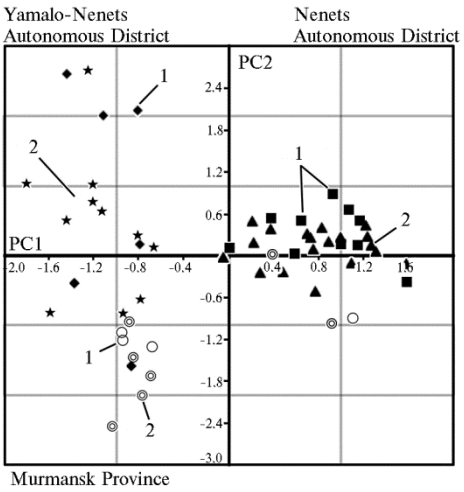

Fig. 2. T-RFLP-based Principal Component Analysis of bacterial rumen community pattern of Nenets reindeer (*Rangifer tarandus*) from Russian Arctic regions: 1 – young animals (up to 2 years), 2 – adult animals (over 2 years old) (2017 год).

Regardless of the region of sampling in the studied animal units of *R. tarandus*, the representatives of the phylum *Firmicutes* were dominant in the rumen content; bacteria of the phyla *Bacteroidetes*, *Proteobacteria*, *Actinobacteria* were less detected, and the proportion of other identified taxa (*Tenericutes*, *Fusobacteria*, *Cyanobacteria*) in the community was minor; a significant number of bacterial sequences could not be identified up to the phylum level.

The greatest interest among researchers in the study of microbial rumen communities of ruminants is associated with microorganisms, the producers of enzymes that are not synthesized by the host organism, primarily cellulolytic ones. Due to the use of various enzymes (cellulases, hemicelluloses, xylanases, glycoside hydrolases, etc.), these microorganisms participate in the degradation of plant biomass with the formation of metabolites, including volatile fatty acids (VFAs): propionate, acetate, butyrate, isobutyrate, valerate, isovalerate and 2-methylbutyric, hexanoic and heptanoic acids [6, 7].

Representatives of the bacterial phyla *Firmicutes* and *Bacteroidetes* comprise the predominant part of the microbial rumen community of the ruminants [26, 27], which suggests an important ecological role of these microorganisms, probably due to their wide metabolic potential, including fermentation of plant polysaccharides. A high proportion of the *Firmicutes*/*Bacteroidetes* phyla, according to a number of authors, is characteristic of various ruminants [28, 29]. Nevertheless, only 33% of *Firmicutes* phylum bacteria were present in the rumen of the North American elk *Alces alces* [29].

It was reported that in reindeer, the bacteria representation of these taxa varies depending on the habitat. In particular, in the subspecies *R. tarandus tarandus* from the continent part of Norway, the representation of *Firmicutes* phylum bacteria (71%) was higher than in the animal units of *R. tarandus platyrhynchus* (55%) living on the Spitsbergen archipelago [1]. The analysis of the rumen microbiota in the studied animal units of *R. tarandus* showed that the smallest percentage of representatives of the phylum *Bacteroidetes* was present in animals from the Nenets Autonomous District, and the largest – in reindeer from the Yamalo-Nenets Autonomous District (Fig. 3). The total share of *Firmicutes* phylum bacteria did not significantly differ in adult animal units from different regions, while in young animals from the Murmansk Region, the number of these microorganisms was significantly lower ($p < 0.05$) than in animal units from the Nenets and the Yamalo-Nenets Autonomous Districts.

The data on the abundance of bacteria involved in fermentation of plant fiber in the rumen of reindeer are consistent with the estimates of nutritional

value of plants from pastures. The composition and nutritional value of multi-component samples repeating the composition of the average summer pasture ration from three regions of the Russian Arctic zone varied. In general, the feed of reindeer was characterized by a high content of crude fiber and a low content of other nutrients. According to the composition of the rumen microbiome, reindeer from the Murmansk Region had the least resemblance to animal units from other regions, which is probably due to the peculiarities of the pasture diet of animals in this region – the composition of vegetation and lower nutritional values (see Table 1).

It was reported that the proportion of the *Firmicutes* and *Bacteroidetes* phyla in the rumen of ruminants depends on the diet type. Thus, the representatives of the phylum *Bacteroidetes* are mainly associated with the presence of easily fermentable carbohydrates (such as starch) and proteins in the diet of animals, while a number of representatives of the phylum *Firmicutes* (bacteria of the genera *Ruminococcus*, *Butyrivibrio*, *Clostridium*, etc.) are associated with the fermentation of plant cellulose [7, 10, 30]. The difference in the enzymatic activity of these microorganisms can also affect the metabolism of the host through the production of VFAs as a result of fermentation of plant polysaccharides.

Interestingly, compared to young animals, adult animals had a wider taxonomic diversity of bacteria associated with fiber enzymes, including the families *Eubacteriaceae*, *Clostridiaceae*, *Lachnospiraceae* of the phylum *Firmicutes* (p 0.05), which indicates an increase in the ability of the microbial community to ferment plant polysaccharides.

We found a statistically significant correlation between the composition of *R. tarandus* rumen microorganisms, involved in the fermentation of plant polysaccharides and nutritional indicators of diets. In particular, the amount of fiber was significantly negatively associated with the presence in the rumen of animals of the families *Bacteroidaceae* ($r = -0.75$, $p < 0.05$), *Rivulariaceae* ($r = -0.88$, $p < 0.05$), *Veillonellaceae* ($r = -0.75$, $p < 0.05$) and significantly positively with the presence of the families *Lachnospiraceae* ($r = 0.89$, $p < 0.05$), *Clostridiaceae* ($r = 0.51$, $p < 0.05$), *Nostocaceae* ($r = 0.52$, $p < 0.05$), *Eubacteriaceae* ($r = 0.46$, $p < 0.05$), and *Prevotellaceae* ($r = 0.56$, $p < 0.05$).

Prevalence of some cellulolytic microorganisms (for example, the families *Clostridiaceae*, *Lachnospiraceae*, *Eubacteriaceae*) was positively interrelated ($p < 0.05$) with the proportion of bacteria from the families *Nostocaceae*, *Enterococcaceae*, *Lactobacillaceae* and negatively ($p < 0.05$) with the representatives of the families *Prevotellaceae*, *Bacteroidaceae*, *Veillonellaceae*, and *Streptococcaceae*. Interestingly, the reindeer, studied by the authors, showed an inverse pattern between the content in the rumen of bacteria utilizing acids of the *Veillonellaceae* family and cellulolytic microorganisms such as *Lachnospiraceae* ($r = -0.60$, $p < 0.05$), *Clostridiaceae* ($r = -0.55$, $p < 0.05$), and *Eubacteriaceae* ($r = -0.60$, $p < 0.05$). Bacteria utilizing acids belong to the physiologically important group of microorganisms for ruminants, since they allow maintaining the necessary acidity in the rumen due to their ability to metabolize acids (including acetic, propionic, butyric, and lactic acids), formed as a result of fermentation of monosaccharides, oligo- and polysaccharides [31].

The patterns we revealed confirm the opinion that during the evolution in the rumen of animals, certain relations were formed between microorganisms, which make it possible to clarify the relationship between the presence of a number of microorganisms in the rumen of reindeer.

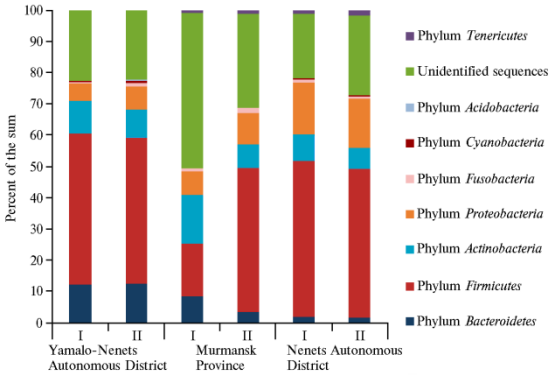

Fig. 3. Bacterial phyla pattern in the rumen of Nenets reindeer (*Rangifer tarandus*) from Russian Arctic regions: 1 — young animals (up to 2 years), 2 — adult animals (over 2 years old) (2017 год).

A significant relationship between the content of potentially pathogenic microorganisms from the phyla *Fusobacteria*, *Tenericutes* (family *Mycoplasmataceae*), *Proteobacteria* (*Enterobacteriaceae*, *Campylobacteraceae* families) and other microorganisms was not found. However, more research isrequired to study deeply this problem. The authors also did not find a direct pattern characterizing age-related changes in the content of potentially pathogenic and pathogenic bacteria in the rumen of *R. tarandus*. Probably, the differences revealed in this case were associated with other factors, i.e. the particularities of the pasture diet and the epizootic situation in the herd.

Thus, our studies showed that in reindeer (*Rangifer tarandus*) of the Nenets breed older than 2 years from the Murmansk Region and the Yamalo-Nenets Autonomous District (but not the Nenets Autonomous District), the number of bacteria increases significantly ($p < 0.05$) compared to calves. Regardless of the region, the *Firmicutes* phylum dominates in the rumen contents; bacteria of the phyla *Bacteroidetes*, *Proteobacteria*, and *Actinobacteria* are less prevalent, and other detected taxa (*Tenericutes*, *Fusobacteria*, *Cyanobacteria*) are minor. Significant changes are identified for a number of taxa of microorganisms in connection with the nutritional value of the pasture diet, depending on the region. A statistically significant relationship is found between the fiber content in pasture feeds and the abundance of the members of families *Veillonellaceae* ($r = -0.75$), *Nostocaceae* ($r = 0.52$), *Rivulariaceae* ($r = -0.88$). For conditionally pathogenic microorganisms from the phyla *Fusobacteria*, *Tenericutes* (family *Mycoplasmataceae*) and *Proteobacteria* (families *Enterobacteriaceae*, *Campylobacteraceae*), such regularities are not observed. In general, the obtained results indicate that habitat conditions are the main factor influencing the microbial community of the reindeer rumen from various regions of the Russian Arctic. Most likely, this is due to the characteristics of feed rations of animals. Other factors (gender, age) have a lesser effect, although they make a certain contribution to the ratio of microorganisms in the rumen. The results of metagenomic analyses (due to their high resolution), allow discovering new patterns and clarifying aspects of the interaction and cohabitation of symbionts in the complex ecosystem of the reindeer rumen of *R. tarandus*.

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