AGRICULTURAL BIOLOGY,
2018, V. 53, № 2, pp. 355-363
(SEL'SKOKHOZYAISTVENNAYA BIOLOGIYA)

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

Northern reindeer herding

UDC 636.294:579.62:579.8

doi: 10.15389/agrobiology.2018.2.35engs
doi: 10.15389/agrobiology.2018.2.355rus

COMPARATIVE ANALYSIS OF RUMEN BACTERIAL COMMUNITY OF YOUNG AND ADULT *Rangifer tarandus* REINDEERS FROM ARCTIC REGIONS OF RUSSIA IN THE SUMMER-AUTUMN PERIOD

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The authors declare no conflict of interests
Acknowledgements:
Supported by a grant from the Russian Science Foundation, the project No. 17-76-20026 for biotechnologies based on fundamental studies of rumen microbiocenosis of the *Rangifer tarandus* from the Russian Arctic
Received December 8, 2017

Abstract

Reindeer husbandry is a strategically important industry in the Arctic regions of Russian Federation due to providing the native population with food stuffs. Observing the characteristics of rumen microorganisms' composition is necessary to deepen the information on the reindeer physiology. In this paper, the results of molecular genetic analysis of the rumen bacterial community composition of young and adult specimen *Rangifer tarandus* individuals from Arctic regions of Russia are presented for the first time. Samples of ruminal contents were collected from 3 animals of each age group in 2017 summer-autumn period in the Yamal-Nenets Autonomous District and the Murmansk Province. The bacterial community composition of the reindeer rumen was analyzed in the laboratory of the «BIOTROF+» company by T-RFLP method (terminal restriction fragment length polymorphism). According to the biodiversity indicators, the Yamal-Nenets Autonomous District reindeer ruminal microorganisms' diversity was significantly higher ($P < 0.05$) than that in the reindeers of Murmansk region. Young reindeers from the Yamalo-Nenets Autonomous District showed lower biodiversity indicators ($P < 0.05$) comparing to adults, whereas in the Murmansk region this was not observed. According to the taxonomic affiliation, it has been established that up to 83.50±5.07 % of the phylotypes belong to four bacterial phylums, the *Firmicutes*, *Bacteroidetes, Actinobacteria* and *Proteobacteria*, while *Tenericutes*, *Fusobacteria*, *Acidobacteria*, *Cyanobacteria* were less frequent. Ruminal microbiome of *Rangifer tarandus* reindeers showed much higher proportion of unidentified bacteria, as well as the *Eubacteriaceae* and *Clostridiaceae* bacteria, as compared to the most studied members of the *Bovidae* family. Note, that several *Eubacteriaceae* and *Clostridiaceae* members are capable of detoxification of usnic acid and other secondary metabolites produced by lichens. During the reindeer ontogenesis, noticeable changes in the ratio of phylotypes and taxonomic groups in rumen microbiota were found. The greatest age changes were noticed in the phylum *Firmicutes* composition. In adult reindeer rumen, the total counts of cellulosolytic bacteria of the *Clostridia* class, especially of the families *Eubacteriaceae*, *Clostridiaceae* and *Lachnospiraceae* potentially capable of hydrolysis of plant carbohydrates with the formation of volatile fatty acids (VFA), were significantly higher than in young group ($P < 0.05$). The inverse pattern was characteristic of bacteria with similar properties from the phylum *Bacteroidetes*, including the genera *Bacteroides*, *Prevotella*. Identification of a significant number of opportunistic and pathogenic microorganisms in the *Rangifer tarandus*

rumen bacterial community, with the dominance of the phylum *Fusobacteria*, families *Campylobacteriaceae* and *Enterobacteriaceae*, is also of interest. Up to date, this issue has been poorly observed. Direct regularity in changing ruminal pathogen profiles in reindeers of different age or from different habitat was not revealed. Perhaps the detected differences in the level of pathogenic and opportunistic microorganisms could be associated with other factors, e.g. specific pasture ration in different regions or the epizootic situation in the herd. Additional research will clarify the issues in question. In general, the obtained results can be used as a basis to develop recommendations for improving the efficiency of animals breeding.

Keywords: rumen microorganisms, molecular-genetic methods, reindeer, *Rangifer tarandus,* Arctic regions

Reindeer (*Rangifer tarandus*) is unique species specifically adapted to life in the context of North and, as such, deer farming serves an important animal breeding industry in arctic regions of Russia supplying food to the population. Reindeer diet has significant seasonal differences. In summer-autumn period, it is based on nearly 300 plant species, including grains, sedge, sallow and dwarf birch leaves. Lichen account for up to 15 %. During winter-spring period it is increase up to 70 %, while the remaining 30 % are represented by residues of green plants, moss, and various admixtures [1, 2].

Digestion of vegetable feed in reindeers occurs just like in other ruminants due to enzymes produced by rumen symbionts. It is known that reindeer rumen is inhabited by symbiont microorganisms: bacteria, fungi, archeas, and protozoans [3-5]. Its microbial community may reflect both regional properties of feeding pastoral diet, as well as total physiological state of animals. By today's estimates, diversity of microorganisms in rumen of ruminant animals reaches several thousand species, of which less than 100 were studied in detail. Majority of them are strictly anaerobic non-cultivated species [6-9] and, at that, the most informative methods of analysis of the microbial community in rumen are molecular-genetic aimed at studying of its structure in general, NGS-sequencing (next-generation sequencing) and T-RFLP-analysis (terminal restriction fragment length polymorphism). They allow detecting and determining the content of low-presented microorganisms in rumen community that is demonstrated in tests on cattle stock [10, 11], sheep [12, 13], deer [14], and goats [15, 16].

Nowadays, rumen microbiocenosis in reindeer is less studied among ruminants, although this is of significant concern due to the assessment of adaption-physiologic and anatomic adaption of a body to unfavorable ecosystem conditions and feeding conditions of such animal species. Only few papers on molecular-genetic analysis of rumen microbiocenosis in reindeers, who live in the territory of Northway [14, 17], as well as rumen microbial flora in other representatives of *Cervidae* family — axis deer [18, 19], were published.

In present paper, we have for the first time carried out molecular-genetic studies of rumen microbiome in reindeer living at the territory of two areas of Arctic Russia — Murmansk Region and Yamal-Nenets Autonomous District. Significant differences in content of bacterial community of rumen were established depending in the region and age of animals. The greatest age-specific changes were found in content of *Firmicutes* gens. No direct regularity characterizing age-specific changes in rumen content of pathogenic microorganisms were found.

Purpose of present study is comparative assessment of taxonomic content of bacterial community in rumen of young and mature *Rangifer tarandus* species from different ecosystems in summer-autumn period.

Technuques. Young (aged 1-2 years) and mature (aged 3-6 years) Nenets reindeer individuals were studied. Samples of rumen content were collected in summer-autumn in 2017 from 3 animals of each age-specific group in Yamal-Nenets Autonomous District (AD) (Harp Township, forest-tundra natural-climate area) and Murmansk Region (Loparskaya Station, tundra natural-climate area).

Content of bacterial community in rumen was analyzed bys T-RFLP method [20]. Total DNA was extracted from the samples by Genomic DNA Purification Kit (Fermentas, Inc., Lithuania) subject to producer recommendations. PCR was carried out at a DNA amplifier Verity (Life Technologies, Inc., USA) with the use of eubacterium primers 63F (3′-CAGGCCTAACACATGCAAGTC-5′) with fluorophore WellRed D4 at 5′-end (Beckman Coulter, USA) and 1492R (3′-TACGGHTACCTTGTTACGACTT-5′). Amplification of 16S rRNA gene fragments was performed in the following mode: 3 minutes at 95 °C (1 cycle); 30 seconds at 95 °C, 40 seconds at 55 °C, 60 seconds at 72 °C (35 cycles); 5 minutes at 72 °C. Final concentration of total DNA in the solution was measured using a fluorimeter Qubit (Invitrogen, Inc, USA) with Qubit dsDNA BR Assay Kit (Invitrogen, Inc., USA) subject to producer recommendations.

Fluorescent labeled amplicones of 16S rRNA gene were purified by standard methodology [21]. Restriction of 30-50 ng DNA by HaeIII, HhaI and MspI was done according to producer recommendations (Fermentas, Inc., Lithuania) during 2 hours at 37 °C. Restriction products were harvested using ethanol. Afterwards, 0.2 μl of molecular weight marker Size Standart-600 (Beckman Coulter, USA) and 10 μl formamide Sample Loading Solution (Beckman Coulter, USA) were added to analyze sample patterns using CEQ 8000 (Beckman Coulter, USA), the device error was no more than 5 %. Peak sizes and area were calculated (Fragment Analysis software, Beckman Coulter, USA), prototypes (phylotypes) were identified with the acceptable study-based error of 1 nucleotide with calculation of their relative portion in microbial community. Bacteria affiliation with certain taxonomic group was determined with the use of databases (http://mica.ibest.uidaho.edu/trflp.php).

Results were processed by dispersion analysis (Microsoft Excel 2010 software). Mean (*M*) values and standard errors of the mean (±SEM) are presented in tables below. Reliability of differences between the mean values was assessed by Student's *t*-test. Shannon's and Simpson's biodiversity coefficients were estimated using software http://folk.uio.no/ohammer/past/.

Results. Data on averaged content of summer-based pastoral diet in reindeer is presented in table 1 below.

1. Composition (%) of summer pasture diet of reindeer (*Rangifer tarandus*) in two areas of Arctic Russia

Diet component	I	II
Lichen *Cladonia*	10	5
Lichen *Nephroma*	–	5
Salix borealis	20	5
Salix polaris	–	15
Blueberry *Vaccinium uliginosum*	–	10
Dwarf birch *Betula nana*	20	25
Ordinary birch *Betula pendula*	20	5
Mixture of longstanding grasses	30	30

Note. 1 — Loparskaya Station, Murmansk Region (forest-tundra), 2 — Harp, Yamal-Nenets Autonomous District (tundra). Dashes mean lack of component in the diet.

The highest death rates in reindeer are during the first years of life that is probably caused by nutritional deficiency in the habitats [22, 23].

Used primers allow us to amplify 16S rRNA gene nucleotide positions 63 to 1492 (numeration for *Escherichia coli* 16S rRNA gene).

By T-RFLP, we revealed in reindeer rumen a significant number of bacterial phylotypes, from 106.0±4.70 to 163.0±7.20 depending on the animal age and habitat (Table 2). This indicator varies during ontogenesis. Maximum phylotypes are in young individuals from Murmansk Region ($P < 0.05$). Animals from Yamal-Nenets Autonomous District show inverse pattern ($P < 0.05$).

Shannon's and Simpson's diversity indices for reindeer from Yamal-Nenets Autonomous District were higher ($P < 0.05$), i.e. these animals are more heterogeneous on rumen bacterial community compared to those of Murmansk Region. Younger deer from Yamal-Nenets Autonomous District had lower diversity ($P < 0.05$) compared to mature individuals. This testifies on less entropy and higher homogeneity of the rumen bacterial community. Diversity indices in *R. tarandus* from Murmansk Region during ontogenesis did not significantly vary (see Table 2).

2. Diversity indicators of rumen bacterial community in young (aged 1-2 years) and mature (aged 3-6 years) reindeer (*Rangifer tarandus*) from Murmansk Region (I) **and Yamal-Nenets Autonomous District** (II) (*M*±SEM, 2017)

Indicator	I		II	
	young	mature	young	mature
Shannon's index	2.89±0.32	2.61±0.23	5.40±0.18*	7.12±0.25*
Simpson's index	0.74±0.03	0.74±0.02	0.88±0.03*	0.90±0.04*
Phylotype number, units	150.0±5.40	106.0±4.70	109.5±4.15	163.0±7.20

N o t e. See description of groups in section "Methodology".
* Regional differences are statistically significant at $P < 0.05$.

3. Bacterial taxa (%) **found in the rumen of young (aged 1-2 years) and mature (aged 3-6 years) reindeer (*Rangifer tarandus*) from Murmansk** Region (I) **and Yamal-Nenets Autonomous District** (II) (*M*±SEM, 2017)

Taxon	I		II	
	young	mature	young	mature
Phylum *Bacteroidetes*	8.20±0.38	3,89±0,13*	18,32±0,84	13,45±0,64*
Phylum *Firmicutes*	17.00±0.75	46,59±2,08*	35,93±1,63	48,65±1,96*
class *Clostridia*	9.08±0.40	32,52±1,65*	15,12±0,65	26,86±1,21*
family *Thermoanaerobacteraceae*	2.42±0.11	1,11±0,04*	0,24±0,01	0,12±0,01
family *Lachnospiraceae*	0.52±0.03	6,83±0,31*	2,30±0,10	2,72±0,35
family *Eubacteriaceae*	1.30±0.05	16,90±0,74*	9,47±0,34	15,34±0,48*
family *Ruminococcaceae*	1.16±0.04	0,80±0,03*	0,19±0,01	–
family *Clostridiaceae*	3.68±0.17	5,16±0,19*	2,61±0,20	8,36±0,38*
genus *Peptococcus*	–	1,72±0,07	0,31±0,02	0,32±0,01
genus *Lactobacillus*	4.16±0.19	4,71±0,27	2,66±0,12	1,12±0,06*
genus *Bacillus*	1.56±0.06	5,97±0,24*	4,37±0,25	5,03±0,22
genus *Staphylococcus*	0.14±0.01	0,86±0,04*	0,10±0,01	0,31±0,02*
class *Negativicutes*	2.06±0.08	2,53±0,14	13,68±0,54	15,33±0,63
Phylum *Actinobacteria*	15.65±0.78	4,47±0,17*	12,20±0,52	7,91±0,30*
genus *Bifidobacterium*	0.25±0.02	0,15±0,01*	1,09±0,06	0,21±0,02*
other	15.40±0.65	4,32±0,16*	11,11±0,36	7,70±0,21*
Phylum *Proteobacteria*	7.63±0.29	13,11±0,63*	4,34±0,21	13,49±0,34*
family *Enterobacteriaceae*	0.64±0.03	7,59±0,12*	1,83±0,09	1,00±0,04*
family *Campylobacteriaceae*	6.08±0.28	3,04±0,10*	1,30±0,05	9,69±0,35*
family *Pseudomonadaceae*	0.91±0.03	1,94±0,43*	0,32±0,05	0,48±0,02
family *Burkholderiaceae*	–	0,29±0,01	0,89±0,04	2,32±0,08*
family *Succinivibrionaceae*	–	0,25±0,01	–	–
Phylum *Tenericutes* (genus *Mycoplasma*)	0.82±0.02	1,48±0,04*	–	–
Phylum *Fusobacteria*	1.05±0.04	0,97±0,06	0,18±0,01	1,65±0,05*
Phylum *Cyanobacteria*	–	–	0,70±0,03	0,75±0,02
Phylum *Acidobacteria*	–	–	0	0,33±0,01
Non-classified sequences	49.65±3.35	29,49±1,32*	28,33±1,12	13,77±0,95*

N o t e. See description of groups in section "Methodology". Dashes mean that values are below those validly determined by T-RFLP method.
* Differences between mature and young species within one region are statistically significant at $P < 0.05$.

Majority of identified phylotypes are of four bacterial phyla, *Firmicutes*, *Bacteroidetes*, *Actinobacteria* and *Proteobacteria*, which in total, depending on the age and habitat, makes 48.48±4.19 to 83.50±5.07 % of the reindeer rumen bacteria community (see Table 3). Bacteria of *Tenericutes*, *Fusobacteria*, *Acidobacteria*, and *Cyanobacteria* phyla are less abundant. Significant part of phylotypes, from 13.77±0.95 to 49.65±3.35 %, could not be identified and refer to any taxon. This necessitates additional studies of their functional role.

Our research results are in line with contemporary understanding of ru-

men microbiota in ruminants [8, 20, 24], and particularly in reindeer [14, 17]. It was earlier reported on large quantity of unidentified taxa in rumen of reindeer *Rangifer tarandus* from Northway as compared to cattle stock and Thompson gazelles [5].

In the samples we collected the percentage of *Eubacteriaceae* and *Clostridiaceae* members of *Clostridia* having cellulose and sacharolythic properties was also significantly higher than that reported for more studied ruminants of *Bovidae*, in particular for cattle [8, 20, 24]. According to researchers, these anaerobic microorganisms, in particular *Eubacterium rangiferina*, in reindeer ensure detoxication of usnic acid and other secondary metabolites produced by lichen of *Cladonia*, *Usnea*, *Lecanora*, *Ramalina*, *Evernia*, *Parmelia*, *Alectoria* genera [25-27]. It is believed that due to specific properties of microbial community of reindeer rumen, consumption of significant quantity of lichen during winter (up to 70 % in total diet) does not have toxic effect on reindeer (as apart from elk or sheep). Note, it was reported on massive death of over 300 elks due to lichen consumption at lack of the alternative feed [25].

Microorganisms of phylum *Cyanobacteria* are present in reindeer rumen [17] that is entirely logic since cyanobacteria refers to lichen symbionts. Lichen cyanobionts are mostly the members of *Nostoc* genus, and to a lesser extent represent *Calothrix*, *Scytonema*, and *Fischerella* genera [28]. In out experiment, the number of cyanobacteria was minor among the individuals from Yamal-Nenets Autonomous District and did not reach the limits of valid determination by T-RFLP method in animals from Murmansk Region that is probably due to regional specific of summer pasture diet of reindeer.

We have identified a number of similar trends in age-specific changes of microbiome structure in reindeer from various Arctic regions. Thus, total percentage of unidentified bacterial phylotypes in mature animals is significantly lower ($P < 0.05$) than in young animals. The greatest age-specific changes among the identified taxa are noted for phylum *Firmicutes*. Total content of microorganisms of *Clostridia* class in rumen of mature species, especially members of *Eubacteriaceae*, *Clostridiaceae*, and *Lachnospiraceae* families potentially able to hydrolyze vegetable carbohydrates to volatile fatty acids, is higher compared to young animals ($P < 0.05$). Bacteria with similar properties from *Bacteroidetes* phylum (including *Bacteroides*, *Prevotella*) which ferment starch, cellulose, several other carbohydrates, proteins and deaminate amino acids shows opposite pattern.

Interestingly, *Ruminococcaceae* family cellulolytic bacteria found in cattle rumen in significant quantity [8, 20, 24] are fully absent in reindeer in our study.

Count of *Negativicutes* bacteria able to utilize acids (including acetic, propione, butyric, lactic, and etc.) after fermentation of mono-, oligo- and polysaccharides had some trend towards growth in mature individuals compared to young animals. Significant abundance of selenomonades in rumen, including *Selenomonas ruminantium*, which differ by appearance and biodiversity from those found in cattle is described by B.V. Tarakanov [7]. It was reported that acid-utilizing bacteria of *Megasphaera*, *Selenomonas*, *Dialister* genera are physiologically significant groups for cattle since they disallow formation of lactate in the rumen. This prevents a drop of pH followed by lactate acidosis [7, 20, 29]. Here, we want to draw attention to reliably low percent found for acid-forming *Lactobacillus* ($P < 0.05$) and high count of acid-utilizing members of class *Negativicutes* ($P < 0.05$) in reindeer from Yamal-Nenets Autonomous District compared to those from Murmansk Region. Identified differences are probably due to regional summer pasture diets in Murmansk Region (tundra) and Yamal-Nenets Autonomous District (forest-tundra area).

Rumen community is widely represented by conventionally pathogenic

microorganisms, majority of which is traditionally related to gastroenteritis. These are bacteria of *Enterobacteriaceae* and *Pseudomonadaceae* families. The percentage of actinomycetes of phylum *Actinobacteria* (*Coriobacteriaceae*, *Corynebacterium*) including causative agents of actinomycosis which affect different organs and tissues [20] was high. Among pathogenic bacteria, we identified agents of campylobacteriosis (*Campylobacteraceae* family), pasteurellosis (*Pasteurellaceae* family), mycoplasmosis (*Tenericutes* gens), necrobacteriosis (*Fusobacteria* genus), and purulo-necrotic infections (*Staphylococcus* genus). Counts of these pathogens in the studied samples were minor, except for enterobacteria, actinobacteria, campylobacteria, and fusobacteria. Importantly, in Northern reindeer only necrobacteriosis causing massive death of young animals is quite fully studied. In cattle, *Fusobacterium necrophorum*, the causative agent of necrobacteriosis, may penetrate into blood and then cause hepatic abscess, injury of hooves, skin, and mucosa [22, 23, 29].

In our study we did not found direct regularity characterizing age-specific changes in rumen composition of pathogenic microorganisms, including *Fusobacteria* gens, *Campylobacteriaceae*, *Enterobacteriaceae* families. The identified differences in emergence of these pathogenic and conventionally pathogenic microorganisms are probably related to nutrition and epizootic situation in stock that requires clarification in additional studies.

Therefore, the results of T-RFLP analysis evidence in notable changes of bacterial community in reindeer rumen during ontogenesis and differences in community composition in animals living in Murmansk Region and Yamal-Nenets Autonomous District. In general, over 80 % of identified microorganisms refer to four bacterial genera, *Firmicutes*, *Bacteroidetes*, *Actinobacteria* and *Proteobacteria*. *Tenericutes*, *Fusobacteria*, *Acidobacteria* and *Cyanobacteria* taxa are less freguent. Total diversity of microorganisms is higher in reindeer from Yamal-Nenets Autonomous District ($P < 0.05$) as compared to animals from Murmansk Region. There are similar trends in composition of bacteria which are potentially able to hydrolyze vegetable carbohydrates. No direct regularity characterizing age-specific changes in rumen composition of pathogenic microorganisms are found. Obtained results extend the available information on reindeer physiology in Arctic Russia conditions. These results may be helpful to improve reindeer herding and decrease animal death.

REFERENCES

1. Orpin C.G., Mathiesen S.D., Greenwood Y., Blix A.S. Seasonal changes in the ruminal microflora of the high-arctic Svalbard reindeer (*Rangifer tarandus platyrhynchus*). *Appl. Environ. Microb.*, 1985, 50(1): 144-151.
2. Sundset M.A., Edwards J.E., Cheng Y.F., Senosiain R.S., Fraile M.N., Northwood K.S., Præsteng K.E., Glad T., Mathiesen S.D., Wright A.D.G. Molecular diversity of the rumen microbiome of *Norwegian reindeer* on natural summer pasture. *Microb. Ecol.*, 2009, 57: 335-348 (doi: 10.1007/s00248-008-9414-7).
3. Mathiesen S.D., Mackie R.I., Aschfalk A., Ringø E., Sundset M.A. Microbial ecology of the gastrointestinal tract in reindeer — changes through season. In: *Microbial ecology of the growing animal; Biology of the growing animals. V. 3.* W. Holzapfel, P. Naughton (eds.). Elsevier Press, Oxford: 73-100.
4. Sundset M.A., Edwards J.E., Cheng Y.F., Senosiain R.S., Fraile M.N., Northwood K.S., Praesteng K.E., Glad T., Mathiesen S.D., Wright A.D. Rumen microbial diversity in Svalbard reindeer, with particular emphasis on methanogenic archaea. *FEMS Microbiol. Ecol.*, 2009, 70(3): 553-562 (doi: 10.1111/j.1574-6941.2009.00750.x).
5. Sundset M.A., Præsteng K.E., Cann I.K.O., Mathiesen S.D., Mackie R.I. Novel rumen bacterial diversity in two geographically separated sub-species of reindeer. *Microb. Ecol.*, 2007, 54: 424-438 (doi: 10.1007/s00248-007-9254-x).
6. Orpin C.G., Joblin K.N. The rumen anaerobic fungi. In: *The rumen microbial ecosystem*. P.N. Hobsen, C.S. Stewart (eds.). Blackie Academic & Professional, London, 1997: 140-195.

7. Tarakanov B.V. *Metody issledovaniya mikroflory pishchevaritel'nogo trakta sel'skokhozyaistvennykh zhivotnykh i ptitsy* [Methods for studying microflora of the digestive tract of agricultural animals and poultry]. Moscow, 2006 (in Russ.).
8. Church D.C. *Ruminant animal: Digestive physiology and nutrition*. Prentice Hall, New Jersey, 1993.
9. Hungate R.E. *The rumen and its microbes*. Academic Press, NY, 1966.
10. Jami E., Mizrahi I. Composition and similarity of bovine rumen microbiota across individual animals. *PLoS ONE*, 2012, 7(3): e33306 (doi: 10.1371/journal.pone.0033306).
11. Veneman J.B., Muetzel S., Hart K.J., Faulkner C.L., Moorby J.M., Perdok H.B. Does dietary mitigation of enteric methane production affect rumen function and animal productivity in dairy cows? *PLoS ONE*, 2015, 10(10): e0140282 (doi: 10.1371/journal.pone.0140282).
12. Snelling T.J., Genç B., McKain N., Watson M., Waters S.M., Creevey C.J., Wallace R.J. Diversity and community composition of methanogenic archaea in the rumen of Scottish upland sheep assessed by different methods. *PLoS ONE*, 2014, 9(9): e106491 (doi: 10.1371/journal.pone.0106491).
13. de la Fuente G., Belanche A., Girwood S.E., Pinloche E., Wilkinson T., Newbold C.J. Pros and cons of Ion-Torrent next generation sequencing versus Terminal Restriction Fragment Length Polymorphism T-RFLP for studying the rumen bacterial community. *PLoS ONE*, 2014, 9(7): e101435 (doi: 10.1371/journal.pone.0101435).
14. Salgado-Flores A., Hagen L.H., Ishaq S.L., Zamanzadeh M., Wright A.-D.G., Pope P.B. Rumen and cecum microbiomes in reindeer (*Rangifer tarandus tarandus*) are changed in response to a lichen diet and may affect enteric methane emissions. *PLoS ONE*, 2016, 11(5): e0155213. (doi: 10.1371/journal.pone.0155213).
15. Han X., Yang Y., Yan H., Wang X., Qu L., Chen Y. Rumen bacterial diversity of 80 to 110-day-old goats using 16S rRNA sequencing. *PLoS ONE*, 2015, 10(2): e0117811 (doi: 10.1371/journal.pone.0117811).
16. Wang L., Xu Q., Kong F., Yang Y., Wu D., Mishra S., Li Y. Exploring the goat rumen microbiome from seven days to two years. *PLoS ONE*, 2016, 11(5): e0154354 (doi: 10.1371/journal.pone.0154354).
17. Zielińska S., Kidawa D., Stempniewicz L., Łoś M., Łoś J.M. New insights into the microbiota of the Svalbard Reindeer *Rangifer tarandus platyrhynchus*. *Front. Microbiol.*, 2016, 7: 170 (doi: 10.3389/fmicb.2016.00170).
18. Gruninger R.J., Sensen C.W., McAllister T.A., Forster R.J. Diversity of rumen bacteria in Canadian cervids. *PLoS ONE*, 2014, 9(2): e89682 (doi: 10.1371/journal.pone.0089682).
19. Zhi-Peng L., Na J., Han-Lu L., Xue-Zhe C., Yi J., Fu-He Y., Guang-Yu L. Analysis of bacterial diversity in rumen of sika deer (*Cervus nippon*) fed different forages using DGGE and T-RFLP. *China Agriculture Science*, 2014, 47(4): 759-768 (doi: 10.3864/j.issn.0578-1752.2014.04.016).
20. Laptev G.YU., Novikova N.I., Il'ina L.A., Iyldyrym E.A., Nagornova K.V., Dumova V.A., Soldatova V.V., Bol'shakov V.N., Gorfunkel' E.P., Dubrovina E.G., Sokolova O.N., Nikonov I.N., Lebedev A.A. *Normy soderzhaniya mikroflory v rubtse krupnogo rogatogo skota* Norms of microflora in rumen of cattle]. St. Petersburg, 2016 (in Russ.).
21. Maniatis T., Fritsch E.F., Sambrook J. *Molecular cloning: A laboratory manual*. Cold Spring Harbor, NY, 1982.
22. Samandas A.M., Laishev K.A., Samoilov S.G. *Sibirskii vestnik sel'skokhozyaistvennoi nauki*, 2011, 5-6: 92-96 (in Russ.).
23. Laishev K.A., Samandas A.M., Mityukov A.S., Prokudin A.V., Silkina E.V. *Izvestiya Sankt-Peterburgskogo gosudarstvennogo agrarnogo universiteta*, 2011, 24: 118-121 (in Russ.).
24. Nelson K.E., Zinder S.H., Hance I., Burr P., Odongo D., Wasawo D., Odenyo A., Bishop R. Phylogenetic analysis of the microbial populations in the wild herbivore gastrointestinal tract: insights into an unexplored niche. *Environ. Microbiol.*, 2003, 5: 1212-1220 (doi: 10.1046/j.1462-2920.2003.00526.x).
25. Roach J.A.G., Musser S.M., Morehouse K., Woo J.Y.J. Determination of usnic acid in lichen toxic to elk by liquid chromatography with ultraviolet and tandem mass spectrometry determination. *J. Agr. Food Chem.*, 2006, 54: 2484-2490 (doi: 10.1021/jf052767m).
26. Luzina O.A., Salakhutdinov N.F. *Bioorganicheskaya khimiya*, 2016, 3(42), 2016: 276 (doi: 10.7868/S0132342316030106) (in Russ.).
27. Sundset M.A., Kohn A., Mathiesen S.D., Præsteng K.E. *Eubacterium rangiferina*, a novel usnic acid-resistant bacterium from the reindeer rumen. 2008. *Naturwissenschaften*, 95: 741-749 (doi: 10.1007/s00114-008-0381-0).
28. Pankratov T.A., Kachalkin A.V., Korchikov E.S., Dobrovol'skaya T.G. *Mikrobiologiya*, 2017, 3(86): 265-283 (doi: 10.7868/S0026365617030156) (in Russ.).
29. Nocek J.E. Bovine acidosis: implications on laminitis. *J. Dairy Sci.*, 1997, 80: 1005-1028 (doi: 10.3168/jds.S0022-0302(97)76026-0).