Comparison of productive and biological characteristics of indigenous and imported cattle in Yakutia

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Abstract. The aim of this research was evaluation of the rumen and faecal bacterial communities with high-throughput sequencing of 16S rDNA in indigenous Yakut and transferred Kalmyk cows, which have potentially valuable gene pools for beef cattle breeding. The obtained results showed a similar composition of the microbiomes in the rumens of Yakut and Kalmyk cows. The dominant bacterial phyla in the rumen microbiocenoses were Firmicutes and Bacteroidetes represented mainly by the Prevotellaceae, Porphyromonadaceae, Ruminococcaceae and Lachnospiraceae families. In the faeces of the cow breeds, compared families Bacteroidaceae, Clostridiaceae, Ruminococcaceae and Verrucomicrobiaceae were the most numerous. Further work is necessary to reveal the role of the identified bacterial taxa in changes of the rumen fermentation.

1. Introduction

The bacteria associated with the intestines of cattle are diverse, numerous, and perform essential functions (synthesis of vitamins, immune defence, etc.) aimed at supporting the functioning of all body systems [1, 2]. However, the main function of the microorganisms inhabiting the digestive tract is the digestion of feed components [3]. The role of microorganisms in the gastrointestinal tract in the case of ruminants is particularly important, since the end products of microbial fermentation in the rumen provide most of the energy that is necessary for the basic vital processes. The diversity of the rumen microbiome is a key feature of ruminants that gives them the ability to adapt to a wide range of changing conditions [4].

Thus, the study of the rumen microbiota is of great interest for the agricultural sphere of human activity, including research aimed at increasing the efficiency of feed use in cattle. This is because amid increasing global demand for the production and quality of meat, especially beef, as the most complete food product, an improvement in feeding efficiency is one of the promising directions in solving this issue [5].

Studies by some authors show that changes in scar microbiome affect cattle productivity (milk quantity, feed efficiency, etc.) [6, 7].

Nevertheless, the microbiome of the rumen of cattle, its characteristics and the possibilities of manipulating it are some of the areas that require more study. Currently, there are a sufficient number of studies on the effect of scar microbiome on feeding efficiency [8]. Nevertheless, for some cattle
breeds such as the Kalmyk and Yakut breeds, which are potentially valuable gene pools for the development of beef cattle breeding, data on scar microbiome are significantly limited [9, 10].

Based on the foregoing, our work was aimed at studying the microbiome of the gastrointestinal tract of cows of the Kalmyk and Yakut breeds, which are of interest to agriculture. These studies will allow us to evaluate the effectiveness of feeding and open the prospect of manipulating the rumen microbiota in order to increase the productive qualities of cattle.

2. Materials and methods

2.1. Experimental animals and their contents

A study of the microbiocenosis of the gastrointestinal tract was carried out in Kalmyk cows breed and indigenous Yakut cattle at the agricultural production complex of the “Serge” farm (Vilyui tract, 27 km, Yakutsk, Russia). The experiment was conducted on animals selected by age (5–6 years) and weight (410–420 kg.). The animals were kept on pasture in conditions with different grasses (*Elytrigia repens*, *Elymus mutabilis*, *Elymus sibiricus*, *Alopecurus*, *Hordeum brevisubulatum*, *Beckmannia syzigachne*, *Puccinellia tenuiflora*, *Festuca rubra*, *Agrostis alba* and *Psathyrostachys juncea*).

Animal services and experimental studies were performed in accordance with the instructions and recommendations of the Russian Regulations, 1987 (Order No. 755 of 08/12/1977, the USSR Ministry of Health) and “The Guide for Care and Use of Laboratory Animals” (National Academy Press Washington, DC 1996).

For the experiment, groups were randomly formed (n = 12), divided into two experimental groups: I – bulls and cows of the Kalmyk breed and II – bulls of the native Yakut breed. All groups of animals were kept under identical conditions.

2.2. Sampling and transportation

The analysis of microbiocenosis was performed in the rumen (ruminal fluid) and faeces. After slaughtering the cattle, sampling was carried out with a sterile instrument in a volume of 0.2–0.3 ml and immediately transferred to a tube with a preservative solution (DNA/RNA Shield, USA) and frozen.

2.3. Extraction of total DNA bacteria and archaea

Total DNA from the rumen fluid was extracted by a combined method (homogenization in combination with subsequent enzymatic lysis and chemical lysis). Total DNA from faeces was isolated using the FastDNA Spin Kit for Faeces (MP Biomedicals, USA). DNA quality was evaluated using electrophoresis in 1 % agarose gel. The purity of the isolated DNA was evaluated photometrically using a Nanodrop 8000 spectrophotometer. The DNA concentration was determined using a Qubit 4.0 fluorometer using the HS dsDNA kit (Life Technologies).

2.4. Library preparation and sequencing

Preparation of DNA libraries, as well as sequencing, was carried out at the Centre of Shared Scientific Equipment “PERSISTENCE OF MICROORGANISMS” of the Institute for Cellular and Intracellular Symbiosis of the Ural Branch of the Russian Academy of Sciences (Orenburg, Russia). 16S DNA libraries were prepared according to the Illumina workflow (http://support.illumina.com/documents/documentation/chemistry_documentation/16s/16s-metagenomic-library-prep-guide-15044223-b.pdf) with primers on the V3 and V4 regions of the SSU rRNA gene, such as direct SD-Bact-0341-bS-17 and reverse SD-Bact-0785-aA-21 [11]. Libraries were sequenced in MiSeq (Illumina) using the MiSeq V3 reagent kit with 2 × 300 base pairs.

Data analysis was performed using USEARCH v8.0.1623_win32 [12] and included merging of paired reads, filtered by quality and size (minimum size 415 base pairs). During filtration, reads with N or a total average Q score of <15 were discarded. Filtering quality assessment was performed using FastQC v 0.11.3. Through dereplication and clustering with USEARCH, operational taxonomic units (OTUs) were formed whereas singletons and doubletons were removed. OTUs were formed using
similarity levels between sequences of at least 97% to classify the microorganisms at the species level. Chimera detection was performed via UCHIME (Edgar, 2011) using USEARCH, and chimeric sequences were removed. Contaminating OTUs, similar to sequences from negative control samples, were found and removed using the USEARCH. A taxonomic classification of sequences was performed using the SILVA reference database [13].

The resulting OTUs, after filtering and assigning taxonomic affiliations, were used to calculate alpha and beta diversity. The calculation of diversity coefficients (Shannon (H) and Simpson) was performed using QIIME 1.8.0 software according to the previously described methods. To calculate the Wilcoxon (Mann-Whitney) rank sum criterion for two independent samples, the Explicit 2.9.4 program was used. Using the STATISTICA program, analyses of variance (one-way ANOVAs) were performed.

3. Results

As a result, 16C metagenomic sequencing of the rumen microbiome and fecal masses of group I cows, 823 (3 Otus unknown type) and 533 Otus (1 Otus unknown type) were classified, respectively. According to the results of sequencing samples of scar tissue and fecal masses of group II cows, 753 (2 Otus unknown type) and 552 Otus (2 Otus unknown type) were classified, respectively.

The study of the microbial profile of the rumen of the native Yakut cows using 16s rRNA as a marker showed that of the 12 classified phyla, Bacteroidetes (48.9 %) and Firmicutes (26 %) dominated (Figure 1).

Among the numerous taxa Proteobacteria (2.36 %), Verrucomicrobia (3.91 %), Fibrobacteres (2.66 %) and Saccharibacteria (2.37 %) were noted; the rest taxa amounted to less than 2% each. The most numerous ones were bacteria of the families Lachnospiraceae (23.3 %), Prevotellaceae (22.7 %), Porphyromonadaceae (10.5 %) and Ruminococcaceae (8.64 %). It should be noted that during the study, 29 genera were classified among which g. Fibrobacter (2.66 %), genus Ruminococcus (2.23 %), genus Succinimonas (2.19 %), genus Prevotella (14.6 %) and genus Paludibacter (6.88 % of the total) were numerically the largest groups of bacteria.
Studying the genetic diversity of the microflora of faeces of cows of the native Yakut breed, 11 phyla were classified, where the most numerous taxa were _Bacteroidetes_ (30.5 %), _Firmicutes_ (58.4 %) and _Verrucomicrobia_ (7.23 %). The most numerous representatives of these phyla were bacteria of the families _Bacteroidaceae_ (5.82 %), _Rikenellaceae_ (3.96 %), _Prevotellaceae_ (2.85 %), _Porphyromonadaceae_ (3.42 %), _Clostridiaceae_ (7.32 %), _Ruminococcaceae_ (44 %), _Lachnospiraceae_ (3.86 %) and _Verrucomicrobiaceae_ (6.31 %). Identification at the genus level and comparison with the SILVA reference database allowed us to classify 42 taxa, of which the dominants were _Bacteroides_ (5.8 %), _Alistipes_ (2.9 %), _Paludibacter_ (2.47 %), _Clostridium_ (6.3 %), _Oscillibacter_ (2.78 %) and _Akkermansia_ (6.31 %).

Analysis of the microbial diversity of the rumen and faeces of Kalmyk cows showed similar results (Figure 2).

The most abundant rumen microbiota of cows was represented by 4 phyla: _Bacteroidetes_ (36.2 %), _Firmicutes_ (28.8 %) _Verrucomicrobia_ (5.93 %) and _Fibrobacteres_ (5.64 %), represented by the families _Clostridiaceae_ (2.24 %), _Ruminococcaceae_ (12.5 %), _Lachnospiraceae_ (6.91 %), _Prevotellaceae_ (16.7 %), _Fibrobacteraceae_ (5.64 %) and _Porphyromonadaceae_ (7.89 %). In the ruminal fluid of Kalmyk breed cows, 52 genera were identified, of which only four were significant: _Fibrobacter_ (5.64 %), _Succinimonas_ (2.11 %), _Prevotella_ (11.6 %) and _Paludibacter_ (4.02 %).

An analysis of the results of 16S rRNA metagenomic sequencing of faecal samples allowed us to classify 11 phyla, of which _Bacteroidetes_ (29.5 %), _Firmicutes_ (55.2 %) _Verrucomicrobia_ (9.01 %) represented the majority. Among the classified families, _Ruminococcaceae_ (42.4 %) occupied a dominant position. The remaining families _Bacteroidaceae_ (4.54 %), _Rikenellaceae_ (4.6 %), _Prevotellaceae_ (3.95 %), _Porphyromonadaceae_ (3.08 %), _Clostridiaceae_ (7.16 %), _Lachnospiraceae_ (2.85 %) and _Verrucomicrobiaceae_ (7.88 %) were less numerous. Assessment of sequencing data showed that the most numerous taxa at the genus level were _Bacteroides_ (4.09 %), _Alistipes_ (3.83 %), _Paludibacter_ (2.43 %), _Clostridium_ (6.24 %), _Oscillibacter_ (4 %), _Akkermansia_ (7.88 %) and _Paraprevotella_ (3.05 %).

As a result, analysis of metagenomic sequencing for 16s rRNA showed similar rumen microbiocenoses of the studied cow breeds. At the same time, the rumen microbiocenosis of cows of
the native Yakut breed was characterised by a higher content of bacterial phylum \textit{Bacteroidetes} (+10 \%), in particular, belonging to the families \textit{Prevotellaceae} (+8 \%) and \textit{Porphyromonadaceae} (+2.61 \%). In the rumen of cows of the Kalmyk breed, a higher content of bacteria of the phylum \textit{Firmicutes} (+2.8 \%) and \textit{Fibrobacteres} (+2.98 \%) was noted. It should be noted that the microbiocenoses of the faecal samples of the analysed cow breeds turned out to be almost identical and the differences between the main taxa abundances were no more than 3 \%.

To assess the biodiversity and complexity of the microbiota of the rumen and faeces of the cattle, the Simpson and Shannon indices were used (table 1). The calculation of the Simpson index indicates the dominance of certain types of community. Since squaring small \( n/N \) ratios results in very small quantities, the Simpson index increases as one or more families in the phylum dominates.

The obtained data on the rumen metagenomes allowed us to calculate these indicators for only two phyla. The values of the Simpson Index of 0.02 and 0.018 for the Yakut and Kalmyk breeds, respectively, indicate a uniform distribution of families represented by the \textit{Firmicutes} phylum without the predominance of any of them. One should note the absence of differences in this indicator among the breeds. However, for the phylum \textit{Bacteroidetes}, another pattern of the indices was found. These indices show that there is a predominance of some families in the Kalmyk breed, which proves the results described above. Comparing these breeds, one can note a greater variety of the rumen microbiota in animals of the Kalmyk breed than that in the native Yakut breed.

Table 1. Calculation of the Simpson and Shannon indices for the microbiocenosis of the rumen and faeces of cows of the Kalmyk and Yakut indigenous breeds

|                | Yakut indigenous breed | Kalmyk breed |
|----------------|------------------------|--------------|
| **Rumen**      |                        |              |
| Phyla          | Simpson Index | Shannon Index | Phylum        | Simpson Index | Shannon Index |
| \textit{Firmicutes} | 0.02          | 2.52         | \textit{Firmicutes} | 0.018 | 2.34 |
| \textit{Bacteroidetes} | 7.98          | 0.83         | \textit{Bacteroidetes} | 2.42  | 0.84 |
| **Faeces**     |                        |              |
| Phyla          | Simpson Index | Shannon Index | Phylum        | Simpson Index | Shannon Index |
| \textit{Firmicutes} | 0.13          | 1.67         | \textit{Firmicutes} | 0.15  | 1.69 |
| \textit{Bacteroidetes} | 0.28          | 1.6          | \textit{Bacteroidetes} | 0.22  | 1.75 |
| \textit{Proteobacteria} | 0.01          | 0.6          | \textit{Proteobacteria} | 0.005 | 0.73 |
| \textit{Verrucomicrobia} | 16.8          | 0.01         | \textit{Verrucomicrobia} | 27.1  | 0.02 |

Faecal masses are characterised by a greater variety of microflora in comparison with the rumen. The most evenly distributed phyla were \textit{Bacteroidetes}, \textit{Firmicutes} and \textit{Proteobacteria}, consisting of the families described above without a specific dominance. The prevalence of phylum \textit{Verrucomicrobia} was observed. It should be noted that there were no large interbreed differences. There was a slight difference concerning the phylum \textit{Verrucomicrobia} in the Kalmyk breed.

The analysis of the Shannon indices calculated for the rumen shows the complexity of the structure of \textit{Firmicutes} phylum in both breeds, while no significant differences between these breeds were revealed. For phylum \textit{Bacteroidetes}, the opposite feature of simplicity was shown. Due to a number of conditions, this phylum is quite simple. For faeces, the maximum values of the Shannon index indicated the average complexity of the microbiota structure, and the minimum values indicated the simplest communities in the stool.

4. Discussion

Assessment of the taxonomic diversity of the rumen microbiocenoses in our study showed a less diverse microbiota for the cow rumens of the native Yakut breed. Therefore, in the rumen microbiocenoses of cows of the Kalmyk breed, a greater number of families (+ 18) and genera (+ 22) of bacteria were identified than those in samples of the ruminal fluid obtained from cows of the Yakut breed. A number of studies have shown that there is a correlation between the diversity of microbiota of the intestine and effective feed intake. Thus, it was noted that the less diverse the microbial status of the gastrointestinal tract of animals, the more number of corresponding metabolites is produced to meet the animal's energy needs [14, 15].
The results of the study showed that the main taxonomic groups in the rumen microbiocenoses of the cows of the studied breeds were *Firmicutes* and *Bacteroidetes*, which were also noted in other works on beef cattle [16]. The ratios of *Firmicutes* to *Bacteroidetes* in the microbiocenoses of cow rumens for the Yakut and Kalmyk breeds were 0.53 and 0.79, respectively. Given the characteristics of ruminal fermentation processes are associated with pronounced glucogenetic dependence, a rather high ratio of *Firmicutes* to *Bacteroidetes* suggests a higher digestibility of feed components and increased body weight [17, 18].

The taxon *Bacteroidetes* represented mainly by the families *Prevotellaceae* and *Porphyromonadaceae* was more numerous in the microbiota of cow rumen. In many studies, the genus *Prevotella* has been observed as the dominant bacterial taxon in the rumen under various nutritional conditions, and it plays a role in the metabolism of polysaccharides and peptides [19-21]. The quantitative content of bacteria of the genus *Prevotella* is associated with numerical differences in the average daily gain of calves. A deterioration in the digestibility of fibres [22], starch, peptides, and pectin [23] has been observed at a lower level of bacteria of the genus *Prevotella*. Thus, the prevalence of the number of representatives of the *Prevotellaceae* family will probably contribute to the numerical differences observed in growth in calves.

Concerning the *Firmicutes* phylum, bacteria of the families *Ruminococcaceae* and *Lachnospiraceae* were observed in the rumens of cows of the studied breeds. Huws et al. (2011) showed that representatives of the families *Ruminococcaceae* and *Lachnospiraceae* play a predominant role in biohydrogenation pathways within the rumen [24]. Also, according to published data, the abundance of bacteria belonging to the families *Lachnospiraceae*, *Ruminococcaceae* and *Clostridiaceae* positively correlates with an increase in feed utilisation and a decrease in the amount of feed consumed [25, 26]. It is likely that an increase in the level of bacteria of the families *Lachnospiraceae* and *Ruminococcaceae* would be reflected in a more complete degradation of food components and an increase in the energy balance in the intestinal cells, which will result in an increase in productivity indicators.

Other groups of rumen bacteria belonging to the *Firmicutes* phylum, such as *Clostridium* (less than 2 % of the total), *Eubacterium* (less than 1 % of the total) and *Butyrivibrio* (less than 1% of the total) were minor and allowed the indirect evaluation of the activity of gas formation processes. More recently, a team of scientists led by Pitta (2016) has used NGS to study the relationship of rumen microbiome associations with gas production in gobies grazing wheat pastures. The scientists noticed that wheat-induced gases were associated with an increase in the number of representatives of several bacterial genera within the phylum *Firmicutes*, including *Clostridium*, *Eubacterium* and *Butyrivibrio*. Studying the dynamics of the rumen microbiome during the development of wheat-induced gas formation, Pitta et al noted that in rumens with normal contents of representatives of the genus *Lactobacillus* and *Prevotella* the number of representatives of the genera *Clostridium*, *Eubacterium* and *Butyrivibrio* increased, which resulted in excessive gas formation. The authors concluded that the latter group of bacteria can use oligosaccharides trapped in the biofilm, which, they suggested, may be associated with a high content of genes that provide carbohydrate metabolism [27]. The results that are similar to other data have suggested that gas production is associated with the activity of the biofilm-forming microorganisms that have a higher potential for the rapid metabolism of soluble carbohydrates trapped in exopolysaccharide mucus [28]. As a result, the low quantitative content of bacteria of the genera *Clostridium*, *Eubacterium* and *Butyrivibrio* in the rumen of cows in our experiment is likely to correlate with a low level of gas generation and is of particular interest for further study.

Analysis of the microbial profile of cow faeces revealed the presence of typical representatives of the microbiota described by other authors without any specific representatives [29-31].

5. Conclusion
The study made it possible to assess in detail the microbial diversity of rumens and faeces of cows of indigenous Yakut and introduced Kalmyk breeds, as well as to characterize their features. As the
results showed, the rumen microbial communities of indigenous Yakut cows have the composition of microbiota and the ratio of bacterial taxa which are very similar to the rumen microbiomes of Kalmyk cows, but nevertheless have a higher content of bacteria belonged to phylum Bacteroidetes. The analysis of microbiomes of cow faeces of the studied breeds showed almost the identical structure and an absence of prominent distinctions between them. Further work is needed to assess the contribution of the identified groups of bacteria in changing the parameters of rumen fermentation and their relationship with the energy and feed efficiency of beef cattle of the studied breeds.

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