Assessment of the microecological status of the rumen of cattle using the 16S Metagenomics method

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Abstract. The paper presents an assessment of the microecological status of the rumen of cattle using the 16S metagenomics method against the background of the introduction of substances with bacteriostatic action. The analysis of the data showed that the introduction of probiotics, prebiotics, antibiotics, both separately and in a composition with a plant extract, decreases the diversity of the bacterial landscape relative to the control group contained in the main diet, but did not have a significant effect on the complexity of bacterial communities. In all groups, the phylum Firmicutes and Bacteroidetes were of the greatest importance; their ratio directly depended on the use of the studied substances in the diet. The range of content of phylum Firmicutes started from 7.31% to 32.4%, phylum Bacteroidetes from 17.9% to 74.5% of the total number of bacteria, depending on the group. The exceptions were the groups that received the probiotic and prebiotic in a composition with the extract. In the first case, the greatest value belongs to the phylum Proteobacteria (67.9% of the total number) relative to Firmicutes, Bacteroidetes (8.01% and 17.9% of the total number), in the second case, the phylum Fibrobacteres (21.3% of the total number) relatively to Firmicutes (7.31% of the total number), Bacteroidetes (70.6% of the total number of microorganisms) was the predominant representative of the taxon.

1. Introduction

The rumen is inhabited by a high density of resident microflora, which consists of bacteria, protozoa, archaea, and fungi that process consumed plant materials [1]. The rumen microbiota of ruminants plays an important role in food digestion due to enzymes produced by symbiotic microorganisms [2]. The variety of rumen microorganisms reaches several thousand species, of which less than a hundred have been studied in detail. Most of them are anaerobic uncultivated species that cannot be studied without the use of metagenomic methods [3]. Therefore, many studies are aimed at studying the effect of plant extracts on the expression level of RNA markers of digestion in poultry and animals, as well as their combined use with synbiotics and feed antibiotics [4]. Sequencing of the genome of rumen microorganisms is an important factor in the correction of cicatricial digestion [5].

The 16S rRNA gene sequencing technology is used as an objective molecular approach in metagenomic analysis, which allows a holistic study of the microecology of the gastrointestinal tract [6]. This method is often used to study the bacterial diversity and biochemical processes of microorganisms in all kinds of ecological niches [7] of fermented products [8] wastewater treatment plants [9] and to study resistomas of humans and animals [10].
Thus, understanding this and the accumulated scientific potential gives us the opportunity to study in detail the microecological status of the rumen when using substances that stimulate and inhibit bacterial groups in the diet of young cattle.

2. Materials and methods
The aim of the research was to study the influence of antibiotics, probiotics and prebiotics, both separately and in a composition with the plant extract, on the microecological status of the rumen of the cattle.

2.1. Object of study
Young cattle, rumen fluid. Animal care and experimental studies were performed in accordance with the instructions and recommendations of Russian Regulations, 1987 (Order No. 755 on 12.08.1977 the USSR Ministry of Health) and “The Guide for Care and Use of Laboratory Animals (National Academy Press Washington, DC 1996). Efforts have been made in the studies to minimize animal suffering and reduce the number of samples.

2.2. Experiment scheme
The studies were carried out in the conditions of the Federal Scientific Center for Biological Systems and Agrotechnologies of the Russian Academy of Sciences (FSC BST RAS). The plant extract Quercus cortex (QC), a prebiotic based on mannanooligosaccharides and beta-glucans, a probiotic based on Bifidobacterium adolescentis and Lactobacillus acidophilus, a feed antibiotic based on chlortetracycline (20%) were used as substances to optimize digestive processes in the rumen of cattle.

During the experiment, the animals were divided into 7 groups (n = 3). Control - basic diet (RR), I experimental group - RR + feed antibiotic (10 g / bird / day 30 days), II - RR + food antibiotic + extract (50 ml / bird), III - RR + probiotic (25 g / bird / day), IV - OR + probiotic + extract, V - OR + prebiotic (15 g / bird / day), VI - OR + prebiotic + extract.

Ruminal fluid was collected through a chronic fistula of the bulls' rumen 3 hours after feeding. The samples were filtered through a four-layer sterile gauze, into sterile cryovials and placed in an ultra-low-temperature laboratory freezer at t = -80 °C, followed by isolation of purified DNA preparations.

Genomic DNA was isolated using a chemical extraction method. DNA concentration was determined using a Qubit 2.0 fluorometer with high sensitivity dsDNA assay (Life Technologies). The preparation of DNA libraries, as well as sequencing, were carried out at the Center for Shared Use of the ICVS Ural Branch of the Russian Academy of Sciences (Orenburg, Russia).

2.2.1. DNA Isolation. After thawing, a mixture of glass beads d = 0.1 and 0.5 mm was added to 400 µl of the suspension in a volume equal to about 1/3 of the volume of liquid in the test tube. The mixture was homogenized using a Tissue Lyser homogenizer for 3 min at maximum speed. The suspension was incubated at 950C for 10 min. It was vortexed and centrifuged for 10 min at 14500 rpm. The supernatant was transferred to a new tube. While the samples were centrifuged, 200 µl of Binding Matrix solution was added to clean 2.0 mL conical tubes. The SPIN Filter column was transferred to a clean 1.9 ml Catch tube, 50 µl TES was added. The mixture was stirred to resuspend the matrix. The samples were centrifuged at 14000 rpm for 2 minutes to elute the purified DNA into a clean Catch tube. The taxonomic composition of the rumen content was determined by NGS sequencing on a MiSeq device (Illumina, USA).

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, targeting the V3 and V4 regions of the SSU rRNA gene, such as forward SD-Bact-0341-bS-17 and reverse SD-Bact-0785-aA-21.
2.3. Statistical processing
Data are expressed as mean values ± standard error of the mean. Statistical analysis was performed using Statistica 10.0 (StatSoft Inc., USA) and Microsoft Excel (Microsoft, USA). Significance of the group differences was estimated using Student’s t-test with p≤0.05 considered as significant.

3. Results
Metagenomic analysis of cicatricial fluid showed that the microecology of the rumen of cattle in the control group (CG) was 82.1% bacteria and 17.9% microscopic fungi. A study of the microecology of the rumen of animals when using an antibiotic in the diet showed a decrease in the number of bacteria by 21.9% and an increase in microscopic fungi by 79.6% of the total.

The use of a probiotic and a mixture of a probiotic with the extract in the diet reduced the number of bacteria by 2.5 and 69.9%, there was an increase in the number of microscopic fungi by the same values relative to the control. Similar values were obtained when prebiotic and probiotic were used in the diet together with the extract, the growth of microfungi increased by 76.5 and 71.0% relative to the reduction in the number of bacteria.

The taxonomic composition of cattle rumen (CG) (was represented by such phyla as: Firmicutes (32.4% of control), Saccharibacteria (18.7% of control), Proteobacteria (12.8% of control), Fibrobacteres (5.04% of control) control) of the total number of microorganisms. The rest together accounted for 1% of the total number of bacteria, where the dominant classes were Bacilli (17.3% of control), Gammaproteobacteria (12.8% of control), Bacteroidia (30.1% of control) and Clostridia (11.1% of control). The species diversity in the rumen contents was represented by bacteria belonging to the genera Lactobacillus (13.9% of the control), Prevotella (18.1% of the control), Escherichia (5.04% of the control), Enterobacter (5.25% of the control), Faecalibacterium, Escherichia, Fibrobacter decreased slightly.

The introduction of an antibiotic into the diet influenced the ratio of gram-negative and gram-positive microflora of the rumen. There was an increase in the number of bacteria of the class Bacteroidia and Clostridia by 55.7 and 2.4% of the total, and a decrease in the class Gammaproteobacteria by 6.07% of the control. Bacteria belonging to the genus Prevotella (Prevotella bryantii) prevailed by 55.9% more than the control, the bacteria of the genera Lactobacillus, Faecalibacterium, Escherichia, Fibrobacter decreased slightly.

The introduction of an antibiotic into the diet in a composition with an extract showed a similar tendency, there is an increase in microorganisms of the phylum Bacteroidetes (by 42.9% from control), Firmicutes (by 13.3% from control) and a decrease in the number of representatives of the Proteobacteria taxon by 6.89% in comparison with control, Saccharibacteria and Fibrobacteres to less than 2% of the total. There was an increase in the number of bacteria of the Clostridia class (by 5.6% of the control) and Bacteroidia (by 42.9% of the control), and a decrease in the number of microorganisms of the classes Gammaproteobacteria (by 6.89% of the control), Bacteroidia (up to 2% of the total numbers) and Bacilli (up to 2% of the total). Species diversity showed an increase in the number of Prevotella bacteria (54.5% of the control), and a decrease in the number of bacteria Lactobacillus, Faecalibacterium, Escherichia, Fibrobacter, and others less than 2% of the total (table 1). The use of a probiotic in the diet led to significant changes in the ratio of the number of gram-negative and gram-positive bacteria in the cicatrical content in relation to the control. There was an increase in the number of bacteria of the phylum Bacteroidetes (32% of the control), in particular of the class Bacteroidia (by 32% of the control), and a decrease in the number of Firmicutes bacteria by 2.9%, Proteobacteria by 19%, Saccharibacteria and Fibrobacteres to 2% of the total number. microorganisms, this was expressed in a decrease in representatives of the classes Bacilli (by 14.9% from the control), Gammaproteobacteria (by 7.88% from the control), and an increase in bacteria of the Clostridia class (by 13.2% from the control). The most common bacterial species compared to the control was Prevotella 37.2% more than the control. A significant decrease in the species relative to the control occurred in the group of bacteria p. Lactobacillus by 11.5%.
Table 1. Taxonomic diversity of the bacterial composition of the rumen when using various additives in the diet.

| Group                                      | phylum          | class               | bloodline                | genus                       |
|--------------------------------------------|-----------------|---------------------|--------------------------|-----------------------------|
| Cattle rumen (CG) bacteria, before feeding |                 |                     |                          |                             |
| Firmicutes (32.4%)                         | Bacilli (17.3%)  | Lactobacillaceae    | Lactobacillus (13.9%)    |
|                                           |                 | Lachnospiraceae     | (6.93%)                  |
|                                           |                 | Clostridia (11.1%)  | Faecalibacterium (4.2%)  |
|                                           |                 | Acidaminococcaceae  | Succinilasticum (3.99%)  |
|                                           |                 | Moraxellaceae (2.52%)| Acinetobacter (2.52%)    |
| Cattle rumen (CG+feed antibiotic) bacteria, 3 hours after feeding |                 |                     |                          |                             |
| Bacteroidetes (30.1%)                      | Bacteroidia (30.1%) | Prevotellaceae    | Prevotella (19.4%)        |
|                                           |                 | Selenomonadaceae    | Selenomonas (3.8%)        |
|                                           |                 | Lachnospiraceae     | (12.5%)                  |
|                                           |                 | Succinivibrionaceae | Succinivibrio (6.73%)     |
| Cattle rumen (CG+feed antibiotic+extract) bacteria, 3 hours after feeding |                 |                     |                          |                             |
| Bacteroidetes (73.0%)                      | Bacteroidia (73.0%) | Prevotellaceae    | Prevotella (72.6%)        |
|                                           |                 | Lachnospiraceae     | (16.6%)                  |
|                                           |                 | Selenomonadaceae    | Selenomonas (2.22%)       |
|                                           |                 | Succinivibrionaceae | Succinivibrio (5.91%)     |
| Cattle rumen (CG+probiotic) bacteria, 3 hours after feeding |                 |                     |                          |                             |
| Bacteroidetes (62.1%)                      | Bacteroidia (62.1%) | Prevotellaceae    | Prevotella (55.3%)        |
|                                           |                 | Ruminococcaceae     | Ruminococcus (6.85%)      |
|                                           |                 | Lachnospiraceae     | Lachnospiraceae (10.1%)   |
The use of a probiotic in feeding in a composition with an extract was marked by an actually opposite ratio of gram-positive and gram-negative microflora of the rumen. There was a decrease in representatives of the classes Clostridia (by 5.74% from control), Bacteroidia (by 12.2% from control), Fibrobacteria by 2.07% and Saccharibacteria to 2% or less of the total number, and an increase in the number of bacteria belonging to the taxon Gammaproteobacteria (by 54.1% of control), this was expressed in a change in the number of bacteria of the families Succinivibrionaceae (an increase of 66.9%), Lachnospiraceae (a decrease of 6.5%), Lactobacillaceae (a decrease of 13.1%), Ruminococcaceae (an increase of 5%), Fibrobacteraceae (a decrease of 2.07%), etc. The predominant species category was Prevotella (by 2.2%), Fibrobacter (by 2.07%), Lactobacillus (up to less than 2% of the total), Faecalibacterium (up to less than 2% of the total), Escherichia (up to less than 2% of the total), etc. compared to the control, respectively.

The introduction of a prebiotic into the diet of cattle increased the number of bacteria in the rumen belonging to the phylum Bacteroidetes (by 24.3% from control), and a decrease in the number of Firmicutes by 17.6%, Saccharibacteria by 16.1% compared to control and Fibrobacteria by 2% of the total. Data analysis showed an increase in bacteria of the Bacteroidia class (by 24.3% from the control), and a decrease in bacteria of the Clostridia class (by 2.92% from the control). Among the identified taxonomic categories, bacteria belonging to the taxon Bacteroidaceae prevailed (by 30% from the control), and a decrease in bacteria belonging to the Prevotellaceae family by 2.6% and the families of Lactobacillaceae, Ruminococcaceae, Clostridiaceae, Lachnospiraceae to 2% and less of the total. The

| Bacteria | Negativicutes | Selenomonadaceae | Selenomonas | Bacilli | Lactobacillaceae | Lactobacillus | Gammaproteobacteria | Succinivibrionaceae | Succinivibrio | Others |
|----------|--------------|-----------------|-------------|--------|-----------------|---------------|---------------------|-------------------|-------------|--------|
| Proteobacteria (6.24%) | Negativicutes (2.76%) | Selenomonadaceae (2.76%) | Selenomonas (2.45%) | Bacilli (2.42%) | Lactobacillaceae (2.42%) | Lactobacillus (2.42%) | Gammaproteobacteria (4.92%) | Succinivibrionaceae (4.92%) | Succinivibrio (4.92%) | Others (2.2%) |

| Cattle rumen (CG+probiotic+extract) bacteria, 3 hours after feeding | Bacteroidetes (17.9 %) | Bacteroidia (17.9%) | Prevotellaceae (17.9%) | Prevotella (15.9%) |
|-----------------|------------------|------------------|---------------------|----------------|
| Firmicutes (8.01%) | Clostridia (7.46%) | Ruminococcaceae (5.46%) | - |
| Proteobacteria (67.9%) | Gammaproteobacteria (66.9%) | Succinivibrionaceae (66.9%) | - |
| Fibrobacteres (2.97%) | Fibrobacteria (2.97%) | Fibrobacteraceae (2.97%) | Fibrobacter (2.97%) |
| Others (3.2 %) | Others (4.8%) | Others (6.8%) | Others (8.12%) |

| Cattle rumen (CG+prebiotic) bacteria, 3 hours after feeding | Bacteroidetes (54.4%) | Bacteroidia (54.4%) | Prevotellaceae (16.8%) | Prevotella (16.8%) |
|-----------------|------------------|------------------|---------------------|----------------|
| Firmicutes (14.8%) | Clostridia (8.18%) | Lachnospiraceae (2.2%) | Clostridiaceae (2.91%) |
| Saccharibacteria (2.58%) | - | - | - |
| Others (28.2 %) | Others (37.4%) | Others (40.5%) | Others (45.6%) |

| Cattle rumen (CG+prebiotic+extract) bacteria, 3 hours after feeding | Bacteroidetes (70.6%) | Bacteroidia (70.6%) | Prevotellaceae (65.3%) | Prevotella (65.3%) |
|-----------------|------------------|------------------|---------------------|----------------|
| Firmicutes (7.31%) | Clostridia (5.75%) | Lachnospiraceae (2.73%) | - |
| Fibrobacteres (21.3%) | Fibrobacteria (21.3%) | Fibrobacteraceae (21.3%) | Fibrobacter (21.3%) |
| Others (0.8%) | Other (2.3%) | Other (6.8%) | Other (13.4%) |

* - this group includes taxa, the number of each of which does not exceed 2% of the total number.

The use of a probiotic in feeding in a composition with an extract was marked by an actually opposite ratio of gram-positive and gram-negative microflora of the rumen. There was a decrease in representatives of the classes Clostridia (by 5.74% from control), Bacteroidia (by 12.2% from control), Fibrobacteria by 2.07% and Saccharibacteria to 2% or less of the total number, and an increase in the number of bacteria belonging to the taxon Gammaproteobacteria (by 54.1% of control), this was expressed in a change in the number of bacteria of the families Succinivibrionaceae (an increase of 66.9%), Lachnospiraceae (a decrease of 6.5%), Lactobacillaceae (a decrease of 13.1%), Ruminococcaceae (an increase of 5%), Fibrobacteraceae (a decrease of 2.07%), etc. The predominant species category was Prevotella (by 2.2%), Fibrobacter (by 2.07%), Lactobacillus (up to less than 2% of the total), Faecalibacterium (up to less than 2% of the total), Escherichia (up to less than 2% of the total), etc. compared to the control, respectively.

The introduction of a prebiotic into the diet of cattle increased the number of bacteria in the rumen belonging to the phylum Bacteroidetes (by 24.3% from control), and a decrease in the number of Firmicutes by 17.6%, Saccharibacteria by 16.1% compared to control and Fibrobacteria by 2% of the total. Data analysis showed an increase in bacteria of the Bacteroidia class (by 24.3% from the control), and a decrease in bacteria of the Clostridia class (by 2.92% from the control). Among the identified taxonomic categories, bacteria belonging to the taxon Bacteroidaceae prevailed (by 30% from the control), and a decrease in bacteria belonging to the Prevotellaceae family by 2.6% and the families of Lactobacillaceae, Ruminococcaceae, Clostridiaceae, Lachnospiraceae to 2% and less of the total. The
species composition was dominated by bacteria: Bacteroides (up to 30% of the total), there was a decrease in the number of microorganisms Prevotella (by 2.6%), Escherichia (to less than 2% of the total), Enterobacter (up to less than 2% of the total), Fibrobacter (up to less than 2% of the total) and Lactobacillus (up to less than 2% of the total).

The use of a composition of a prebiotic and an extract in the diet favorably influenced an increase in the number of bacteria Bacteroidetes and Fibrobacteres by 40.5 and 16.3% of the control, and a decrease in the number of phyla Firmicutes by 2.51% and Saccharibacteria to less than 2% of the total number. Changes in the ratio of bacteria were associated with the predominance of the class Bacteroidia (by 40.5% from the control), Fibrobacteria by 16.3%, and a decrease in representatives of the class Bacilli to less than 2% and Clostridia by 5.35% from the control, respectively. Within the Clostridia taxon, a decrease in the number of bacteria Lachnospiraceae (by 4.2%) and Clostridiaceae (to less than 2% of the total number) was observed, while the number of microorganisms Bacteroidia and Fibrobacteria increased, respectively, the number of microorganisms Bacteroidia (by 47.2 %). In the species composition, the number of Escherichia bacteria (to less than 2% of the total number), Enterobacter (to less than 2% of the total number), etc. decreased, and the number of microorganisms Fibrobacter (by 16.3%), Prevotella (by 47.2 %).

4. Discussion
About 90% of the total microflora in the cattle rumen consists of Firmicutes and Bacteroidetes, with a large interindividual variability, with an inverse correlation between the abundance of both types [11]. In our studies in all groups the phyla Firmicutes and Bacteroidetes were of greater importance. Their ratio directly depended on the use of the studied substances in the diet. The representatives of the taxon Firmicutes started from 7.31% to 32.4%, the phylum Bacteroidetes - from 17.9% to 74.5% of the total number of bacteria. Another value was in the groups that received the probiotic and prebiotic in a composition with the extract; in the first group, the phylum Proteobacteria (67.9% of the total number) occupies the greatest value relative to Firmicutes, Bacteroidetes (8.01% and 17.9% of the total number), in the second phylum Fibrobacteres (21.3% of the total number) relatively to Firmicutes (7.31% of the total number), the predominant representative of the taxon was Bacteroidetes 70.6% of the total number of microorganisms. Bacteroides is a gram-negative microorganism, it uses plant glycans as its main energy sources, is one of the main bacteria involved in the production of short-chain fatty acids [12], and also plays an important role in the breakdown of complex compounds into simple molecules necessary for animal growth [13].

Analysis of the data showed that the introduction of probiotics, prebiotics, antibiotics, both separately and in a composition with a plant extract, decreases the diversity of the bacterial composition relatively to the control group. Scientists have found that low levels of microbes in the rumen are closely associated with higher feed efficiency in dairy cows [14]. The authors suggested that lower rumen saturation in animals leads to a simpler metabolic chain, and this leads to high concentrations of certain enzymatic substances that are used to support the energy requirements of animals [15]. In our experiment, in groups where a plant extract was used as a feed additive, the bacteria responsible for the digestion of fiber, in particular Prevotellaceae, increased, this is probably due to the fact that the tannins that make up the extract reconstruct the bacterial ecosystem of the rumen, especially the niche of fiber and starch decomposition [16].

The advent of next-generation sequencing technologies, such as 16S rRNA gene sequencing, has made it possible to characterize the structure of the gut microbiome without the need for culturing [17], as well as to the widespread use of metagenomic analysis to study complex intestinal ecosystems such as the rumen [18].

5. Conclusion
The results obtained indicate that the 16S Metagenomics method is promising for studying the microecological status of the cattle rumen. The topic requires further research to maximize the potential for correcting the microecological status of the rumen of ruminants.
Acknowledgments
This research was performed with the financial support from the Russian Science Foundation (project No. 16-16-10048).

References
[1] Jami E and Mizrahi I 2012 Composition and similarity of bovine rumen microbiota across individual animals PLoS One 7(3) e33306
[2] Morgavi D P, Kelly W J, Janssen P H and Attwood G T 2013 Rumen microbial (meta)genomics and its application to ruminant production Animal 7(1) 184-201
[3] Henderson G, Cox F, Ganesh S, Jonker A and Young W 2015 Rumen microbial community composition varies with diet and host but a core microbiome is found across a wide geographical range Sci. Rep. 5 14567
[4] Duskaev G K, Rakhmatullin S G, Kazachkova N M, Sheida Y V, Mikolaychik I N, Morozova L A and Galiev B H 2018 Effect of the combined action of Quercus cortex extract and probiotic substances on the immunity and productivity of broiler chickens Veterinary World 11(10) 1416-22
[5] Hall A B, Tolonen A C and Xavier R J 2017 Human genetic variation and the gut microbiome in disease Nat. Rev. Genet. 18(11) 690-9
[6] Schären M, Frahm J, Kersten S, Meyer U, Hummel J, Breves G and Dänicke S 2018 Interrelations between the rumen microbiota and production, behavioral rumen fermentation metabolic and immunological attributes of dairy cows J. Dairy Sci. 101(5) 4615-37
[7] Li F and Guan L L 2017 Metatranscriptomic profiling reveals linkages between the active rumen microbiome and feed efficiency in beef cattle Appl. Environ Microbiol 83(9) e00061-17
[8] Myer P R, Smith T P L, Wells J E, Kuehn L A and Freetry H C 2015 Rumen microbiome from steers differing in feed efficiency PLoS One 10(6) e0129174
[9] Deryabin D, Galadzhieva A, Kosyan D and Duskaev G 2019 Plant-derived inhibitors of AHL-mediated quorum sensing in bacteria: Modes of action International Journal of Molecular Sciences 20(22) 5588
[10] Yanan Wang, Yongfei Hu and George Fu Gao 2020 Combining metagenomics and metatranscriptomics to study human, animal and environmental resistomes Medicine in Microecology 3 100014
[11] Nawab A, Li G, An L, Nawab Y, Zhao Yi, Xiao M, Tang S and Sun C 2020 The potential effect of dietary tannins on enteric methane emission and ruminant production, as an alternative to antibiotic feed additives – a review Annals of Animal Science 20(2) 355-88
[12] Kaakoush N O, Sodhi N, Chen J W, Cox J M, Riordan S M and Mitchell H M 2014 The interplay between Campylobacter and Helicobacter species and other gastrointestinal microbiota of commercial broiler chickens Gut. Pathog. 6 18
[13] Lan P T N, Sakamoto M, Sakata S and Benno Y 2006 Bacteroides barnesiae sp. nov., Bacteroides salanitronis sp. nov. and Bacteroides gallinarum sp. nov., isolated from chicken caecum Int. J. Syst. Evol. Microbiol. 56 2853-9
[14] Shabat S K, Sasson G, Doron-Faigenboim A, Durman T, Yaacob Y, Miller M B, White B, Shitzer Z and Mizrahi I 2016 Specific microbiome-dependent mechanisms underlie the energy harvest efficiency of ruminants ISME J 10 2958-72
[15] Rivera-Méndez C, Plascencia A, Torrentera N and Zinn R A 2017 Effect of level and source of supplemental tannin on growth performance of steers during the late finishing phase J. Appl. Anim. Res. 45 199-203
[16] Duskaev G K, Karimov I F, Levakhin G I, Nurzhanov B S, Rysaev A F and Dusaeva K B 2019 Ecology of ruminal microorganisms under the influence of Quercus cortex extract International Journal of GEOMATE 16(55) 59-66
[17] Lima J, Auffret M D, Stewart R D, Dewhurst R J, Duthie C A, Snelling T J, Walker A W, Freeman T C, Watson M and Roehe R 2019 Identification of rumen microbial genes involved in
pathways linked to appetite, growth, and feed conversion efficiency in cattle *Front. Genet.* 10 701

[18] Atlanderova K, Makaeva A, Miroshnikov S and Ivanishcheva A 2019 Changes in rumen microbiota of cattle with the simultaneous introduction of iron and copper nanoparticles and quorum sensing suppressants *FEBS Open Bio* 9(S1) 415-6