Evaluation of the effects of plant extracts on cattle rumen microbiome

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Abstract. The prohibition of antibiotic drugs in animal husbandry to stimulate animal productivity has provoked an active study of alternative substances, including plant extracts. No less interesting for a detailed study of the extracts of plants Eucalyptus viminalis and Scutellaria baicalensis, as information about their effect on ruminant microbiome is extremely scarce. According to the results of phytochemical analysis, 14 small molecules were found in the extract of Scutellaria baicalensis and 13 in the composition of the extract of the leaves of Eucalyptus viminalis. Analysis of metagenomic sequencing when plant extracts are included in the feed indicates a significant change in the rumen microbiome. The absence of Proteobacteria and Firmicutes phyla, and an increase in Bacteroidetes by 12.0–17.0% and an increase in Firmicutes (p≤0.05) and Saccharibacteria (p≤0.05) were noted. An increase in the class Clostridia (p≤0.05), due to the family Ruminococcaceae (p≤0.05) and the genus Ruminococcaceae, was found. In the Bacteroidia class in all groups, the family Prevotellaceae, the genus Prevotella, prevailed in most of the groups. Additional inclusion in the diet of plant extracts of Eucalyptus viminalis and Scutellaria baicalensis have a significant impact on the rumen microbiome of the cattle, the taxonomic identification of microorganisms indicates the prevalence of phyloma Firmicutes, Clostridia class and the Ruminococcaceae family, which are responsible for the separation of imperfect migration. Further evaluation of plant matter is needed in the direction of the overall effect on fermentation of feed in the rumen.

1. Introduction
The prohibition of antibiotic drugs in animal husbandry to stimulate animal productivity provoked an active study of alternative substances, including plant extracts. Recent studies have focused on the search for substances capable of causing inhibition or activation of the production of substances by the microbiome [1–4]. Vegetable substances (essential oils, saponins, tannins, etc.) have been found to have an effect on the metabolism in the rumen of the ruminant [5] and have an “antiquorum” effect [6].

It is noted that a detailed study of plant products that affect the microbiota of the rumen and the fermentation profile [7] is necessary to reduce the production of toxins by pathogenic bacteria [8], to study the anthelmintic effect (Lantana Camara plant extracts, Bocconia frutescens, Piper auritum, Artemisia mexicana and Cajanus cajan) [9].
The recent screening of plant essential oils in vitro confirms their influence on fermentation characteristics and methane production in the rumen [10], bioactive compounds of essential oils can act as feed additives, however, further studies are needed to clarify their exact effect and use in animal husbandry [8].

Evaluation of thirteen plant leaf extracts for antimicrobial susceptibility to bacteria causing cattle diarrhea, found that an aqueous extract of Caryocar brasiliense Cambess is more active against strains of E. coli, Schinopsis brasiliensis - against Staphylococcus spp. Flavonoids and tannins were the major metabolites found in extracts [12]. Colophospermum mopane, S. persica, and D. cinerea exhibited antibacterial activity against the clinical isolates Staphylococcus aureus and Escherichia coli [13].

In our opinion, extracts of plants Eucalyptus viminalis and Scutellaria baikalensis are no less interesting for a detailed study, since information about their effect on the ruminant microbiome is extremely scarce. It is noted that the additives of Scutellaria baikalensis did not affect the biomarkers of inflammation and metabolism in the body against the background of an increase in milk yield during lactation of cows [14], positively influenced the prevention of oxidative metabolism in dairy cows [15], the oil of this plant has the potential to increase immune response in poultry [16]. In turn, the flour Eucalyptus Camaldulensis can modify the fermentation of the rumen ruminant [17].

2. Materials and methods
Cattle, bull-calves with rumen fistula, age - 12 months. Animal maintenance and experimental studies were performed in accordance with the instructions of the Russian Regulations, 1987 (Order No.755 on 12.08.1977) and "The National Academy Press Waschington, D "S. 1966". In doing research, efforts were made to minimize the suffering of animals and reduce the number of samples used. Keeping animals - on a leash in the room, feeding - twice a day. Three groups of animals (n = 5). An experimental study was carried out based on the center for collective use of scientific equipment of the Federal Scientific Center of the BSE RAS.

The control group of animals - the main ration (MR), consisting of hay (70%), grain feed (30%), 1 experimental group - OR + Eucalyptus viminalis extract (100 ml / goal), 2 experimental group-PA - OR + extract Scutellaria baikalensis (200 ml / goal).

Extracts of plant substances were obtained in accordance with the recommendations of the manufacturer, followed by filtration.

The phytochemical analysis of the extract was carried out on a gas chromatograph with a mass selective detector GCMS 2010 Plus (Shimadzu, Japan). For identification of compounds, a set of libraries of spectra CAS was used. The number of components was estimated by the value (%) - the ratio of the peak area to the total extract area. The extract was administered orally through the fistula of the scar.

Scar fluid samples were taken 3 hours after feeding. The content of the scar or chymus was filtered immediately after collection.

Metagenomic analysis of rumen microorganisms. The analysis of microflora was carried out by the method of metagenomic sequencing (Illumina MiSeq, Illumina, USA) with the MiSeq® Reagent Kit v3 reagent kit (600 cycle). For bioinformatical processing of the results, the PEAR program (Pair-End Assembler, PEAR v0.9.8) was used.

Statistical processing was performed using the IBM "SPSS Statistics Version 20" program, the average value (M), the standard deviation (σ), and the standard deviation error (m) were calculated. The significance level was considered significant at p <0.
**Table 1.** Evaluation of the microbial biodiversity of the rumen when fed in the rations of young cattle extracts of eucalyptus eucalyptus (Eucalyptus viminalis) and Baikal skullcap (Scutellaria baicalensis)

| Group | phylum          | class           | family                | genus                        |
|-------|-----------------|-----------------|-----------------------|------------------------------|
|       | **Firmicutes**  | **Bacteroidetes** | **Porphyromonadaceae** |                              |
|       | Bacilli (17.3±0.42 %) | Bacteroidia (30.1±1.35 %) | Lactobacillaceae (13.9±0.36 %) | Lactobacillus (13.9±0.36 %) |
|       | Clostridia (11.1±0.25 %) | Bacteroidia (30.1±1.35 %) | Clostridiales (2.2±0.06 %) | Clostridium (3.6±0.11 %) |
|       | Negativicutes (3.99±0.12 %) | Bacteroidia (30.1±1.35 %) | Acidaminococcaceae (3.99±0.12 %) | Succinibacter (2.2±0.023 %) |
|       | **Proteobacteria** | **Fibrobacteres** | **Porphyromonadaceae** |                              |
|       | Bacteroidetes (12.8±0.78 %) | Bacteroidia (3.4±0.34 %) | Bacteroidales (2.5±0.14 %) | Bacteroides (2.2±0.023 %) |
|       | Bacteroides (12.9±0.41 %)* | Bacteroidia (2.6±0.027 %) | Bacteroides (2.6±0.014 %) | Bacillus (4.8±0.17 %) |
|       | Saccharibacteria (18.7±0.89 %) | Saccharibacteria (2.7±0.07 %) | Bacillus (2.9±0.012 %) | Bacillus (2.2±0.023 %) |
|       | Other (1±0.02 %) | Other (19.7±0.86 %) | Other (23±0.87 %) | Other (42±1.22 %) |
|       | Ruminococcus (34.0±0.52 %) | Ruminococcus (3.6±0.11 %) | Ruminococcus (5.8±0.10) |                              |
|       | Clostridiales (2.2±0.06 %) | Acidaminococcaceae (2.2±0.023 %) | Prevotellaceae (6.1±0.21 %) | Prevotella (4.8±0.17 %) |
|       | Negativicutes (2.64±0.027 %) | Prevotellaceae (6.1±0.21 %) | Prevotellaceae (5.7±0.06 %) |                                |
|       | Bacteroides (3.4±0.34 %) | Bacteroides (3.4±0.34 %) | Bacteroides (3.4±0.34 %) |                                |
|       | Saccharibacteria (18.7±0.89 %) | Saccharibacteria (2.7±0.07 %) | Saccharibacteria (2.7±0.07 %) |                                |
|       | Other (1±0.02 %) | Other (19.7±0.86 %) | Other (23±0.87 %) | Other (42±1.22 %) |
|       | Ruminococcus (24.7±0.19 %) | Ruminococcus (12.6±0.12 %) | Ruminococcus (4.8±0.06) |                              |

* - p<0.05, significance of differences compared to control
3. Research results and discussion

According to the results of phytochemical analysis, small molecules were found in the composition of the extract of Baikal skullcap (Scutellaria baicalensis): 3,5-dihydroxy-6-methyl-2,3-dihydropyran-4-one; Lactone G; Dianhydroglucitol, TBS 2x; 7,8-dihydroxy-2h-cromen-2-one; 4H-1-Benzopyran-4-one, 5,7-dihydroxy-6-methoxy-2-phenyl-; Flavone, 5,7-dihydroxy-8-methoxy- 5,7-dihydroxy-8-methoxy-2-phenylethromen-4-one; 5,7-Dihydroxy-3,8-dimethoxyflavone; 5,6,7-Trihydroxy-2-phenethylchromen-4-one; 2,4-Dihydroxy-; 2,5-dimethyl-3(2H)-furan-3-one; 1,4-Dioxin, 2,3-dihydro-5,6-dimethyl; 2-Furanone, 3,4-dihydroxytetrahydro.

Small molecules identified as part of eucalyptus leaf extract (Eucalyptus viminalis): 2,4-dihydroxy-2,5-dimethylfuran-3-one; 1-Methyl-4-(1-methylethyl)benzene; 3-Hydroxy-2-methyl-4H-pyran-4-one; 3,4-dihydroxyxolan-2-one; 2-ethyl-5-methyl-1,3,2-dioxaborolan-4-one; 5-ethylxolan-2-one; 2(3H)-Furanone, 5-butylidihyro; [(3S,3aS,6R,6aS)-3-nitrooxy-2,3,3a,5,6,6a-hexahydrofuro[3,2-b]furan-6-yl] nitrate; 4-hydroxy-3-methyl-6-propan-2-ylcyclohex-2-en-1-one; 2-hydroxy-3-phenylpropanoic acid; Shiikimic acid /r(3R,4S,5R)-3,4,5; (6S,7aR)-6-hydroxy-4,4,7a-trimethyl-6,7-dihydro-5H-1-benzofuran-2-one; 2’-(2R,4aS,6R)-4a,8-dimethyl-3,4,5,6,7,8-hexahydro-2H-naphthalen-2-yl)propan-2-ol; 4H-1-Benzopyran-4-one, 5,7-dihydroxy-2-methyl.

Analysis of the metagenomic sequencing with the inclusion of extracts of plants Eucalyptus viminalis and Scutellaria baicalensis in the feed indicates a significant change in the rumen microbiome. The absence of Proteobacteria and Fibrobacteres phyla in the experimental groups was noted, the decrease in the content of Bacteroidetes by 12.0 - 17.0% and the increase in Firmicutes by 10.5 - 20.3% (p≤0.05) and Saccharibacteria (by 4.8 - 17.7%) (p≤0.05). An increase in the class Clostridia (by 29.1 - 41.1%; p≤0.05) was found due to the Ruminococcaceae family (p≤0.05) and the genus Ruminococcaceae. In the Bacteroidia class in all groups, the family Prevotellaceae, the genus Prevotella, prevailed in most of the groups.

The data obtained by Eucalyptus viminalis (1st experimental group) coincides with the studies performed earlier [17], where using eucalyptus (E. Camaldulensis) leaf flour in buffaloes resulted in a decrease in the concentration of ammonia nitrogen in the rumen; in our experiment, the relative absence of the phylum Proteobacteria was found responsible for protein metabolism.

In addition, depending on the origin of the essential oil [18], it can either increase the number of microorganisms in the Firmicutes phyla, especially in the Clostridia class, as in our experiment (Eucalyptus viminalis is rich in essential oils), or reduce their values.

In another experiment, the extract of Humulus lupulus [19], contributed to the reduction of representatives of the class Clostridia, in our case, the extracts, on the contrary, increased their number.

The extract of Scrophularia striata [20] increased the number of representatives of Fibrobacteres, in our experiment this trend was not observed. In addition, the use of secondary plant substances can increase the relative size of the bacterial community in herbivorous mammals [21].

The fact that microbial diversity is reduced by adding plant extracts is confirmed, including by previous studies, when using isoflavones in the diet, the changes affected bacteria belonging to the type Bacteroidetes, Proteobacteria, Firmicutes [22, 23, 24, 25], which is consistent with the results of our research.

4. Conclusion

Additional inclusion in the diet of plant extracts of Eucalyptus viminalis and Scutellaria baicalensis have a significant impact on the rumen microbiome of cattle, taxonomic identification of microorganisms indicates the prevalence of phylum Firmicutes, the class Clostridia and the Ruminococcaceae family, which are responsible for splitting laboriously, for the splitting of the Ruminococcaceae class, which are responsible for splitting the labor of Ruminococcaceae, which are responsible for the splitting of the organism, for the cleavage of the Ruminococcaceae class, and for the Ruminococcaceae family, which are responsible for splitting the organism. Further evaluation of plant matter is needed in the direction of the overall effect on fermentation of feed in the rumen.
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