Changing of the composition of the rumen microflora to improve the efficiency of feed use by ruminants

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Abstract. Ruminant animals use their symbiotic microorganisms in the rumen to hydrolyze plant fibers and generate energy and other nutrients, while the eukaryotic community (fungi) makes up about half of the total microbial biomass and plays a critical role in the effectiveness of use of lignocellulosic particles of feed components. The aim of the study was to develop a way to increase the efficiency of feed use by ruminants through the introduction of a composition of promising substances into the diet. The assessment of biodiversity included the following: sampling, outflow, purification, measurement of DNA concentrations, PCR, validation and normalization of libraries, followed by sequencing on the platform of high-performance sequencer MiSeq Illumina (USA). Introduction of new substances in the diet of cattle contributes to a change in the eukaryotic composition of the rumen microflora towards an increase in Ascomycota and Neocallimastigomycota phylums (when used together with Quercus cortex extract), which have cellulose and ligninolytic properties, more active development of Chytridiomycota (separate use of substances).

1. Introduction
Ruminant animals use their symbiotic microorganisms in the rumen to hydrolyze plant fibers and generate energy and other nutrients [1]. The rumen ecology consists of bacteria, archaea, protozoa, fungi, and bacteriophages [2]. In the microbial consortium, it was shown that the eukaryotic community (protozoa and fungi) accounts for approximately half of the total microbial biomass [3] and is believed to play a critical role in the degradation of lignocellulosic particles of feed components [4, 5]. In addition, cicatricial microbiomes have a great influence on ruminant health and productivity, and various plant extracts are able to modulate microbiomes [6, 7] improving digestion and feed fermentation [8, 9]. Some plant secondary compounds affect the methane content, ammonia production [10, 11] and the population of protozoa and bacteria in the rumen [12, 13].

The use of food ingredients and live microorganisms in animal husbandry is interesting; some of them have shown that they can selectively stimulate the growth of endogenous lactic acid and bifidobacteria in the intestine, improving host health [14, 15]. Food ingredients selectively stimulate the growth and activity of one or a small number of bacterial species already living in the colon and, thus, improving the animal’s health [16]. In agriculture, live microorganisms are used as animal feed, as well as potential alternatives to antibacterial substances, as growth stimulants, and in some cases, they are used to control specific intestinal pathogens [17, 18].

At the same time, there is insufficient knowledge of the impact of these substances on certain types of rumen microflora in ruminants, due to their complex structure of the stomach.
The aim of this study was to evaluate the ecological profile of eukaryotes (fungi) of cattle rumen against the background of additional feed ingredients to increase the efficiency of animal feed use.

2. Materials and Methods

Object of Study. Bulls of red steppe breed (dairy) with scar fistula, (n=3, in 5 repetitions). The selection of ruminal fluid for analysis was carried out 3 hours after feeding.

Feeding ration: 70 % of roughage (alfalfa and Sudan grass) and 30 % of granules containing barley grain, wheat bran, waste sunflower oil, limestone, salt, vitamins and premix. Chemical composition of the diet: (g/kg DM (dry matter) was 165 non-degradable protein, 485 neutral detergent fiber, 47 raw fats, 85 ash and metabolic energy 10.00 MJ/kg CB. The feed was set twice a day and the animals had free access to drinking water.

Substances. Quercus cortex extract – extraction in water 50 g/200 ml (30 min/70 С), followed by drying to dry matter (60 С, within 24 hours) – dose 5 g/head/day.

The live microorganisms (LM) are based on Bifidobacterium adolescentis – 80.0 CFU and Lactobacillus acidophilus – 1.0 million CFU (20 g/head/day for 30 days).

A food ingredient (FI) is based on mannanoligosaccharides (20 %) and beta-glucans (8 %) – 15 g/head/day throughout the experiment, 30 days.

Study Flow. Control – the main diet (MD), group I – MD+probiotic, group II – MD+probiotic+extract, group III – MD+prebiotic, IV – MD+prebiotic+extract.

Experimental studies were conducted in the center for collective use of scientific equipment of Federal Scientific Center, RAS.

Microbial Rumen Analysis. Assessment of microbial biodiversity included the following: sampling, outflow, purification, measurement of DNA concentrations, PCR, validation and normalization of libraries, followed by sequencing on the platform of high-performance sequencer MiSeq Illumina (USA). Bioinformatic processing of the results was carried out using PEAR program (Pair-End AssembeR, PEAR v0.9.8, April 9, 2015) (Zhang et al., 2014).

3. Research Results and Discussion

The analysis of data showed that the rumen microbiocenosis before cattle feeding was represented by 82.1 % of bacteria and 17.9 % of microscopic fungi. The use of LM and a mixture of LM with the extract in the diet after 3 hours of exposure contributed to a decrease in bacterial content by 2.5 and 69.9 % and an increase in the number of unicellular fungi by the same values, respectively. Similarly to probiotics, the use of FI and FI with the extract in the diet resulted in an increase in the number of fungi by 76.5 and 71 % 3 hours after feeding and a decrease in the number of bacteria.

The study of taxonomic diversity of microscopic fungi of cattle rumen before feeding showed that the majority of identified microorganisms belonged to the departments of Ascomycota (43.3 % of the total number) and Chytridiomycota (16.9 % of the total number), represented mainly by the classes Saccharomycetes (43.3 % of the total number) and Chytridiomycetes (16.9 % of the total number). The analysis of species diversity showed that the most numerous were microorganisms belonging to the cattle. Vanderwaltozyma (19.2 % of the control group), the rest microorganisms, which were identified belonged to taxa, the percentage of which was less than 2 % of the total number (Table 1).

The use of LM in the diet was accompanied by a decrease in the number of microorganisms in the rumen belonging to Chytridiomycota taxa by 3.9 % of the control group, and a significant increase in the size of the Ascomycota by 22.9 % of the control group and Neocallimastigomycota to 16.5 % of the total number, which was reflected in a percentage change in the microbiocenosis of representatives of the Saccharomyce classes (+6.3 %), Dothideomycetes (increase to 16.5 % of the total), Chytridiomycetes (–4.3 %) and Neocallimastigomycetes (increase to 16.5 % of the total number). In the species composition, there was observed an increase in the number of p. Vanderwaltozyma by 9.2 % of control, Davidiella and Cyllamyces up to 16.6 and 16.5 % of the total number.
| Group          | Phylum             | Class             | Family            | Genus               |
|---------------|--------------------|-------------------|-------------------|---------------------|
| Control (MD)  | Ascomycota         | Saccharomyces     | Saccharomycetaceae| Vanderwaltozyma     |
|               | (43.3±2.81 %)      | (43.3±2.81 %)     | (43.3±2.81 %)     | (19.2±1.13 %)       |
|               | Chytridiomycota    | Chytridiomycetes  | Chytriomycetaceae | Other*              |
|               | (16.9±0.83 %)      | (16.9±0.83 %)     | (16.9±0.83 %)     | (24.1±1.68 %)       |
|               | Other*             | Other*            | Other*            | Other*              |
|               | (39.8±2.52 %)      | (39.8±2.52 %)     | (39.8±2.52 %)     | (39.8±2.52 %)       |
| MD+ LM        | Ascomycota**       | Saccharomyces     | Saccharomycetaceae| Vanderwaltozyma     |
|               | (66.2±4.33 %)      | (49.6±3.89 %)     | (49.6±3.89 %)     | (28.1±1.93 %)       |
|               | Other*             | Other*            | Other*            | Other*              |
|               | (16.9±0.83 %)      | (16.9±0.83 %)     | (16.9±0.83 %)     | (16.9±0.83 %)       |
|               | Other*             | Other*            | Other*            | Other*              |
|               | (39.8±2.52 %)      | (39.8±2.52 %)     | (39.8±2.52 %)     | (39.8±2.52 %)       |
| MD+ LM + extract | Ascomycota**       | Saccharomyces     | Saccharomycetaceae| Vanderwaltozyma     |
|               | (46.5±3.05 %)      | (44.4±2.97 %)     | (44.4±2.97 %)     | (9.27±0.5 %)        |
|               | Other*             | Other*            | Other*            | Other*              |
|               | (0.4±0.02 %)       | (0.4±0.02 %)      | (0.4±0.02 %)      | (0.4±0.02 %)        |
| MD+ FI        | Neocallimastigomyces | Neocallimastigomycetes | Neocallimastigaceae | (16.5±1.1 %)       |
|               | (16.5±1.1 %)       | (16.5±1.1 %)      | (16.5±1.1 %)      | (16.5±1.1 %)        |
|               | Other*             | Other*            | Other*            | Other*              |
|               | (4.3±0.23 %)       | (4.3±0.23 %)      | (4.3±0.23 %)      | (4.3±0.23 %)        |
| MD+ FI + extract | Ascomycota**       | Saccharomyces     | Saccharomycetaceae| Vanderwaltozyma     |
|               | (59.2±4.04 %)      | (57.2±3.95 %)     | (57.2±3.95 %)     | (25.3±1.42 %)       |
|               | Other*             | Other*            | Other*            | Other*              |
|               | (0.8±0.06 %)       | (0.8±0.06 %)      | (0.8±0.06 %)      | (0.8±0.06 %)        |

* This group includes taxa the number of which does not exceed 2 % of the total number

Table 1. Taxonomic diversity of microscopic fungi of rumen of cattle when using various additives in the diet, n = 3
** P≤0.05 in comparison with the control group

The use of a LM in the diet together with the extract was accompanied by an increase in the number of representatives of the Chytridiomycota taxon by 32.6 % of the control, Ascomycota by 3.2 % and Neocallimastigomycota up to 2.47 % of the total number, which was reflected in the percentage change in the microbiocenosis of the representatives of the families Chytridiomycetaceae (–2.79 %), Chytridae up to 4.72 % of the total number) and Neocallimastigaceae (up to 2.47 % of the total number). The analysis of species diversity showed a decrease in the number of microorganisms of p. Vanderwaltozyma by 9.93 % of control group, and an increase in the number of representatives of p. Saccharomyces and p. Cyllamyces up to 7.0 and 2.47 % of the total number.

When introduced into the diet, a FI caused an increase in the number of representatives of the Saccharomycetes classes by 13.9 % of the control group, Chytridiomycetes by 19.3 % of the control group and Neocallimastigomycetes up to 4.46 % of the total number, which was expressed in an increase in the number of representatives of the Chytridiaceae families (up to 19.3 % of the total number), Saccharomyceace (by 13.9 % of the control group) and Neocallimastigaceae (up to 4.46 % of the total number). The changes at the genus level were mainly expressed in an increase in the number of representatives of the p. Vanderwaltozyma by 23.1 % and p. Saccharomyces (Saccharomyces cerevisiae) up to 14.9 % of the total number.

The use of a FI together with the extract in the diet showed a similar trend to the individual use. An increase in the number of representatives of the Saccharomycetes classes (by 10.4 % of the control group), Chytridiomycetes by 8.4 % of the control group, and Neocallimastigomycetes up to 18.1 % of the total number was noted. The study of the species composition showed an increase in the number of representatives of p. Vanderwaltozyma at 8.2 % of control, p. Saccharomyces (Saccharomyces cerevisiae) and p. Cyllamyces up to 26.0 and 18.1 % of the total number.

In the available literature there is little information about the impact of FI and LM on the composition of eukaryotes in the rumen of cattle. At the same time, there is evidence that the breed of cattle affects the eukaryotic composition of the rumen [19]. Thus, six fungal taxa (Neocallimastigomycota, Basidiomycota, unclassified fungi: Mucoromycota, Ascomycota, and Chytridiomycota) with a relative abundance of more than 0.01 % and at least 50 % of animals in each breed were identified, the Neocallismastigomyae family prevailed, with the Orpinomy genus being the most common. In our case, the study of dairy breed was carried out and in the experimental groups there were found three fungal taxa – Ascomycota, Chytridiomycota and Neocallimastigomycota; the Saccharomycetaceae family and the genus Vanderwaltozyma prevailed.

The low content of Ascomycota in the rumen of cattle was observed by Wang et al. in their studies [20]; Neocallimastigomycota was found in large numbers; the dominant genera were Piromyces, Anaeromyces, Cyllamyces, Neocallimastix, and Orpinomy in four cattle breeds. It should be noted that the main factors in the studies were the inclusion of FI and LM, and plant extract, which could contribute to an increase in Ascomycota. There is an evidence that some plant extracts (Rosmarinus officinalis L.) do not change the quantitative values of eukaryotes in the rumen [21], but the inclusion of probiotic substances has a positive effect on rumen fermentation [22, 23] against the background of the known fibrolytic properties of fungi [24] and antiquorum activity of extracts [25].

4. Conclusion

The inclusion of FI and LM in the diet of cattle contributes to a change in the eukaryotic composition of the rumen microflora towards an increase in Ascomycota and Neocallimastigomycota phylums (especially against the background of the FI + Quercus cortex extract), which have cellulose and ligninolytic properties, and more active development of Chytridiomycota (with LM and Quercus cortex extract). Thus, this composition can be effective for increasing the productivity of cattle and better utilization of feed components.
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Ethical approval: All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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