

**TITLE:** RUMEN FUNGI COMMUNITIES OF HEIFERS GRAZING NATURAL GRASSLANDS OF PAMPA BIOME

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## **ABSTRACT**

Natural grasslands of the Pampa Biome are the main food source of beef cattle in the South of Brazil. In addition to their economic importance, they also have ecological relevance for the preservation of a broad floristic diversity but are usually characterized by low nutritional value due to the high fiber content. In this regard, the rumen's fungal community presents a relevant role in degradation of low quality diets due to the initial breakdown of recalcitrant fibers. Although of recognized importance, little is known about the diversity of fungi in these natural grasslands or about the fungal's community variability between animals. Thus, the present study aimed to evaluate the diversity of fungi in a population of beef cattle raised in natural grassland of São Gabriel municipality, RS. Ruminal fluid samples were collected with an orogastric tube from 12 Braford heifers with  $350 \pm 50$  kg and two years of age. The DNA was extracted and purified through QIAGEN® QIAmp Fast DNA Stool kit. The ITS2 region was amplified and sequenced using the Ion Torrent PGM technology. Sequences were grouped into taxonomic unities at 97% similarity cutoff and taxon assignments were performed by blast search against the UNITE database. After quality control, a total of 83,917 high quality sequences were obtained. Following the removal of singletons, library comparisons were performed with a rarefied dataset (2,500 sequences per sample) and only OTUs present in 80% of the heifers were considered. The most abundant known phyla were *Ascomycota* (15.7%), *Basidiomycota* (53.2%) and *Neocallimastigomycota* (6.0%). A total of 25.1% of the sequences were not assigned to any known phylum. From the *Ascomycota*, the most abundant genera were *Cercospora* (3.0%) and *Nigrospora* (0.3%). From the *Basidiomycota* the main genera were *Rhodotorula* (2.7%), *Hannaella* (1.6%) and *Kockovaella* (0.6%). The remaining 48.3% of the sequences were not assigned to any known genera. In the *Neocallimastigomycota*, frequently reported as a characteristic fungus of the ruminal environment, the distribution of genera occurred as follows: 0.5% of *Caecomyces*, 2.9% of *Cyllamyces*, 0.6% of *Neocallimastix*, 0.5% of *Orpinomyces* and 1.4% of sequences remain unclassified. The fungal community variability between animals was high (number of observed OTUs varied from 55 to 177) and might be associated to dietary intake and selection patterns or to intrinsic factors of each animal and should be thoroughly evaluated.

**Keywords:** gut microbiome, herbivore microbiome, Neocallimastigomycota

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