**TITLE: ISOLATION AND MORPHOLOGICAL CHARACTERIZATION OF CELLULOLYTIC FUNGI FROM BOVINE RUMEN**

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**Abstract:**

The fungi found in ruminal microbiota play an important role in the digestion of ruminant animal, mainly because of the production of enzymes capable of degrading vegetable fibre. The fibrolytic enzymes produced are also promising in generating new technologies for ethanol production. The aim of this study was to isolate and characterize cellulolytic fungi from the bovine ruminal content. Samples of ruminal fluid were collected from four cannulated cows, fed four types of diets: Diet A, composed by star grass hay (7.0kg); Diet B, star grass hay (7.0kg) and isolated soy protein (260g); Diet C, star grass hay (7.0kg), isolated soy protein (135g) and urea (50g); Diet D, star grass hay (7.0kg) and urea (100g). Aliquots of 1 mL of ruminal fluid were transferred to Hungate tubes containing Joblin's medium, filter paper strips as a sole carbon source and a mix of antibiotics (chloramphenicol, streptomycin, ampicillin, tetracycline, and neomycin). The tubes were incubated at 39°C, in aerobic and anaerobic conditions. After 7 days of growth, aliquots were plated in selective growth media containing 1% carboxymethylcellulose (CMC). The enzymatic assay of Congo Red was performed to assess the cellulase production and to determine the enzymatic index. Identification was performed examining colony morphology and microscopic characteristics after microcultures. Out of 11 filamentous fungi recovered, 10 aerobic isolates were obtained (Diet A, n = 1; Diet B, n = 2; Diet C, n = 4; Diet D, n = 3) and one facultative anaerobe was isolated from Diet A. The average of enzymatic index obtained was 1,19mm (ranging from 1,07mm to 1,44mm). Macroscopically, aerobic fungi showed pale/yellowish colonies, with velvety texture and rough surface, while the facultative anaerobic fungi showed colonies with light green olive reverse side, cottony texture, small and compact mycelium and rough surface. Zoospores in monocentric thallus were identified from the microculture of the facultative anaerobic fungus, suggesting the isolation of *Neocallimastix* spp. On the other hand, the aerobic isolates showed structures that are characteristic of *Aspergillus* spp. According to the results obtained, species of *Aspergillus* were the most commonly isolated fungi from the diets evaluated, and only poor-quality fibrous diet (Diet A) enabled the isolation of a facultative anaerobic strain. Additional studies are needed to optimize and quantify the cellulase production by the cellulolytic fungi identified.

**Keywords:** cellulolytic fungi, cellulases, rumen

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