

TITLE: Partial Biochemical Characterization and *in vitro* Digestibility Assessment of Proteases Produced by *Geomyces pannorum*

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ABSTRACT:

The dietary protein of non-ruminant feed is one of the main factors responsible for the rationing of feed, and it is necessary to use alternatives to enhance its use. In this context, the use of exogenous protease, especially microbial, has been efficient in the use of this ingredient. This object was to characterize and evaluate the *in vitro* digestibility of proteases produced by *Geomyces pannorum* isolated on the Antarctic continent. The enzymatic production was carried out from *G. pannorum* submerged fermentation in a medium composed of 5 g L⁻¹ of soybean meal and 20 g L⁻¹ of wheat bran in 1L bioreactor for 96 h at 14 °C and 120 rpm. The enzyme was biochemically characterized at the optimum temperature and pH, temperature and pH stability, and the enzymatic extract was subsequently submitted to gastric, intestinal, enteric and complete *in vitro* digestion assays. The enzyme showed optimum pH and optimum temperature around 8.0 and 50 °C, respectively. The enzyme was very stable in the studied pH ranges (pH 3-10) during 180 minutes, with relative activity higher than 95%. While for temperature stability, the enzyme was stable at 20-40 °C for 2 hours, losing activity from 50 °C, however, maintained relative activity above 50%. The proteases produced by *G. pannorum* showed about 25% inhibition in the presence of pepsin; in contrast, they showed a 50% increase in their final relative activity. In the presence of pepsin, pancreatin, bile and trypsin, a 24% increase in total activity was observed in the complete digestion assay. In conclusion, the proteases present in the crude extract show stability over a wide range of temperature and pH, in addition, the enzymes that resisted the *in vitro* digestion process potentiate their activity, which makes the application in the diet of non-ruminants attractive.

Keyword: Filamentous fungus, Animal nutrition, Extremozyme

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