

## Quantification of Archaea and Bacteria domains in the sheep rumen incubated under two diet conditions

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Ruminants occupy distinct ecological niches and their survival depends on the symbiotic association with the rumen microorganisms. The rumen microbiome is a dynamic system where the microbial diversity and community structure are shaped when exposed to different diet compositions. In this study, the total abundance of Archaea and Bacteria domains was accessed by qPCR in ruminal fluid incubated under different conditions. Fistulated sheep ("Santa Inês" breed) were fed for four weeks with a corn-based diet (60% of concentrate and Tifton-85 *Cynodon* spp. 40%). In order to evaluate the effect of diet, 25 mL of the ruminal fluids were sampled and anaerobically incubated *in vitro* at 39°C for 24 hours including 50 mL of buffer solution and 0.5 g of the diet composition used during animal feeding or the same diet in which the concentrate was fully replaced by *Parkia platycephala* (Fabaceae). Moreover, in order to evaluate the effect of ruminal fluids storage before *in vitro* incubation 300 mL of ruminal fluid and 600 mL of nutritive solution and 12 g of corn-based diet and CO<sub>2</sub> was stored in glass flasks at 39°C for 72 hours. After this storage period the material was submitted to the same treatments described for the *in vitro* incubation considering different diets. Afterwards, total DNA isolation was performed and qPCR using universal primers was applied to quantify the total abundance of Bacteria and Archaea. No significant differences were observed in the total abundance of Bacteria or Archaea under different diets; however, the relative abundance of subgroups within Bacteria and Archaea may be changed. On the other hand, the storage period of 72 hours affected the total microbial relative abundance where Archaea was significantly reduced after storage. This information is very important considering that in many cases, for practical reasons, the storage of ruminal fluid is needed before sample processing, consequently the pre-storage effect should be taken into account for data interpretation on microbial community analysis.