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Using high throughput sequencing to describe protozoal communities in RUSITEC fermenters fed two different diets

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Novel techniques of sequencing have given researchers a useful tool to assess the composition of microbial populations. In the case of ruminant's gastrointestinal tract, these techniques are widely used to study bacteria and archaeal populations but the rest of microbial are less studied. Olive cake is a by-product of oil extraction that contains bioactive compounds with antimicrobial and antioxidant activities that could be beneficial for the animals, but might also affect the ruminal microbial communities. The aim of this study was to assess protozoal populations in Rusitec fermenters when they received either a conventional diet for dairy sheep (50:50 forage:concentrate) or a diet including 16.7% of olive cake (OC) in replacement of corn silage. Diets were incubated in four Rusitec fermenters in a cross-over design with two 14-day incubation periods. Fermenters were given daily 30 g of diet, and in each period half of them received the diet with no olive cake (CON) and the other half received the OC diet. Samples of solid and liquid digesta were collected from four donor sheep and from the fermenters at the end of the incubation period, DNA was extracted, and protozoal profiling was performed using 18S rRNA gene amplicon sequencing. The reads generated were processed using the FROGS pipeline. Only reads that correspond to protozoa were kept for further analysis using R and the Phyloseq package. Protozoa were detected only in sheep inocula and liquid samples from CON fermenters. In the inocula, number of reads was more than 20 times higher in liquid samples comparing to the solid ones and more than 99% of reads in the solid inocula were unidentified protozoa. Six genera (*Ophyroscolex*, *Entodinium*, *Polyplastron*, *Isotricha*, *Dasytricha* and *Enoploplastron*) were detected in the liquid inocula, and only 4 were present in the CON-fermenters liquid samples. *Ophyroscolex* was the most abundant (55.40% of reads, median values) genus in liquid inocula samples followed by *Polyplastron* (10.70%). In CON-fermenters *Entodinium* was the most abundant genus (57.44%) followed by *Ophyroscolex* whose abundance was lower than in the inocula (30.49%). In summary, a loss in protozoal diversity was detected in Rusitec fermenters over the incubation period and a defaunation effect was observed when OC was included in the diet. High throughput sequencing seems to be an efficient tool to characterise protozoal populations in ruminal samples.