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Study of the *in vivo* periodic fluctuation of microbiome composition and enzymatic activity of rumen fluids used as inocula

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Aim of the present work was to evaluate the microbiome composition (MC) and the relative enzyme activities (EAs) to assess individual, daily and weekly fluctuations of rumen fluids (RFs) inocula degradative capacity. MC and cellulase (Ce), amylase (Am) and xylanase (Xy) activities were tested on the liquid fraction of RFs sampled before –T0–, and after 4 and 8 hours from the main meal –T4 and T8 respectively – in 3 days with one-week interval for a total of 3 weeks. RFs were collected from 4 Holstein cows (2 heifers, 2 adults) fed a hay-based diet. The liquid fraction of each sample was divided in 2 aliquots: one was centrifuged and filtered through 0.45µm filter porosity for the EAs tests; the other was freeze-dried for the MC analysis. Bacteria were extracted using the Repeated Bead Beating Plus column method followed by PCR amplification of the V3-V4 hypervariable regions of the 16S rRNA gene. Libraries were sequenced on a Illumina MiSeq instrument with a paired-end 2×300 run. MC data were analyzed by QIIME pipeline using Greengenes as reference database. The EAs were tested using the radial enzyme diffusion method using substrate concentrations of 0.5%, 0.5% and 0.1% for Ce, Am and Xy respectively, and expressed as area of the halos surface (mm²). EAs statistical analyses was performed using the Linear mixed model. MC did not differ daily, but individual ($p \leq .001$) and weekly ($p \leq .001$) differences in bacterial composition (beta-diversity) were found. The individual differences were more marked among heifer and adult cows. Overall, individual, weekly and daily EAs variations were found ($p \leq .001$) while not significant daily differences for Am were measured in analogy with MC findings. The lowest Xy was found in the 1st week (177.86 vs. 193.34 and 196.50 mm², $p \leq .001$) while Ce showed a depression in the 2nd week (239.32 vs. 315.50 and 276.35 mm², $p \leq .001$) and Am was depressed in the 3rd (110.75 vs. 136.36 and 153.35 mm², $p \leq .001$) week. Regarding the daily variation, Xy generally increased starting from T0 to T4 and T8 (172.62, 192.44 and 202.64 mm² respectively) while Ce showed different results at T4 increasing in two cows and decreasing in the other two. Nevertheless, MC did not change over the day while weekly and individual variations appeared especially when heifer and adult cow were compared. Enzymatic activity of rumen fluids varied individually, weekly and daily, therefore degradative capacity could vary widely among inocula used for *in vitro* procedures.