

Changes in rumen bacterial communities from cattle receiving immature oak (*Quercus pyrenaica*) leaves determined by fluorescence in situ hybridization and real-time PCR.

By A. BELENGUER¹, G. HERVÁS¹, P. DE PAZ², C. EZQUERRO³ and P. FRUTOS¹,

¹Estación Agrícola Experimental, CSIC, Finca Marzanas, 24346 Grulleros, León, Spain; ²Cell Biology and Anatomy, and ³Molecular Biology, Genomics and Proteomics Institute, University of León, Campus de Vegazana, 24071 León, Spain.

In the hill areas of northern Spain intoxication of beef cattle occur recurrently in the spring when the animals graze in Pyrenean oak (*Quercus pyrenaica*) areas and consume immature oak leaves (OL). These leaves are rich in hydrolyzable tannins that are inhibitory to the growth and activity of many rumen microorganisms and may cause poisoning in animals if sufficiently large amounts are consumed. The aim of the study was to investigate the impact of the administration of hydrolyzable tannin-rich oak leaves on the rumen bacterial communities in cattle. Three ruminally cannulated Brown Swiss bulls were fed grass hay for an adaptation period before a progressive feed restriction 10-day period. Afterwards, they were offered daily 14 g dry matter (DM) of grass hay/kg metabolic weight ($LW^{0.75}$), and 14 g DM of OL/kg $LW^{0.75}$ were administered through the cannula for 6 days. Then, all bulls received again grass hay. *In situ* determinations of OL and alfalfa hay (AH) degradation by rumen microbes and rumen samplings for microbial population studies (real time PCR - total rumen contents - and fluorescence in situ hybridization, FISH - rumen fluid -) were performed before and after the feed restriction, after 5 days of OL administration, and 4 days after stopping the administration.

Inter-individual differences in bacterial profile were observed initially. Proportions of Gram-positive bacteria, Prevotella-Bacteroides, and other bacterial groups relative to total Eubacterial count (GP:Bac:Others) in the rumen fluid were 28:16:56 in animal 1, 30:9:61 in animal 2, and 27:6:67 in animal 3. The effect of feed restriction and OL administration on the studied microbial groups was similar in the three animals. Total eubacterial counts (Eub) were reduced by the feed restriction, but members of Bac and *Streptococcus* groups (by FISH) maintained or even increased their numbers. After 5 days of OL administration, Eub were recovered, the numbers of *Streptococci* were reduced and those of Bac did not change, whereas GP were stimulated, specially in bulls 2 and 3 (over 3-fold increase). These animals showed signs of intoxication and a lower microbial degradation of OL and AH in the rumen, did not ingest any hay and presented a 3-fold higher abundance of *Streptococci* in the rumen fluid after the OL administration than bull 1. On the contrary, the latter animal consumed all the offered hay and showed lower proportions of the *Streptococcus bovis* group relative to total eubacteria in total rumen contents (by real time PCR: 0.03 vs 1.13 and 0.99 %) than the intoxicated animals. Four days after stopping the OL administration, proportions of GP: Bac:Others were again different not only between animals but also from the initial values: 35:37:28 in animal 1, 20:58:22 in animal 2, and 17:19:64 in animal 3.

The administration of tannin-rich oak leaves affected the composition of the bacterial population in the rumen, but their effects depended probably on the individual microbial profile. Further research is necessary to understand the role of the ruminal microbiota to prevent intoxications in cattle unadapted to the intake of tannin-rich plants.