

which is typical of the rumen environment. However, large postprandial variations in rumen pH may occur under different dietary conditions. The objective of this study was to compare enzyme activity measurements for the solid-associated microorganisms (SAM) made at pH 6.5 with those made at actual rumen pH in relation with the diet and sampling time. Four cows were restricted-fed once a day at the level of 7 kg DM/d and received 3 diets: 100% cocksfoot hay, (diet **H**), 65% hay and 35% pelleted ground barley (diet **HB**) and diet **HB** with a continuous intraruminal infusion of bicarbonate salts (diet **HBB**). These diets were chosen for their pronounced differences in the diurnal variations in rumen pH. SAM enzymes were extracted by sonication under anaerobic conditions from rumen contents collected 1 h before feeding, and 2 and 5 h after feeding on 2 consecutive days. Polysaccharidase and glycosidase activities were determined at pH 5.5, 6.5 and 7.5 by measuring the amount of reducing sugars or *p*-nitrophenol released from appropriate substrates. The results were analysed by a split-plot analysis of variance. Glycosidase activities in the SAM decreased ( $P < 0.001$ ) when the pH increased. A specific activity of 20.1, 15.3, and 12.3 nmol *p*-nitrophenol released/mg protein/min for the  $\beta$ -D-glucosidase was measured at pH 5.5, 6.5 and 7.5, respectively. A similar pH effect was observed for polysaccharidase activities, but the variations were not significant ( $P > 0.05$ ) for xylanase which had a specific activity of 180, 176 and 136 nmol reduced sugars released/mg protein/min at pH 5.5, 6.5 and 7.5, respectively. Nevertheless, variations in the enzyme activity of the SAM with diet and sampling time were similar ( $P > 0.05$ ) for measurements made at pH 6.5 and at actual rumen pH. Thus, measurements of enzyme activity realized at pH 6.5 remain valid irrespective of ruminal physico-chemical conditions at sampling time.

**Contribution of the rumen ciliate *Polyplastron multivesiculatum* to the degradation and fermentation of crystalline or soluble celluloses.** JP Jouany<sup>1</sup>, T Michalowski<sup>2</sup>, S Toillon<sup>1</sup>, J Sénaud<sup>3</sup> (<sup>1</sup>INRA-Theix, Station de Recherches sur la Nutrition

*des Herbivores*, 63122 Saint-Genès-Champagne, France; <sup>2</sup> Department of Vertebrate Animal Physiology, Zoological Institute University of Warsaw, 93 Zwirki i Wigury Str, 02-089 Warsaw, Poland; <sup>3</sup> Université Blaise-Pascal, UACNRS 138, 63177 Aubière Cedex, France)

Suspensions of about  $2 \times 10^5$  *Polyplastron multivesiculatum* were prepared from the rumen contents of sheep harbouring this single ciliate genus. Each suspension was incubated for 24 h in anaerobic conditions with 40 ml of a buffer solution and chloramphenicol ( $50 \mu\text{gml}^{-1}$ ) in a fermentor. The formation of the end products of the digestion and fermentation of the celluloses tested was measured by comparison it with control fermentors that received no substrate (2 fermentors per series) and fermentors to which 80 mg of cellulose were added (4 fermentors with soluble celluloses and 6 fermentors with crystalline celluloses per series). Incubations were performed during 4 separate series of measurements.

*P. multivesiculatum* did not ferment the soluble celluloses but had an effect on the degradation and fermentation of the 3 different crystalline celluloses tested (8–12 mg fermented in each fermentor in 24 h). The total production of volatile fatty acids (VFA) was  $0.50 \mu\text{mol}/1\ 000$  ciliates, made up of 72% acetate and 28% butyrate. About  $10 \mu\text{l}$  of gas/ $1\ 000$  cells/h was produced during the first 4 h; the mean production over 24 h was only  $2 \mu\text{l}/1\ 000$  cells/h. The gas mixture was composed of  $\text{CO}_2$  (70–85%) and  $\text{H}_2$  (15–30%).

Compared to the rare previously published findings, our results show that *P. multivesiculatum* has an ability to degrade and ferment crystalline celluloses that is greater than that of *Eudiplodinium maggii* (2 200 vs 25 pg cellulose/ ciliate/h). However, although *P. multivesiculatum* degraded the soluble celluloses (HEC, CMC), it was not able to ferment them. The absence of methane in the gases and of propionate in the VFA shows that the bacteria were eliminated during washing and decontamination of the ciliates, and that the results obtained are evidence of the direct action of enzymes produced by *P. multivesiculatum* on the degradation of crystalline celluloses.