Quatre fourrages sont expérimentés : du feuillage de chêne Kermès (% MS : 5,5 MM, 8,3 MAT, 29 CB), du feuillage d'arbousier (% MS : 6,1 MM, 7,1 MAT, 16,7 CB), du marc de raisin (% MS : 10,8 MM, 16,2 MAT, 29,3 CB) et de la paille de blé (% MS : 4,1 MM, 5,8 MAT, 40,7 CB).

Les vaches et les moutons, placés dans le même local, reçoivent, distribué 2 fois par jour, le même régime composé de 70% de bon foin de luzerne/dactyle (%MS : 8,2 MM, 14,9 MAT, 60,3 NDF, 46,5 ADF, 11,3 ADL) et de 30% de concentré (43% orge, 40% pulpes, 11% tourteau de soja et 6% protéinal). Les fourrages broyés à la grille de 0,8 sont introduits dans des sachets de toile de nylon (F100 Tripette et Renaud) en respectant le même rapport poids/surface (20 mg/cm^2) pour les 2 espèces. Chaque mesure comprend 6 points de cinétique (2, 4, 8, 16, 24 et 48 h) avec 6 valeurs par fourrage et par point de cinétique (2 pour chaque vache, 1 par mouton).

Pour 2 des fourrages (le marc de raisin et le feuillage de chêne Kermès), la dégradation de la matière sèche est pratiquement terminée au bout de 8 h et il n'apparaît aucune différence au bout de 48 h avec respectivement, pour les moutons et pour les vaches, 33 et 34,9% de disparition pour le marc de raisin et 48,7 et 48,1% pour le feuillage de chêne Kermès.

Il n'en est pas de même pour les 2 autres fourrages. L'arbousier est pratiquement totalement dégradé à 16 h chez la vache et est beaucoup moins dégradé après 48 h que chez le mouton : 43,9% contre 73,5%. L'inverse est constaté pour la paille de blé qui est pratiquement dégradée à 16 h chez le mouton avec une disparition à 48 h de 32,5% contre 54,5% chez la vache.

Une analyse approfondie, en cours, des composants polyphénols de ces fourrages devrait nous permettre d'expliquer ces différences.

Preliminary study on the cecal fungal flora in the donkey: enumeration, isolation and identification. V Julliand, A de Vaux, A Zidane *(Unité Associée de Recherches Zootechniques INRA/ENESAD, BP 1607, 21036 Dijon Cedex, France)*

The fungal flora in the hindgut of donkey has rarely been studied. Our objectives were firstly to establish the presence and the number of fungi in the cecal ecosystem, and secondly to isolate and characterize the main fungal strains.

Cecal contents were collected every 2 h after the distribution of the meal from 3 cecally fistulated donkeys fed a maintenance alfalfa hay diet. Fungi were enumerated according to the MPN method on a modified Joblin liquid medium. The main fungal strains were isolated by the roll-tube method on a modified solid Joblin medium. The morphology was characterized by photonic microscopy. The end products of fermentation were analysed by gas chromatography and the enzymatic Boehringer method.

A large variation in the fungal numbers appeared both between the 3 donkeys and the time of sampling. No significant difference could be observed. A mean enumeration of total fungal flora was 239 CFU/ml of cecal content with extreme variations from 3 to 1 600 CFU/ml. Most of the strains showed a monocentric branched thallus with mono- or biflagellated zoospores. They are classified in the genus *Piromyces.*

Strain AVA1a was cultivated on a liquid Lowe medium using the following substrates: glucose; cellobiose; glucomannan; MN300; and Avicel celluloses. It did not grow on mannitol, sucrose, raffinose, pectin, arabinogalactan, polygalacturonic acid, dextran 60 or CMC. After 3 d fermentation, the main end-products of strain AVA1a on glucose or cellobiose Lowe media were succinate (6.00 and 2.67 mmol/100 mmol fermented hexose, respectively), formate (107.00 and 63.00 mmol/100 mmol), acetate (65.26 and 31.10 mmol/100 mmol), ethanol (30.40 and 13.10 mmol/100), H_2 and CO_2. Lactic-acid production could be detected on neither glucose nor cellobiose substrates.

In conclusion, fungi are present in the cecum of donkey with a great variation in number. They mainly belong to the genus *Piromyces.* Strain AVA1a was the sole non-lactate-producing fungus isolated up to now.

Acknowledgments to F Bonnemoy and L Millet (INRA-Theix).

Effect of pH on fibrolytic activity in rumen solid-adherent microorganisms. C Martin, L Genestoux, B Michalet-Doreau *(INRA-Theix, Station de Recherches sur la Nutrition des Herbivores, 63122 Saint-Genès-Champanelle, France)*

Measurements of fibrolytic activity of rumen microorganisms are generally made at pH 6.5,

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. Glycosidases 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 β-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 [1], T Michalowski [2], S Toillon [1], J Sénaud [3] *([1] INRA-Theix, Station de Recherches sur la Nutrition des Herbivores, 63122 Saint-Genès-Champanelle, France; [2] Department of Vertebrate Animal Physiology, Zoological Institute University of Warsaw, 93 Zwirki i Wigury Str, 02-089 Warsaw, Poland; [3] Université Blaise-Pascal, UACNRS 138, 63177 Aubière Cedex, France)*

Suspensions of about 2 x 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 $\mu g ml^{-1}$) 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 µmol/1 000 ciliates, made up of 72% acetate and 28% butyrate. About 10 µl of gas/1 000 cells/h was produced during the first 4 h; the mean production over 24 h was only 2 µl/1 000 cells/h. The gas mixture was composed of CO_2 (70–85%) and 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.