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# **Influence of the nature of the substrate in rumen bags on fibrolytic activity of microorganisms associated with bag residues \***

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It has been suggested that the activity of microorganisms inside rumen bags might vary with the nature of the feed in the bags. This can be characterized by measuring the fibrolytic activity of solid-adherent microorganisms (SAM) from bag residues. The aim of this study was to compare this fibrolytic activity on bag residues of 5 feeds. Three fistulated dry cows were given a diet (60% hay, 10% straw, 30% barley) twice a day at the level of 7 kg DM /d. Two cereals (barley and maize), 2 hays (highly (H+) or less (H-) digestible), and beet pulp were incubated in rumen bags at feeding time for 2 and 23 h on 2 successive days. Enzymes of SAM were extracted from bag residues by sonication under anaerobic conditions. Polysaccharidases (xylanase, CMCase, avicelase) and glycosidases ( $\beta$ -D-cellobio-,  $\beta$ -D-galacto-,  $\beta$ -D-xylo-,  $\beta$ -D-glucosidases) activities were expressed in  $\mu$ mol reducing sugars and nmol *p*-nitrophenol released/g DM, respectively, over 1 h.

All activities were higher at 23 h than at 2 h ( $P < 0.001$ ), and varied with the nature of feed ( $P < 0.01$ ). Fibrolytic activities were higher in high-fibre feeds than in cereals, and they were higher in barley than in maize. This could be explained by the presence of an horny endosperm in maize which is resistant to microbial colonization and digestion. Significant differences between high-fibre feeds were observed only at 23 h. Polysaccharidases were more active when the corresponding polysaccharides were highly represented in feed. Xylanase was higher in hays than in beet pulp (266 vs 76); CMCase and avicelase were lower in H+ and beet pulp than in H-. This could be related to differences in the nature and the amount of fibre in feeds. Glycosidase activities were inversely related to polysaccharidases, and were probably dependent on the amount of substrates easily available for microorganisms. Modulations in fibrolytic activity of SAM from different feeds incubated in a same ruminal environment can thus be observed, according to the nature of feed.

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# **Diurnal variations of ruminal microflora in buffalo and cattle fed fibrous diets.**

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Total viable, cellulolytic and xylanolytic bacteria plus fungi were counted in rumen samples of 2 buffaloes and 2 Friesian bulls at different times after feeding. The animals were fed at maintenance level once daily (9 am) 2 different diets A: 75% wheat straw + 25% concentrate, B: 75% cocksfoot hay + 25% concentrate, according to a factorial plan. Samples of the whole rumen content were withdrawn at 8.00 am, 2.00 and 8.00 pm. The total viable bacteria were determined on petri dishes in complete medium and the xylanolytic bacteria in the differential carbohydrate medium of Leedle and Hespell. The cellulolytic bacteria were counted according to the MPN procedure in liquid medium of Hungate and the cellulolytic fungi in Joblin medium. An anaerobic glove-box (atmosphere 95% CO<sub>2</sub> / 5% H<sub>2</sub>) was used. The incubation at 39°C lasted from 5 to 15 d according to different microorganisms. The microflora of the 2 animal species increased after feeding diet A or diet B, but the trend was slightly different for the various microorganisms according to diet and animal species. Only buffaloes fed on diet B showed significant differences among the withdrawals (buffaloes = total bacteria: 127.80, 150.45, 655.60  $\times 10^8$ , 1 vs 3,  $P \leq 0.01$  and 2 vs 3,  $P \leq 0.01$ ; cellulolytic bacteria: 20.75, 2.50, 109.60  $\times 10^8$ , 1 vs 3,  $P \leq 0.01$  and 2 vs 3,  $P \leq 0.01$ ; xylanolytic bacteria: 22.25, 36.28, 80.61  $\times 10^8$ , 1 vs 3,  $P \leq 0.03$ , 2 vs 3,  $P \leq 0.09$ ; fungi: 749.08, 56.43, 149.09  $\times 10^3$ , 1 vs 2  $P \leq 0.01$ , 1 vs 3,  $P \leq 0.01$  as cells/g dry rumen content; cattle = total bacteria: 108.18, 143.42, 268.13  $\times 10^8$ ; cellulolytic bacteria: 24.15, 10.06, 28.49  $\times 10^8$ ; xylanolytic bacteria: 21.97, 31.91, 23.84  $\times 10^8$ ; fungi: 105.62, 74.93, 252.34  $\times 10^3$  as cells/g dry rumen content). Moreover, buffalo species had significantly higher counts in diets B as compared to diet A for each microbial population (total bacteria: 311.28 vs 81.01  $\times 10^8$ ,  $P \leq 0.01$ ; cellulolytic bacteria: 77.41 vs 2.36  $\times 10^8$ ,  $P \leq 0.0$ ; xylanolytic bacteria: 46.38 vs 6.25  $\times 10^8$ ,  $P \leq 0.01$ ; fungi: 318.20 vs 31.27  $\times 10^3$ ,  $P \leq 0.01$  as cells/g dry rumen content). On the contrary cattle did not show significant differences between diets.