

**Comparison of in vitro digestion of hays with horse caecal and sheep rumen fluids.** D Macheboeuf, M Jestin, M Chenost (*INRA, Station de Recherches sur la Nutrition des Herbivores, Centre de Clermont-Theix, 63122 Saint Genès-Champanelle, France*)

*In vitro* fermentations of hays with horse caecal (HCL) or sheep rumen (SRL) fluids as inocula were compared using the gas production method [1]. Four types of substrate were used: two grass hays (GH1, GH2) and two alfalfa hays (AH1, AH2). The hays fermented with horse caecum fluid were not the same as hays fermented with rumen fluid but their chemical composition and their in vivo organic matter digestibilities measured in sheep were very similar. Gas production was measured after 3, 6, 12, 24, 48, 72, 96 and 120 hour fermentations. For each sample, 6 runs were fitted to the model:  $G = a + b(1 - e^{-ct})$  [2]. Volatile fatty acids (VFA) were determined at the end of the fermentation.

The potential gas production (a+b) and the gas production rate (c) were lower with HCL and represented respectively 93.5% and 53.5% of rumen fluid values for grass hays and 82.0% of rumen fluid

values for both alfalfa hays. The amounts of total VFAs were similar for caecal and rumen fluids (Table). Fermentation with HCL led to lower proportions of acetate (60.7% vs 67.6%) and higher proportions of propionate and butyrate (25.8% vs 21.1% and 10.0% vs 7.7% respectively) than with SRL.

The difference between the inocula depended on the nature of the forage. In the case of grasses, changing the inoculum mainly affected the c value while with legumes the potential gas production (a+b) was also modified. The horse fluid might be more susceptible, at least at the beginning of the fermentation process, than the sheep fluid to the differences in cell wall characteristics between grasses and legumes. Comparisons of rumen and caecal fermentation characteristics with the in vitro gas technique are in progress in our laboratory, using the same grass and legume samples, the in vivo digestibility of which had been measured simultaneously with sheep and horses.

1. Menke KH, Raab L, Salewski A, Steingass H, Fritz D, Schneider W (1979) *J Agric Sci, Camb* 93, 217-222

Digestion and fermentation by sheep rumen and horse caecal fluids

Nature of substrate Nature of inoculum	GH1		GH2		AH1		AH2	
	HCL	SRL	HCL	SRL	HCL	SRL	HCL	SRL
CP (DM%)	9.1	8.0	19.8	11.5	15.1	19.2	19.7	19.7
CF (DM%)	33.5	34.2	29	27.5	35.1	34.5	26.7	25.2
OMD (%)	53.7	54.1	65.8	65.0	54.3	55.2	62.6	62.5
(a+b) (ml 200mg DM <sup>-1</sup> )	52.1	54.2	49.8	54.6	35.2	42.2	38.0	46.7
c (hours <sup>-1</sup> )	0.022	0.040	0.035	0.068	0.057	0.071	0.075	0.089
VFA (mM l <sup>-1</sup> )	38.54	35.46	38.72	39.32	28.92	26.76	33.38	34.08

CP: crude protein; CF: crude fiber; OMD: sheep in vivo digestibility of organic matter; DM: dry matter. HCL: horse caecal fluid, SRL: sheep rumen fluid, VFA: volatile fatty acids.

2. Ørskov ER, McDonald I (1979) *J Agric Sci, Camb* 92, 499-503

**Microbial metabolism of plant 4-hydroxycinnamic and hydroxyalicyclic acids in vitro.** JH Pagella, XB Chen, WJ Shand, PS Dewey, ER Ørskov (Rowett Research Institute, Bucksburn, Aberdeen AB21 9SB, UK)

Benzoic acid (BA) is a common xenobiotic present in ruminant urine. It is the metabolic product of 3-phenylpropionic (PPA) and cyclohexanecarboxylic (CHCA) acids absorbed from the rumen. PPA is produced from cinnamic acid (CA) and from 4-hydroxycinnamic [*p*-coumaric (PCA), ferulic (FA), sinapic (SNA) and caffeic (CFA)] acids and CHCA from hydroxyalicyclic [quinic (QA) and shikimic (SKA)] acids during the microbial fermentation of plant feeds. This study examined the production of PPA and CHCA from their precursors and the stability of PPA and CHCA using an in vitro incubation technique. Ten mg (45-78 µmol) of each of the substrates (PPA, CHCA, PCA, FA, CFA, SNA, CA, QA and SKA) was mixed with 90ml of incubation media in 100-ml conical flasks. The media consisted of 60ml of buffer solution and 30ml of rumen fluid from either rumen-defaunated

or normal sheep. The flasks were flushed with CO<sub>2</sub>, sealed with a rubber stopper with a Bunsen valve and incubated at 39°C. All incubations were done in duplicate. Samples (4ml) of the incubation mixture were collected at 0.25, 2, 4, 8, 12, 24 and 48h after the start of incubation. The samples were analysed by gc.

Each of the PPA and CHCA precursors examined disappeared completely after 4h incubation. However, the recovery as PPA and CHCA were not complete. With the hydroxycinnamic acids, the recovery was higher when incubated with faunated than with defaunated rumen fluid, but with hydroxyalicyclic acids, the recovery was higher with defaunated rumen fluid. SNA was not a precursor of PPA. The production of PPA from CA, which only involves a hydrogenation reaction, was rapid and reached a plateau at about 4h, indicating that hydrogenation would not be a limiting step causing the incomplete conversion of hydroxycinnamic acids to PPA. When PPA and CHCA were incubated, their concentrations in the media remained unchanged over the 48h period, indicating that PPA and CHCA are not further metabolised by rumen microorganisms. The results indicate that PCA,

Production of PPA and CHCA from their precursors after incubation with defaunated and faunated rumen fluid for 48h.

Precursor	PCA	FA	CFA	SNA	CA	QA	SKA
End product	PPA	PPA	PPA	PPA	PPA	CHCA	CHCA
Recovery (%)							
Defaunated rumen fluid	34(±6)	29(±2)	14(±2)	0(±0.2)	68(±0.3)	16(±0.3)	29(±0.01)
Faunated rumen fluid	67(±0.1)	64(±6)	54(±0.3)	0(±0.2)	96(±0.4)	6(±0.5)	7(±0.6)