Ciliate numbers ($x10^3$ ml^{-1}), pH and activities of protozoal enzymes (nmol mg^{-1} protein h^{-1})

	Hay			*Hay + barley*			
	DS	*VS*	*AS*	*DS*	*VS*	*AS*	*S*
pH	6.68	6.83	6.87	6.27	6.47	6.60	0.16
Entodiniomorphs	69	56	47	1192	912	874	227
Xylanase	3413	2511	2091	2605	2746	2319	1173
Avicelase	903	713	707	1204	1009	1021	323
β-D-Xylosidase	230	187	169	259	235	212	91
β-D-Glucosidase	2869	1866	2234	2035	1865	1879	815

microbial populations which form the content of the reticulo-rumen generally is observed. The bacterial concentration is higher in rumen samples taken from the dorsal sac than from the ventral sac. However, information is not available on the location of protozoa and, more particularly, on the distribution of their enzyme activities in the rumen. The objective of this experiment was therefore to determine effects of sampling site within the rumen on the concentration and fibrolytic activity of protozoa.

Four ruminally cannulated cows fed twice daily were used to compare rumen contents obtained from three sampling sites in the rumen when animals were fed two different diets (7 kg DM d^{-1}) of 100% hay or, 40% hay plus 60% barley. Samples were collected from the dorsal (DS), ventral (VS) and anterior (AS) sacs 1h prior to and 3h after morning feeding on two sampling days with a two-day interval. Rumen contents were strained to obtain a liquid phase from which pH measurements, protozoal counts and extraction of protozoal enzymes by sonication under anaerobic conditions were made. Polysaccharidase and glycosidase activities were measured respectively by the amount of reducing sugar released from xylan or Avicel (cellulose) or *p*-nitrophenol from the nitrophenol derivatives of xylose and glucose. Results reported here are means of the two sampling times.

The distribution of protozoa in term of concentration and fibrolytic activity was different in the three sampling sites of the reticulo-rumen (Table). Irrespective of diet and sampling time, the major proportion of entodiniomorphs, representing the major population of protozoa, was found in the dorsal sac (40% mean; $P < 0.01$) compared to the bottom of the reticulo-rumen. The specific activity of all studied fibrolytic enzymes was correspondingly significantly greater ($P < 0.05$) in the top of rumen than in the ventral or anterior sacs. The low pH values ($P < 0.001$) observed in the dorsal sac indicated a higher fermentative activity in this site than in the other parts of the rumen. Consequently, the protozoa may contribute more than was previously thought to the great digestive potential present at the top of the rumen classically attributed to bacteria.

Effect of rumen protozoa on the degradation of neutral sugars from plant cell walls. JP Jouany, A Cornu, F Mathieu, J Senaud (*INRA, Station de Recherches sur la Nutrition des Herbivores, Centre de Clermont-Theix, 63122 Saint Genès-Champanelle, France)*

Although some studies have indicated that faunated animals are less efficient fibre digesters than their defaunated counterparts [1], it is now more generally acknowledged that the presence of protozoa has a positive effect on the ruminal degradation of plant cell walls. The present work specifically analyses the effect of addition of protozoa into a defaunated rumen on the main neutral sugars that comprise the hemicellulose and cellulose fractions of grass hay.

Ground fescue hay was introduced into nylon bags (50 µm mesh size) and placed in the rumen of six sheep for 1, 3, 6, 12, 24, 48 and 72h. Sheep were defaunated during period 1 and refaunated with a mixed B-type protozoal population during period 2. Animals were fed a mixed diet of the same fescue hay (450g kg^{-1}) + barley (450g kg^{-1}) + soybean meal (100g kg^{-1}) which favours the growth of protozoa (6 x 10^5 ml^{-1}). Six kinetic studies were carried out during each period in each sheep. Proportions of degraded DM, OM, cell wall residue [2] and their neutral sugars [3] were calculated.

Refaunation increased the degradation of all the measured fractions after 48 hr in the rumen, but differences were only significant for the cell wall and its components. *In situ* digestibility of cell wall residues was improved by seven units when protozoa were present. Xylose and glucose, which are the most representative sugars of hemicellulose and cellulose respectively, displayed the highest increase in digestibility (+10, +15 units). Arabinose, galactose and rhamnose, as secondary sugars of hemicellulose, were better degraded in refaunated rumen but to a lesser extent (+12, +7, +5 units respectively).

These results indicated that protozoa stimulate to the same extent the digestion of the hemicellulosic and cellulosic fractions of grass hay in the rumen.

Loss of organic matter and cell wall material in the presence and absence of protozoa

Fraction	*Degradation (%) after 48h*	
	Defaunated	*Refaunated*
OM	42	44
Cell wall residue	27	34*
Glucose	26	41*
Xylose	21	31*
Arabinose	38	50*

* Significant differences between defaunated and refaunated animals ($P<0.05$)

1. Romulo BH, Bird SH, Leng, RA (1986) *Proc Aust Soc Anim Prod* 156, 327-330
2. Jarrige R (1961) *Ann Biol Anim Biochim Biophys* 1, 163-212
3. Englyst HN, Cummings JH (1984) *Analyst* 109, 937-942 (adapted by Hoebler, personal communication)

Effect of addition of *Isotricha* spp or a mixed fauna to defaunated rumen contents on methane production measured in vitro.

JP Jouany, S Toillon *(INRA, Station de Recherches sur la Nutrition des Herbivores, Centre de Clermont-Theix, 63122 Saint Genès-Champanelle, France)*

Hydrogen produced by protozoa is used by rumen methanogens associated with the surface and in the cytosol of ciliates. It has been shown that differences between defaunated and refaunated animals in methane production are increased when animals are fed mixed diets with starch which favour the growth of protozoa in the rumen