

Variation in the *in vitro* hydrolytic activity of rumen and faecal inocula

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Introduction Considerable efforts have been made regarding the use of faecal material to provide a microbial inoculum for *in vitro* feed evaluation systems. However total gas production, rate of gas release and the extent of degradation of feeds incubated using faecal inoculum are lower than those incubated in a rumen fluid medium. It has been suggested that this is due to lower microbial activity, a consequence of the different microflora and reduced microbial numbers (e.g. Mauricio, 1999). Microbial populations are dynamic so, as their enzyme activity profiles change rapidly, little information is obtained from examining these. However, their hydrolytic activity as reflected by their ability to degrade specific substrates can be simply measured and provides a potential method with which to assess the quality of inocula with respect to their use in *in vitro* systems. The data presented here are from a larger study in which the differences between the hydrolytic activity of faecal material and rumen contents as influenced by the time of sampling were assessed *in vitro*.

Materials and methods Rumen fluid (hand squeezed contents) and faecal samples (*per rectum*) were obtained one hour before feeding at 07.00 or 15.30 h, from two dry cows offered a diet of hay and grass silage, on two occasions. The faecal material was blended (30 seconds) with an equivalent volume of reduced buffer and strained twice through a single cloth. The rumen fluid was strained through a double layer of cloth and both inocula held under CO₂ at 39 °C until used (<1 h after sampling). The sacchrolytic, amylolytic and fibrolytic activity of the inocula were examined by incubating xylose (*X*), starch (*S*) and cellulose (*C*), respectively over a 48 h period at 39 °C using the Reading Pressure Technique. An estimate of cell wall degradation kinetics was provided by a fourth substrate, washed hay (*WH*). Head-space gas pressure readings were taken 2, 4, 6, 8, 10, 12, 15, 19, 24, 30, 36 and 48 h post-inoculation and three replicates per substrate plus negative controls were withdrawn at 6, 12, 19, 24 and 48 h to determine degradability. SAS GLM procedures were used to generate LS means and significances of difference.

Results The extent of *WH* degradation was significantly reduced when the faecal, compared to rumen fluid, was used, (Table 1). Although there was a tendency for the pm rumen inoculum to show a reduced rate of degradation up to 24 h this effect was not significant. Both inocula ranked the four test substrates in a similar order with respect to cumulative gas production (*S* > *X* > *C* > *WH*), however the 48 h gas release values for the faecal inocula were significantly lower and generally equal to the 24 h values obtained with the rumen fluid (Table 2). Not only were lag times extended when faeces were used, but unlike rumen fluid, the least fermentable substrates (*C* and *WH*) could not be differentiated even at 48 h. As expected little difference between sampling time, was identified with respect to activity of the faecal material. In contrast rumen samples obtained at 15.30 h showed significantly lower rates of gas release than those taken at 07.00 h. Due to similarities between the *S* fermentation curves it can be concluded that the amylolytic activity of rumen fluid and faeces were, in this study, equivalent. However marked differences in the rate of gas release when *X* and to a lesser extent *C* were incubated suggest compositional differences in the rumen and faecal microflora. This lends support to the idea that the faecal microflora tends to be facultative characterised by fewer, opportunistic microorganisms, while the rumen microflora is highly specialised, exhibiting considerable diurnal variation due to substrate availability.

Table 1 Influence of inoculum and sampling time on washed hay degradation (g kg⁻¹OM)

Inoculum	Time (h)	Degradation g kg ⁻¹		
		12 h	24 h	48 h
Rumen	07.00	39a	299a	523a
	15.30	35ab	241a	521a
Faeces	07.00	15b	62b	407b
	15.30	23b	82b	399b
se means	-	2.7	8.1	5.8

Table 2: Cumulative gas release by substrate as influenced by inoculum

Inoculum	Substrate	Cumulative gas release (ml g OM ⁻¹)				
		6h	12h	19h	24h	48h
Rumen	Cellulose	5.0d	12.4e	44.8d	92.6d	260.7bc
	Starch	78.1c	230.0a	297.9a	323.5a	356.8a
	Xylose	24.6d	101.1c	216.0b	255.6b	294.0b
Faeces	Cellulose	1.2d	3.4e	6.3e	16.1e	114.0a
	Starch	34.2a	202.3b	278.5a	298.3a	325.1ab
	Xylose	3.5b	59.4d	161.8c	188.3c	233.3c
se means	-	0.90	2.87	3.51	4.28	6.49

LS Means in columns without similar letters are significantly different (P<0.05)

Conclusions The study has confirmed the lower rates of fermentation and degradation associated with the use of faecal material as an inoculum for *in vitro* feed evaluation. A significant effect of sampling time on the quality of the inoculum with respect to rumen fluid but not faeces, was identified. The identified variation in hydrolytic activity suggests compositional differences in the microflora, indicating that care should be exercised when extrapolating results, obtained *in vitro* using faecal inocula, to the practical situation.

References

Mauricio, R.M. 1999. *Comparison of bovine rumen liquor and bovine faeces as sources of microorganisms for an in vitro gas production technique for evaluating forages*. PhD thesis, University of Reading, Reading, U.K.