Microbiology of feed samples incubated in nylon bags in the rumen of sheep

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Nylon bags of different mesh size (5–53 μm), containing ground lucerne, were incubated in the rumen of sheep fed lucerne hay. Counts of total culturable, proteolytic and cellulolytic bacteria were performed on the bag contents after 16 h of incubation and also on a ruminal ingesta sample in an anaerobic cabinet using the plating technique. Microscopic counts of ciliate protozoa were also made. Total culturable counts in the 5 and 10 μm bags were <10% and in the 53 μm bag only 60% of the values in ruminal ingesta. However, counts of the ciliate protozoa were higher in the 30 and 53 μm bags than in the ruminal ingesta probably due to their greater ability to move into the bags with larger mesh size. The results show that the microbiology inside the bag differs from that of the surrounding ingesta and care should be taken in interpreting results on feed evaluation and degradation obtained using this technique.

Nylon sakkies van verskillende stofdigthede (5–53 μm) en wat gemaalde lusern bevat het, is in die rumen van skape, wat lusernhooi gevoer is, geïnkubeer. Tellings van totale kweekbare, proteolitiese en sellulolitiese bakterieë is gedoen op die inhoud van die sakkies na 16 h inkubasie, asook op 'n monster van die verteringsmateriaal in die rumen, in 'n anaerobiesekabinet deur gebruik te maak van die plaattegniek. Mikroskopiese tellings van die siliaat protosoë is ook gedoen. Totale kweekbare tellings in die 5 en 10 μm sakkies was <10% en in die 53 μm sakkie slegs 60% van die waardes in rumen verteringsmateriaal. Tellings van die siliaat protosoë was egter hoër in die 30 en 53 μm sakkies as in die rumen verteringsmateriaal, waarskynlik weens hul groter vermoë om in die sakkies met die laer stofdigtheid in te beweeg. Die resultate dui aan dat die mikrobiologie binne-in die sakkie verskil van dié in die omliggende verteringsmateriaal en versigtigheid moet aan die dag gelê word tydens die interpretasie van resultate van die waardebepaling en afbraak van voer wat deur gebruik van hierdie tegniek verkry is.

Keywords: Nylon bag technique, mesh size, microbiology of feed samples, ruminal bacteria, viable counts, ciliate protozoa

Introduction

The *in situ* nylon bag technique has been used extensively for feed evaluation and estimating rates of degradation of different dietary components in the rumen. Although this technique has the advantage of giving very rapid estimates of digestion of different nutrients in the rumen, it is subject to a large amount of variation (Mehrez & Ørskov, 1977; Playne, Khumnualthong & Echevarria, 1978; Lindberg, 1981). The technique assumes that the microbiology of the feed sample inside the bag is the same as, or similar to, that in the surrounding ruminal ingesta. This assumption was investigated in sheep fed on lucerne hay, with nylon bags of different mesh size in order to explain some of the large variation found when using this method of feed evaluation.

Methods

Three mature Merino-type sheep fitted with ruminal cannulae (83 mm ID) were used in the experiments. The animals were kept in individual pens and fed 600 g of milled lucerne hay twice daily, at 08h00 and 16h00. Counts were repeated on 4–5 separate occasions. Nylon bags of 6 different mesh sizes were studied (5; 10; 12,7; 20; 30; 53 μm). The 12,7 μm mesh size was a woven nylon filter cloth (115013; Henry Simon, Special Products Division, Stockport, Cheshire, England, SK3 ORT) which on microsopic examination had pores ranging from 5–75 The remaining bags were a defined-aperture polyester

material manufactured by Swiss Silk, CH 9425, Thal SG, Switzerland.

A bag of each mesh size containing a 7 g sample of ground lucerne hay was placed in the rumen just after the afternoon feed. The nylon bags were removed from the rumen together with a representative sample of ruminal ingesta just before the morning feed i.e. a 16 h incubation. The samples were taken to the laboratory, diluted exactly 1/10 with anaerobic diluent and processed with the Ultra-Turrax homogenizer for 1 min (Mackie, Therion, Gilchrist & Ndhlovu, 1983). Bacterial counts were made using the media reported by Mackie, Gilchrist, Robberts, Hannah and Schwartz, (1978) and agar plates prepared, inoculated and incubated in an anaerobic cabinet (Forma Model 1024, Marietta, Ohio; 30% CO_2, 5% H_2, 65% N_2 gas phase). Microscopic counts of ciliate protozoa were made on unprocessed samples preserved with 14% (w/w) formalin solution.

A preliminary time-course study was also conducted on bacterial and protozoal concentrations in the 12,7 and 30 μm mesh size bags after 4, 8, 12 and 16 h of incubation. One bag of each mesh size was placed on the sheep's rumen immediately after the afternoon feed and at 4 h intervals thereafter. All the bags and a representative sample of ruminal ingesta, were again removed immediately prior to the morning feed and the counting procedure repeated on three separate occasions.

Results and Discussion

The results presented in Table 1 show that <10% of the total culturable bacteria were present in the samples incubated in the 5 and 10 μm mesh size bags when compared to the count in the surrounding ruminal ingesta. Even with the largest mesh size studied (53 μm) the counts were only 60% of those in ruminal ingesta. This effect was even greater when comparing the cellulolytic bacteria where there were, in the 53 μm bag, only 18% of the corresponding count in ruminal ingesta. This is probably due to the fact that cellulose digesting bacteria are attached to plant particles in the rumen and are unable to enter the nylon bags as easily as unattached bacteria, especially when using small mesh size.

Counts of ciliate protozoa inside the nylon bags were also markedly influenced by mesh size (Table 2). Small entodinia where able to pass into the nylon bags through the small mesh (5 and 10 μm) and ciliate counts inside the bags of 30 and 53 μm mesh were actually higher than the surrounding ruminal fluid. Although the ciliate protozoa have a greater ability, possibly related to motility, to enter the nylon bags than do most of the ruminal bacteria, some of the larger organisms (*Ophryoscolex* and *Polyplastron*) are unable to enter the bags of large mesh size (53 μm). Large holotrichs (*Isotricha*) were able to enter the 12,7 μm mesh bag because of the wide range of pore size found with this material.

The results of the time course study showed that after 4 h of incubation protozoal counts were *ca* 50% of the maximum and had reached a maximum after 12 h of incubation in both the 12,7 and 30 μm mesh size bags (Table 3). In the 12,7 μm mesh bag, total culturable counts increased markedly between 12 and 16 h of incubation, while proteolytic counts more than doubled. Counts of total culturable bacteria in the 30 μm mesh bag after 12 h of incubation were 60% of the 16 h value, while counts of proteolytic bacteria increased 6-fold between 12 and 16 h of incubation. Thus bacterial counts require at least 16 h incubation and probably longer to reach a maximum. These results could have been influenced by

Table 1 Counts of total culturable, proteolytic and cellulolytic bacteria in lucerne hay samples incubated in nylon bags of different mesh size suspended in the rumen of sheep fed lucerne hay

Nylon bag mesh size (μm)	Bacterial count/g contents			
	Total culturable ($\times 10^8$)	(% of RI)	Proteolytics (% of RI)	Cellulolytics (% of RI)
5	4,6	7,7	2,5	0,3
10	4,2	7,1	2,5	0,9
12,7	17,7	29,7	12,5	4,8
20	25,7	43,2	17,3	6,3
30	27,7	46,5	21,6	6,9
53	37,1	62,3	45,2	17,7
[a]RI	59,5	100,0	100,0	100,0

[a] RI = Ruminal ingesta.

Table 2 Counts of ciliate protozoa in lucerne hay samples incubated in nylon bags of different mesh size suspended in the rumen of sheep fed lucerne hay

Nylon bag mesh size (μm)	Ciliate protozoa $\times 10^5$/g contents					
	Entodinium	*Holotricha*	*Ophryoscolex*	*Polyplastron*	Total	Total (% of RI)
5	0,3	0	0	0	0,3	7,9
10	1,1	0	0	0	1,1	29,0
12,7	2,7	0,06	0	0	2,8	73,7
20	2,5	0,04	0	0	2,5	65,8
30	4,8	0,08	0	0	5,0	131,6
53	4,5	0,26	0,08	0	4,8	126,3
[a]RI	3,2	0,26	0,2	0,06	3,8	100,0

[a] RI = Ruminal ingesta.

Table 3 Effect of incubation time on bacterial and protozoal counts in lucerne hay samples incubated in 12,7 and 30 μm mesh nylon bags suspended in the rumen of sheep fed lucerne hay

Incubation time (h)	Protozoal count/g contents					Bacterial count /g contents	
	Entodinium	*Holotricha*	*Ophryoscolex*	*Polyplastron*	Total	[a]TC ($\times 10^8$)	[b]P ($\times 10^6$)
12,7 μm mesh size							
4	1,1	0,11	0	0	1,2	1,9	4,1
8	1,4	0,08	0,02	0	1,5	1,5	5,7
12	2,5	0,04	0	0	2,5	1,7	7,4
16	2,6	0,11	0	0	2,6	8,8	17,9
30 μm mesh size							
4	1,5	0,18	0	0	1,7	2,5	8,9
8	1,3	0,14	0	0	1,5	1,7	2,1
12	3,2	0,33	0	0	3,5	8,9	7,1
16	3,0	0,25	0,01	0	3,3	15,0	41,8

[a] TC = total culturable. [b] P = Proteolytic.

the introduction into the rumen of sample bags containing fresh material at different times resulting in a strong chemotactic effect when soluble nutrients are depleted in the surrounding ruminal ingesta.

In conclusion, the present results show that for sheep fed lucerne hay the microbiology inside the bags differs considerably from that of the surrounding ruminal ingesta and care should be taken when interpreting results on feed evaluation and especially on rates of degradation obtained using this technique. The counts of bacteria and ciliate protozoa indicate that a mesh size of 30–53 μm would be optimal, although this could be compromised by the influx and efflux of feed particles.

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