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Comparison of two solid-phase markers for measuring the flow of digesta components in the duodenum of sheep

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Abstract

In an experiment studying the effect of defaunation and refaunation of the rumen of sheep on duodenal passage and some rumen digestion parameters, for reasons of certainty, two marker combinations were used for application of the double-marker method for intestinal flow measurements. A combination of Cr_2O_3/PEG was used and both markers were mixed in the concentrate portion of the ration. The other combination was Ru-phenanthroline/PEG; the former was continuously infused in the rumen. The sheep had T-piece cannulae in the proximal duodenum and were fed hourly. This experimental set-up permitted comparison of the results for the flow of digesta components, obtained with both marker combinations. Differences in flow of duodenal digesta and digesta components, calculated with both marker systems, were rare and rather small, indicating that Cr_2O_3 was at least in this experiment an adequate marker of the solid phase of the digesta for application of the double-marker method.

Keywords: sheep, rumen digestion, digesta flow, markers

Introduction

During the last years, the use of re-entrant cannulae in proximal duodenum and distal ileum of ruminants for determination of flow of digesta and digesta components has decreased in favour of the use of T-piece cannulae, mainly as the result of development of satisfactory dual-phase markers (Table 1). The latter method offers several advantages, e.g. there is no need for transsection of the small intestine, influencing intestinal peristaltic activity and digesta flow, although still some disturbances of normal flow are observed. Also, the animals are less under stress and can probably be maintained for a longer period, while the method requires considerably less labour (Wenham, 1979; Wenham & Wyburn, 1980).

With T-piece cannulae in the gastro-intestinal tract, the spot sampling technique instead of total collection of digesta is used. It has been shown that samples are not

Table 1. Evolution of the use of re-entrant versus T-piece cannulae in the gastro-intestinal tract of ruminants.*

Year	Re-entrant	T-cannulae
1982	5	8
1983	1	4
1984	4	6
1985	8	7
1986	1	10
1987	2	4
1988	1	7
Total	22	46

* Papers published in British Journal of Nutrition, 1982-1988.

representative for the whole digesta flowing along the sampling point (Hogan & Weston, 1967; Faichney, 1980). Using two markers, one for the liquid and one for the solid phase of digesta, reconstitution of the true digesta can be done by the double-marker method (Faichney, 1975; 1980).

In an experiment investigating the effect of defaunating the rumen on microbial growth efficiency and other rumen fermentation parameters, we used two combinations of markers. The first combination was polyethylene glycol (PEG)/Cr_2O_3, marker for the liquid and solid phase of digesta, respectively, and both mixed in the feed, while the second combination was PEG/ruthenium-phenanthroline, the first mixed in the feed and the latter continuously infused in the rumen of sheep, fitted with a T-piece cannulae in the proximal duodenum (Kayouli et al., 1986). Flows of digesta and digesta components at the duodenum, calculated with both combinations, were compared.

Materials and methods

Animals and markers

Two adult wethers (Nos 1 and 2), weighing 59.5 and 73.0 kg, respectively, were provided with a permanent rumen cannula and a T-piece cannula in the proximal duodenum, prior to the biliary and pancreatic duct (Decuypere et al., 1977). The ration was fed in hourly portions and consisted of 600 g of concentrates and 600 g of hay. The composition of the ration is given in Table 2. The sheep ate all the feed offered. The first marker combination was mixed in the concentrates: polyethylene glycol (PEG 4000, 1.32 % v/w) as marker of the liquid phase of the digesta and Cr_2O_3 powder (0.81 % w/w) as marker of the solid phase. As second marker of the solid phase, ruthenium-phenanthroline was used, dissolved in water and prepared as described by Tan et al. (1971). It was continuously infused in the rumen (10 ml per h; ca 7 mg of Ru per 24 h).

Table 2. Composition of the experimental feed.

	DM[1] (%)	OM (%)	CP (% in DM)	EE (% in DM)	CF (% in DM)	NFE (% in DM)
Concentrate	90.2	82.3	17.2	2.9	4.9	66.3
Hay 1[2]	87.2	75.6	17.5	2.9	31.2	35.1
Hay 2	84.3	74.9	20.6	2.5	30.7	35.2
Hay 3	86.0	76.0	23.0	3.4	27.8	34.1

[1] DM = dry matter; OM = organic matter; CP = crude protein; EE = ether extract; CF = crude fibre; NFE = nitrogen-free extract.
[2] Hay fed during P1, P2 and P3 (see text), respectively.

Sampling and analysis

Chemical composition of the ration was determined following the Weende scheme, using the CEC methods.

Sampling and analysis of rumen contents, duodenal digesta and faeces was as described in detail by Kayouli et al. (1986). Ruthenium in lyophilized samples of duodenal digesta (total digesta and liquid phase after filtration) was determined by neutron activation analysis. The samples were irradiated for 21 h at a neutron flux of 1.6×10^{12}n cm^{-2} s^{-1}. The induced activities were measured by Ge(Li) gammaspectrometry after a decay time of 20 days. The 497.8 keV gamma ray of the isotope ^{103}Ru ($T_{1/2}$ = 39.1 days) was measured. The net peak area was compared to that of a coirradiated standard (214 μg ruthenium).

Flows of digesta and digesta components were calculated by the double-marker method, as outlined by Faichney (1975; 1980), using mathematical reconstitution of the true digesta.

Experimental scheme and statistical methods

Originally, the experiment was planned to consist of three periods with two sheep: a first control period (P1) with the sheep having a normal rumen fauna, which was followed by a defaunated period (P2) and a second control or refaunated period (P3). However, as one animal died during the defaunation procedure, another sheep was used in P2 and P3 (Kayouli et al., 1986). Each period lasted three weeks, on seven days of which, rumen and duodenum contents were sampled (100 ml of duodenal contents at 8.30 h, 12.30 h and 16.00 h). The three samples were pooled and then analysed to obtain the daily flow. The daily flow of digesta and digesta components, calculated by using the two marker combinations, were compared and subjected to a *t*-test on paired observations (Snedecor & Cochran, 1971). Each animal thus acted as its own control.

Table 3. Comparison of the flow of digesta components in the duodenum and some rumen fermentation parameters calculated with both marker combinations: mean values per sheep and per period with their standard deviation.

Digestion[1] parameter	Sheep 1						Sheep 2			
	P1 (n = 7)[2]		P2 (n = 7)		P3 (n = 7)		P2 (n = 7)		P3 (n = 5)	
	Cr/PEG	Ru/PEG	Cr/PEG	Ru/PEG	Cr/PEG	Ru/PEG	Cr/PEG	Ru/PEG	Cr/PEG	Ru/PEG
Digesta flow ($l\ d^{-1}$)	13.95	14.05	13.10	13.15	12.77	12.59*[3]	11.49	11.47	9.08	8.82
	1.43[4]	1.40	1.36	1.36	1.12	1.21	0.82	0.83	0.23	0.13
DM flow ($g\ d^{-1}$)	726.9	717.6	747.2	703.1	585.3	618.2*	638.4	662.0	506.8	578.2
	94.5	128.7	41.2	61.6	38.1	23.0	56.1	57.0	14.9	67.2
OM flow ($g\ d^{-1}$)	552.9	544.1	615.7	573.1	469.2	500.8*	510.4	532.2	398.4	465.2
	74.7	109.3	41.2	52.9	37.3	20.3	42.9	45.0	14.4	60.7
TN flow ($g\ d^{-1}$)	24.90	25.00	31.40	30.49	24.50	25.19*	28.83	29.45	20.78	22.99
	5.44	6.44	1.88	2.50	2.90	2.61	3.64	3.70	1.63	1.77
NAN flow ($g\ d^{-1}$)	23.13	23.21	29.95	29.03	22.94	23.65*	27.28	27.93	19.44	21.67
	5.40	6.42	2.01	2.56	2.90	2.57	3.69	3.80	1.68	1.72
ADF flow ($g\ d^{-1}$)	122.6	116.9	148.1	132.4	99.2	110.0*	117.8	125.5	93.7	119.2
	19.8	12.4	19.5	18.0	15.6	12.0	43.4	43.1	10.0	27.7
Bacterial N flow[5] ($g\ d^{-1}$)	13.08	12.86	16.27	15.51	16.56	17.13*	17.66	18.02	10.06	11.22
	1.35	1.28	3.53	2.70	5.79	5.78	2.41	2.55	0.70	0.85
% OM digested in rumen	41.7	42.6	34.7	38.6	50.60	47.3*	45.9	43.9	58.1	51.0
	7.9	11.5	4.4	6.0	3.9	2.1	4.6	5.2	1.5	6.4
% ADF digested in rumen	50.1	52.4	36.1	42.9	54.5	49.5*	49.2	45.8	57.0	45.3
	8.1	5.1	8.4	7.8	7.2	5.5	18.7	18.6	4.6	12.7
Bacterial growth efficiency[5] $g_{N_i}kg^{-1}_{OM_f}$	33.27	31.95	49.63	42.15	34.94	38.34*	40.66	43.04	18.23	20.66
	4.67	3.62	11.34	1.72	13.80	13.57	5.00	4.77	1.46	3.72

[1] DM = dry matter; OM = organic matter; TN = total nitrogen; NAN = non-ammonia nitrogen; ADF = acid detergent fibre; $g_{N_i}kg^{-1}_{OM_f}$ grams of nitrogen incorporated in rumen bacterial matter per kg of organic matter fermented in the rumen.

[2] Number of observations.

[3] * Mean values are significantly different from Cr/PEG values ($P < 0.05$) (t-test on paired observations).

[4] Standard deviation.

[5] Only 3 replicates.

Results and discussion

In Table 3, comparison of flow of digesta and digesta components as well as some rumen fermentation parameters, calculated with both marker combinations, are shown. Each period for each sheep is given separately, and only for one period (sheep 1, period 3), significant differences were apparent for all parameters calculated. It must be said, however, that the differences were often rather small; the majority of Ru/PEG values for the flow of digesta components were slightly higher than the corresponding Cr/PEG values.

These results are in good agreement with some recent literature data, reporting comparison of different marker combinations with sheep and cows. Poncet & Al Abd (1987) studied three marker combinations, Cr_2O_3 on cellulose/PEG, Cr_2O_3 mordanted/PEG and $^{169}Yb/^{51}Cr$-EDTA in sheep with T-piece cannulae in duodenum. The animals were fed two different rations in eight portions per day, and PEG, Yb and Cr-EDTA were solubilized and continuously infused in the rumen. The other markers were added at feeding time. There were no differences in flow of organic matter or N at the duodenum, calculated with the three marker combinations as outlined by Faichney (1980). Peyraud (1987) worked with cows and tested Yb/PEG versus Cr_2O_3/PEG. Yb and PEG were continuously infused in the rumen, while Cr_2O_3 was brought in the rumen three times a day, at feeding time. Again, no differences in flow of NAN (non-ammonia N) at the duodenum were observed, as calculated with both marker systems, but the data obtained with Cr_2O_3/PEG showed a much greater variability (higher standard deviation) than the Yb/PEG combination. This does not agree with our results, probably due to the fact that Cr_2O_3 was given only three times daily while our sheep received hourly portions, creating conditions closer to a steady-state system. Higher variability could also be linked to the use of cows instead of sheep. From Table 3, it can be seen that flows in sheep 2 were consistently lower than in sheep 1, demonstrating variability in flows between animals, as observed earlier (Borhami et al., 1980).

It can be argued that it is not surprising that no differences in digesta flow were observed, as a same marker for the liquid phase was used, and liquid accounts for 95 % of digesta. However, the experiment allowed to compare the usefulness of Cr and Ru as solid-phase markers applying the double-marker technique for measuring flows of the different nutrient components of digesta (Faichney, 1975). It has been suggested that Cr_2O_3 does not associate satisfactorily with the solid phase of the digesta in the gastro-intestinal tract, but behaves rather independently, making it not suitable for use in combination with a liquid-phase marker for application of the double-marker method (Faichney, 1972; 1980). Our experimental data do not support this suggestion, unless we assume that the behaviour of Ru-phenanthroline in our experiment was comparable with Cr_2O_3, as far as its shortcomings are concerned. However, the use of Ru-phenanthroline as a solid-phase marker for the double-marker method has been investigated by several authors and its accuracy and reliability have been found to be satisfactory (Tan et al., 1971; Faichney, 1972; MacRae & Ulyatt, 1972; MacRae & Evans, 1974).

It is also noteworthy to mention an experiment done by McAllan & Smith (1983)

with steers fitted with T-piece cannulae in abomasum or duodenum and fed twice daily. They used Cr_2O_3-impregnated paper and PEG as markers, directly introduced in the rumen at feeding time. Flows in the duodenum, calculated with Cr_2O_3 or PEG as single marker, did not differ significantly, indicating that the sample taken from the cannula was representative for the digesta passing through the duodenum. It is possible that chemical and physical (particle size) characteristics of the ration fed and the level of feeding play an important role in the applicability of several marker methods. Furthermore, basic information about the importance of specific gravity of marked particles, formation of a raft in the rumen which functions as a differential retaining mechanism specially for larger particles, and finally the role of reticulum and omasum in the control of particle flow can be found in a recent paper by Sutherland (1988).

In conclusion, in this experiment the results of duodenal digesta flow obtained with the Cr_2O_3/PEG combination were very good comparable with those obtained with the Ru/PEG combination. However, we must emphasize that the sheep were hourly fed. The use of Cr_2O_3 offers some advantages. It can easily be mixed in the concentrate part of the ration and it can be analysed by simple atomic absorption spectrophotometry, a technique which is cheaper and more accessible for the nutritionist than neutron activation analysis or other techniques for ruthenium determination.

Use of single markers in combination with T-piece cannulae at a section of the intestinal tract is strongly discouraged as considerable errors in flow of digesta and digesta components can be introduced (MacRae, 1974; Faichney, 1980).

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