Studies on intestinal digestion in the sheep

I. The use of chromic oxide as an indigestible marker

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1. The use of chromic oxide (Cr_2O_3) as an indigestible marker for studies on intestinal digestion in sheep has been examined. The sheep used were equipped with a cannula into the rumen and a re-entrant cannula in the proximal duodenum; some also had a re-entrant cannula in the terminal ileum. The marker was administered twice daily in the form of Cr_2O_3-impregnated paper, through the rumen cannula.

2. Recovery of Cr_2O_3 in the faeces, based on 7-day collection periods, was satisfactory, the mean recovery for all seven experiments being 99·6 ± 0·7 %. In seventeen 24 h collections of digesta entering the proximal duodenum, the mean recovery of the daily dose of marker was 83·7 % (range 63·6–148·4 %); in eleven such collections at the terminal ileum the mean recovery was 77·3 % (54·0–90·0 %).

3. Detailed examination of the concentrations of Cr_2O_3 in dry matter was made with individual samples taken during single 24 h periods for five duodenal and three ileal collection periods. There were always marked variations in these concentrations. It is concluded that use of short collection periods to give mean values for the flow of digesta throughout the 24 h, at particular points along the tract, may lead to large errors.

Quantitative aspects of digestion in the intestinal tract of the ruminant animal are being studied in this laboratory. For these studies sheep have been equipped with re-entrant cannulas into the proximal duodenum and the terminal ileum.

In view of the relatively short periods over which collections of duodenal and ileal digesta are made, great care must be taken to ensure that the results obtained with such animals are a valid reflection of the processes occurring in intact animals. Hogan & Phillipson (1960) demonstrated that by re-introducing either more or less digesta than was collected, it was possible to affect the flow from the abomasum. Re-introduction of more digesta resulted in a reduced flow, probably due to distension of the duodenum (Phillipson, 1952). Goodall & Kay (1965) showed that when continuous 3-day collections of ileal digesta were made, there was a reduced flow in the first 24 h with compensation in the flow on the 2nd and 3rd days.

In order to minimize the errors arising from a single 24 h collection, and those arising from the collection procedures used, it is the practice to use an indigestible marker as a reference substance. This practice enables the result obtained in the collection period to be used to obtain an average value for 24 h.

Lignin, polyethylene glycol (PEG) and chromic oxide (Cr_2O_3) have all been used as reference substances. The last mentioned has been preferred for the present studies since it has been shown to be associated with the solid phase of intestinal digesta (Harris & Phillipson, 1962) and can be easily and accurately determined. PEG

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associates itself with the liquid phase of digesta (Hydén, 1956) and lignin suffers the disadvantage that it is an ill-defined entity the estimation of which is empirical. Cr_2O_3 can be administered as a powder, as an oil suspension in gelatin capsules or impregnated onto paper. For studies with ruminants Cr_2O_3-impregnated paper appears the most satisfactory, no doubt owing to the slow and sustained release of the oxide as the paper undergoes microbial digestion (Corbett, Greenhalgh, McDonald & Florence, 1960).

In the present paper the validity of using Cr_2O_3 as an indigestible marker, in studies on intestinal digestion in sheep, is discussed in the light of results obtained when sheep equipped with duodenal and ileal re-entrant cannulas were fed on hay, barley and hay plus cereal rations.

EXPERIMENTAL

Sheep. Halfbred × Suffolk wether sheep, 1–3 years old and weighing 45–55 kg, were used for all the experiments. Sheep Br, E, K, W and S were each fitted with a rumen cannula and re-entrant cannulas were placed in the proximal duodenum; sheep B, C, R, Rg, Ri and Ro were each equipped with a rumen cannula and re-entrant cannulas in the proximal duodenum and terminal ileum. Re-entrant cannulas of the type described by Ash (1962) were used and were fitted to each sheep in a single operation (Brown, Armstrong & MacRae, 1968).

Management of sheep. The sheep were housed in metabolism crates. They had free access to water at all times and were subjected to alternating 12 h periods of light and darkness except when duodenal or ileal collections were being made, when the lights were kept on for the whole 24 h period. Environmental temperature varied over the range 15–20°. The sheep were fed twice daily at 9.00 and 16.30 h, receiving equal quantities of ration at each feed.

Shredded paper impregnated with Cr_2O_3 (kindly made available by Dr J. F. D. Greenhalgh, Rowett Research Institute, Aberdeen) was used as the source of Cr_2O_3. Pellets of the paper weighing 3 g (approximately 1 g Cr_2O_3) were made up and one was introduced at each feed, into the rumen of the sheep through the fistula, throughout the experimental period and for at least 14 days before its start.

Collection of samples. For those sheep fitted with duodenal and ileal re-entrant cannulas, each 7-day digestibility trial was followed by a 24 h ileal collection and some 2 days later by a 24 h duodenal collection. The intestinal collections were performed in this order so that no possible contamination of ileal contents could occur from donor material introduced at the duodenum. This precaution was probably unnecessary in view of the limited retention times of digesta in the small intestine of sheep (2·25–4·50 h) reported by Coombe & Kay (1965). For those animals not fitted with re-entrant cannulas in the terminal ileum, the 24 h duodenal collections were made immediately after the completion of the 7-day faecal collection. During the faecal collection period subsamples of faeces (approximately 10% of the daily faecal outputs) were dried separately at 103°, ground through a 1 mm sieve in a Christy and Norris hammer mill and stored for analysis. During the continuous 24 h duodenal collections, the cannulas were disconnected and digesta leaving the abomasum were

collected for consecutive 90 min periods. At the end of a period the digesta collected were weighed and homogenized and a sample (approximately 100–150 g) was taken for subsequent analysis; smaller duplicate samples (each approximately 20 g) were also taken from each 90 min collection for the determination of dry matter. The surplus digesta was then made up to its original weight with duodenal contents collected from a donor animal given the same ration (Harris & Phillipson, 1962) and then re-introduced slowly over a period of approximately 1 h, while the next 90 min sample was being collected. All samples for dry-matter determination were immediately dried to constant weight at 103°. The larger samples for analysis, one from each 90 min period, were immediately stored at −5°. They were subsequently dried under reduced pressure at 50° and ground through the 1 mm sieve in a Christy and Norris hammer mill. A similar procedure was adopted for the 24 h collections at the terminal ileum except that, owing to the reduced flow at this point, 3 h collection periods were used. In all the experiments accumulated 24 h samples of duodenal and of ileal digesta were prepared from the 24 h dried samples on a dry-weight basis.

Analyses. In all the experiments, faecal samples and accumulated 24 h samples of duodenal and of ileal digesta were analysed for their content of Cr_2O_3 by the method of Christian & Coup (1954) as modified by Stevenson & De Langen (1960). In certain experiments the vacuum-dried samples obtained for each 90 min (duodenal) or 3 h (ileal) period within a 24 h collection were also analysed for their content of Cr_2O_3. A random selection of the marker pellets was also analysed to determine the daily intake of Cr_2O_3.

RESULTS

Recoveries of Cr_2O_3 in the faeces

The recoveries of Cr_2O_3 in the faeces of sheep given rations of rolled barley, whole barley, hay and hay plus rolled barley are shown in Table 1. It can be seen that the

Table 1. *Daily recoveries of Cr_2O_3 in the faeces of sheep given rations of hay or barley. The marker was administered twice daily through the rumen fistula*

		Cr_2O_3 (g/24 h)									
			Quantity in faeces								
Sheep	Ration	Intake	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Mean	Recovery as % of intake
K	1050 g rolled barley	1·950	1·001	1·411	2·672	2·199	2·557	2·213	1·647	1·957	100·3
S		1·950	2·112	1·801	3·138	1·608	1·910	1·910	1·212	1·986	101·7
S	1050 g whole barley	1·950	1·879	1·563	2·841	1·377	2·356	1·718	—	1·956	100·2
B	900 g hay	2·161	2·446	1·780	2·595	1·897	1·897	2·219	2·045	2·125	98·3
Rg		2·161	2·341	2·429	2·173	2·102	2·058	2·065	2·086	2·179	100·8
B	600 g hay +	2·161	1·943	2·042	2·076	2·161	2·875	2·213	1·335	2·092	96·8
C	300 g rolled barley	2·161	2·371	2·152	2·079	1·910	2·458	1·924	2·426	2·188	101·2

mean daily recoveries of Cr_2O_3 taken over the duration of 7-day digestibility trials were very close to 100%. The mean recovery, with its standard error for all seven experiments was $99{\cdot}6 \pm 0{\cdot}7$ % of the intake. However, in the light of the very considerable day-to-day variation in weight of oxide excreted, this value may be regarded as somewhat fortuitous. If the values for the last day's collection were omitted the mean recovery would be $102{\cdot}4 \pm 1{\cdot}2$%.

Recoveries of Cr_2O_3 at the proximal duodenum and at the terminal ileum

Table 2 gives the recoveries of Cr_2O_3 found in the 24 h accumulated samples from the duodenal and ileal collections, in experiments in which the rations given comprised all hay, all barley or mixtures of hay with either rolled barley or flaked maize.

Table 2. *Recoveries of Cr_2O_3 in the accumulated samples from 24 h collections of duodenal and ileal digesta from sheep, expressed as percentages of the quantity introduced into the rumen per 24 h*

Ration	Sheep	Recovery of Cr_2O_3 (% of intake)	
		Duodenal collection	Ileal collection
1050 g rolled barley	K*	90·6	—
	S*	148·4	—
1050 g whole barley	K*	90·0	—
	S*	46·2	—
900 g hay	B	71·0	72·7
	Rg	92·2	90·0
600 g hay + 300 g rolled barley	B	87·8	74·7
	C	89·1	83·8
	R	91·5	75·4
300 g hay + 600 g rolled barley	B	78·8	72·3
	C	94·1	80·7
900 g rolled barley	B	63·6	54·0
	Br*	67·4	—
300 g hay + 600 g flaked maize	C	79·8	75·3
	E*	83·8	—
240 g hay + 470 g flaked maize	Ri	68·4	84·5
	Ro	79·8	86·6

* Fitted only with a duodenal re-entrant cannula.

It can be seen that in only one instance was the recovery of the marker over a 24 h period greater than the daily intake. The mean recovery for the seventeen duodenal collections was 83·7 % and for the eleven collections at the terminal ileum 77·3%.

Variations in the concentration of Cr_2O_3 in dry matter

Another important aspect of the use of Cr_2O_3 as a marker in studies of intestinal flow is the possibility that its use may allow short collection periods to be used for obtaining representative samples of duodenal and ileal contents. If the concentration

of Cr_2O_3 were constant throughout the 24 h period, a small sample of digesta collected at any one time could be used to obtain information relating to mean 24 h values; this would obviate the need for continuous 24 h collection periods.

Certain experiments were therefore selected in which the concentration of Cr_2O_3 in dry matter was determined in each of the individual samples taken within any one 24 h period. Table 3 shows the Cr_2O_3 concentration in the individual samples taken during 24 h collections of duodenal and of ileal contents from two sheep given an all-hay ration and one sheep given an all-barley ration; similar values relating to the duodenal collections made on two sheep receiving only barley are also given. The concentrations of Cr_2O_3 determined in the accumulated 24 h samples are also shown.

Table 3. *Variations in the concentration of Cr_2O_3 in dry matter found within collections of duodenal and ileal digesta from sheep given hay or rolled barley rations*

(It was intended that duodenal and ileal contents would be collected in 1·5 and 3 h periods respectively; thus for any one 24 h collection the total number of samples should be 16 and 8 respectively. The small number of samples taken during certain 24 h collections indicates a lengthening in certain periods of collection occasioned by reduced flow)

	Cr_2O_3 (mg/g dry matter)							
	900 g hay				600 g barley		1050 g barley	
	Sheep B		Sheep Rg		Sheep B		Sheep K	Sheep S
Sample no.	Duodenum	Ileum	Duodenum	Ileum	Duodenum	Ileum	Duodenum	Duodenum
1	4·72	6·84	4·59	6·28	7·31	15·38	4·46	5·69
2	3·58	5·81	4·24	5·11	7·23	13·23	5·11	5·02
3	4·67	6·57	5·51	4·16	8·12	9·31	4·97	5·16
4	4·48	5·79	6·31	3·36	8·03	12·98	6·00	5·50
5	4·11	4·47	4·26	4·27	7·40	—	5·47	6·61
6	4·67	5·24	4·31	6·25	7·89	—	6·06	6·34
7	4·03	6·68	3·94	6·74	9·42	—	5·31	4·95
8	4·10	7·25	4·14	7·64	8·61	—	4·81	5·92
9	4·25	—	4·48	—	9·04	—	4·30	6·09
10	3·89	—	4·43	—	10·28	—	4·62	5·21
11	4·42	—	4·73	—	7·61	—	5·53	5·15
12	4·56	—	4·75	—	6·87	—	6·53	2·99
13	5·04	—	4·04	—	—	—	6·08	2·60
14	—	—	—	—	—	—	7·57	6·29
15	—	—	—	—	—	—	7·30	6·08
16	—	—	—	—	—	—	6·71	—
Cumulative 24 h sample	4·18	5·63	4·76	6·25	8·28	13·40	5·62	5·61

It can be seen from Table 3 that for any one 24 h collection the concentration of Cr_2O_3 varied very considerably between different periods. The extent of the variation was greater with the all-barley ration than with the all-hay ration, and when both duodenal and ileal collections were made it was greater for the ileal digesta than for the duodenal digesta.

Reference has been made to the possibility of calculating 24 h flow values from

results obtained with short-term collection periods and applying the concentration of Cr_2O_3 found to the equation:

$$\text{Total dry-matter flow (g/24 h)} = \text{dry-matter flow during period } x \text{ (g)} \times \frac{Cr_2O_3 \text{ intake (g/24 h)}}{Cr_2O_3 \text{ collected during period } x \text{ (g)}}.$$

From the concentrations of Cr_2O_3 given in Table 3 for individual collection periods and the dry-matter flows during these periods, mean 24 h flows of dry matter have been calculated and are shown in Table 4. For each experiment the mean 24 h dry-matter flow is also given; this has been calculated from the total dry-matter flow measured during the 24 h period of collection and the concentration of Cr_2O_3 in the dry matter of the accumulated sample for the collection period.

Table 4. *Calculated 24 h flows of dry matter in the duodenal and ileal digesta of sheep receiving diets of hay or rolled barley. The values were obtained from the Cr_2O_3 concentration in the dry matter of samples collected for different periods during the 24 h (given in Table 3) and the measured flows of dry matter during these periods*

	Calculated dry-matter flow (g/24 h)							
	900 g hay				600 g barley		1050 g barley	
	Sheep B		Sheep Rg		Sheep B		Sheep K	Sheep S
Sample no.	Duodenum	Ileum	Duodenum	Ileum	Duodenum	Ileum	Duodenum	Duodenum
1	459	316	470	345	296	139	482	380
2	609	373	515	423	300	163	423	432
3	462	330	392	518	267	233	436	418
4	484	373	342	643	267	166	361	393
5	529	483	515	506	292	—	395	328
6	461	412	501	346	274	—	357	341
7	536	324	549	321	229	—	412	438
8	526	298	524	283	251	—	447	368
9	508	—	482	—	239	—	507	356
10	554	—	486	—	210	—	466	416
11	489	—	456	—	285	—	393	420
12	473	—	454	—	313	—	331	726
13	428	—	537	—	—	—	359	835
14	—	—	—	—	—	—	285	344
15	—	—	—	—	—	—	297	354
16	—	—	—	—	—	—	323	—
Maximum value	609	483	549	643	313	233	507	835
Minimum value	428	298	342	283	210	139	297	328
Cumulative 24 h sample	517	384	451	361	261	161	348	348

It can be seen from Table 4 that in any one experiment the calculated 24 h flow rates for dry matter varied considerably. Thus with sheep S on an all-barley ration, 24 h flow rates for dry matter based upon samples taken over periods of approximately 90 min varied from a minimum value of 328 g to a maximum value of 835 g. The

value based on the Cr_2O_3 analysis of the accumulated 24 h sample gave a corrected flow rate of 348 g dry matter passing in 24 h. Despite the fact that with ileal digesta the individual collection periods within any one 24 h collection were at least of 3 h duration, similar variations in flow rate were also found.

Table 5. *Comparisons of the summation of the Cr_2O_3 found in the individual samples taken within 24 h collections of duodenal and ileal digesta from sheep on diets of hay or rolled barley (a) with the Cr_2O_3 found in the cumulative 24 h samples from the same experiments (b). The ratio, a:b × 100 is shown for each experiment*

	Cr_2O_3 content (g)							
	900 g hay				600 g barley		1050 g barley	
	Sheep B		Sheep Rg		Sheep B		Sheep K	Sheep S
Sample no.	Duodenum	Ileum	Duodenum	Ileum	Duodenum	Ileum	Duodenum	Duodenum
1	0·140	0·163	0·124	0·351	0·141	0·316	0·050	0·402
2	0·047	0·263	0·069	0·211	0·143	0·359	0·128	0·090
3	0·180	0·176	0·185	0·421	0·214	0·143	0·140	0·290
4	0·053	0·072	0·293	0·182	0·154	0·205	0·131	0·282
5	0·070	0·164	0·055	0·167	0·254	—	0·084	0·084
6	0·581	0·254	0·121	0·159	0·178	—	0·147	0·176
7	0·111	0·084	0·119	0·262	0·191	—	0·058	0·115
8	0·105	0·355	0·155	0·252	0·199	—	0·047	0·068
9	0·184	—	0·153	—	0·110	—	0·069	0·433
10	0·082	—	0·183	—	0·290	—	0·048	0·120
11	0·194	—	0·184	—	0·097	—	0·115	0·244
12	0·224	—	0·205	—	0·076	—	0·190	0·094
13	0·150	—	0·160	—	—	—	0·566	0·030
14	—	—	—	—	—	—	0·277	0·257
15	—	—	—	—	—	—	0·153	0·116
16	—	—	—	—	—	—	0·157	—
Total	1·621	1·531	1·953	2·005	2·047	1·023	1·860	2·810
Cumulative 24 h sample	1·535	1·572	1·978	1·946	2·063	1·053	1·773	2·898
Ratio, a:b × 100	105·6 %	97·4 %	98·7 %	103·0 %	99·2 %	97·2 %	104·9 %	97·2 %

In the preparation of an accumulated digesta sample it is of paramount importance that the subsamples should be representative of the digesta passing the cannula if the results to be obtained from its analysis are to have any significance. In Table 5 the weights of Cr_2O_3 passing in a 24 h collection period, derived from a summation of the weights of the marker found in each of the short collection periods, are compared with the values determined by analysis of the accumulated sample. There appears to be no systematic error in the preparation of the accumulated samples. These results suggest that the method used in preparing the accumulated samples is adequate.

DISCUSSION

The virtually complete recovery of Cr_2O_3 in the faeces obtained here over 7-day collection periods is in agreement with the findings of Putnam, Loosli & Warner

(1958) in experiments with cows given the oxide in gelatin capsules and of Cowlishaw & Alder (1963) in experiments with steers given Cr_2O_3-impregnated paper.

Certain workers have reported incomplete recoveries of Cr_2O_3. Thus although Johnson, Dinusson & Bolin (1964) found 101·8% recovery with Cr_2O_3-impregnated paper in 10-day digestibility trials with cows, there was only a 93·3% recovery when Cr_2O_3 powder was used. While Corbett, Greenhalgh, Gwynn & Walker (1958) reported recoveries of 100% over a 5-day collection period, when Cr_2O_3 was incorporated into dried grass cubes made from October-cut herbage and fed to lactating cows, they found only 94·4% in similar experiments with dried grass cubes prepared from June-cut herbage. Pigden & Brisson (1956) obtained recoveries in the faeces of 101, 94 and 87% of the administered dose in three separate 4-day trials, each with four sheep. Deinum, Immink & Deijs (1962) obtained recoveries of 97·5, 98·6 and 98·4% in experiments with cows given 50 g Cr_2O_3-impregnated paper daily and found traces of the oxide in liver, lymph glands and kidneys. They suggested that some absorption of the marker must have occurred.

Donaldson & Barreras (1966) showed that ^{51}Cr when given orally to human subjects in the trivalent form, $[^{51}Cr]Cl_3$, gave almost complete recovery in the faeces (99·6 ± 1·8%), although when given by duodenal intubation to two patients the recovery was slightly reduced (93·7 ± 4·5%). On the other hand, when chromium was administered orally in the hexavalent form ($Na_2[^{51}Cr]O_4$) the faeces yielded only 89·4 ± 2·6%, which fell to 56·5 ± 11·7% when it was given by duodenal intubation. These workers showed that there was considerable binding of the trivalent form with neutralized gastric juice but none with the hexavalent form, although some binding of the hexavalent form did occur with acidic gastric juice. They suggested that such binding reduces absorption and concluded that absorption of the trivalent form, when given orally, is less than 1%. It would certainly appear from the results obtained in the present study that Cr_2O_3 can be considered as an indigestible marker and, as such, can reasonably be used for correcting flow rates of digesta obtained at various points along the digestive tract and measured over 24 h periods to give mean 24 h values.

From the values presented in Table 2 it is clear that the continuous collection of digesta for periods of 24 h tends to suppress flow rate. This is not, perhaps, surprising in view of the fact that during collections of digesta the animals are disturbed. As already mentioned, Goodall & Kay (1965) found that the reduced flow at the ileum observed during the first 24 h was compensated for by increased flow rates during the succeeding 48 h. Even in intact animals, however, the flow rate of digesta past a given point in the tract is unlikely to be constant between consecutive 24 h periods. Certainly the variations in daily excretion of Cr_2O_3 shown in Table 1 support this view.

The very considerable variation found in the concentration of Cr_2O_3 in the dry matter within any one 24 h collection period in the present study is in agreement with the results reported by Harris & Phillipson (1962). It is evident that the use of Cr_2O_3 ratios, derived for digesta obtained in short-term collection periods, to calculate mean 24 h values may give rise to very considerable error.

Finally, it must be noted that a basic assumption in the calculation of corrected 24 h values for the flow of dry matter or other constituent of the digesta from the

observed collection values is that the flow of digesta and of marker is affected to the same extent in any 24 h period. The results presented here do not give any indication as to the correctness of this assumption.

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