Measurement of the rate of flow of dry matter in digesta passing through the duodenum of sheep

By J. D. OLDHAM* and J. R. LING†

University of Nottingham, School of Agriculture, Sutton Bonington, Loughborough, Leics. LE12 5RD

(*Received* 5 *December* 1975 – *Accepted* 20 *August* 1976)

1. A total of seven sheep fitted with re-entrant cannulas in the proximal duodenum were used in three experiments to measure the passage of dry matter (DM) in duodenal digesta.

2. Fifty-three 24 h measurements and ten 48 h measurements of DM flow were made in Expt 1. Coefficient of variation (CV) of these measurements was 22·1 % which was similar to variability reported for chromic oxide 'corrected' flows and for an estimate of day-to-day variation. DM flow on day 1 was 103·2 % (SE = 8·94) of flow on day 2 in 48 h measurements.

3. In two 3 × 3 Latin-square design experiments, duodenal DM flow was measured continuously over 72 h and on days 1, 2 and 3 DM flow was 100·4, 102·6 and 96·9 % (SE = 5·82) of mean flow (Expt 2) and 95·8, 101·2 and 103·0 % (SE = 6·70) of mean flow (Expt 3) respectively.

4. It was concluded that no depression in duodenal DM flow occurred during the first 24 h of collection in these experiments and that the variability of repeated 24 h measurements of flow was within day-to-day variation.

Re-entrant cannulas have been placed at specific points in the digestive tract of ruminants to allow measurement of the rate of flow of digesta past these points. Most reports have described flow measurements of 24 h duration (for example Bruce, Goodall, Kay, Phillipson & Vowles, 1966; MacRae & Armstrong, 1969; Nicholson & Sutton, 1969; Thomson, Beever, Coelho da Silva & Armstrong, 1972; Thompson, 1973; Ulyatt & MacRae, 1974) but there have been a few studies over longer collection periods (Goodall & Kay, 1965; van 't Klooster, Rogers & Sharma, 1969; Thompson & Lamming, 1972; van 't Klooster, Kemp, Geurinck & Rogers, 1972). The length of the collection period has been limited by the labour-intensive nature of manual or semi-automated collection procedures. Fully automated measuring and sampling equipment has been described (Axford, Evans & Offer, 1971; Corse, 1974) although only short reports of studies with these machines have appeared in the literature (Offer, Axford & Evans, 1972; Offer, Evans & Axford, 1972; Tas, Offer, Evans & Axford, 1974; Sutton, Youssef & Oldham, 1976).

Because collection periods have been mostly short (24 h) many workers have used an indigestible reference substance to allow correction for inevitable day-to-day variations in measured digesta flow. The most commonly used marker in ruminant digestion studies has been chromic oxide, despite the inadequacies of this material as a marker (Drennan, Holmes & Garrett, 1970; Faichney, 1972; MacRae, 1974).

* Present address: Department of Feeding and Metabolism, National Institute for Research in Dairying, Shinfield, Reading RG2 9AT, Berkshire.

† Present address: Department of Biochemistry and Agricultural Biochemistry, University College of Wales, Aberystwyth SY23 2AX.

Furthermore, it has been found by several groups that, for continuous digesta collections of 24 h, Cr_2O_3 recovery in digesta averaged only about 85 % (Bruce *et al.* 1966; Topps, Kay & Goodall, 1968; MacRae & Armstrong, 1969; Nicholson & Sutton, 1969; MacRae, Ulyatt, Pearce & Hendtlass, 1972). It was considered that this reflected a depression in digesta flow, perhaps as a result of the stress of experimental procedure. Support for this was given by Goodall & Kay (1965) in 72 h measurements of ileal digesta flow. They suggested that flow in the first 24 h of collection was less than the average flow for 3 d, but that this was compensated by an increased flow on the second day. No markers were used in that study, but van 't Klooster *et al.* (1969) measured duodenal digesta flow and daily marker (Cr_2O_3) recoveries over 72 or 120 h periods and confirmed that Cr_2O_3 recovery was reduced in the first 24 h of collection, although over-all recovery was invariably close to 100 %.

In contrast, Thompson & Lamming (1972) could find no difference between days in 72 h continuous duodenal digesta collections for either dry matter (DM) or digesta flow. This was used by them to justify the measurement of digesta flow over 24 h without marker correction although no marker was used in the 72 h study.

We have adopted the experimental procedure of Thompson & Lamming (1972), and have measured digesta flow over 24, 48 and 72 h in sheep fed on a variety of diets with the purpose of studying the effect of the duration of measurements on measured DM flow and to obtain an estimate of the reliability of repeated 24 h determinations of flow.

We found no difference in DM flow between the first and subsequent 24 h periods of a duodenal digesta collection and also concluded that the variability of repeated 24 h measurements of flow was within day-to-day variation.

METHODS

Animals and feeding

Seven crossbred wethers were used in these experiments. Each was fitted with a re-entrant cannula in the proximal duodenum by the technique used by Thompson & Lamming (1972). The sheep in Expts 1 and 2 were fed once daily at 11.00 hours and those in Expt 3 were fed hourly from a continuous belt feeder. All sheep had continuous access to water. Between digesta collection periods the sheep were kept in individual pens with wire mesh floors. The day before collection they were moved to standard metabolism crates and remained there until all collections for a particular dietary treatment had been completed.

Diets

Diets 1–9 (Table 1) were used in Expt 1, diets 10–12 in Expt 2 and diets 13–16 in Expt 3.

Change-over from one diet to another was completed in 4 d and at least a further 10 d on full ration was allowed before any duodenal collections were made.

Diets 3, 6, 9 and 12 were fed as loose mixes since they had a high molasses content and could not be pelleted. All other diets were pelleted.

Table 1. *Composition (g/kg) and daily allowance (g) of the experimental diets given to sheep*

Diet ...	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Ingredient																
Barley straw	710	110	—	800	150	—	755	115	—	760	110	—	—	150	150	150
Wheat straw	—	—	308	—	—	362	—	—	326	—	—	282	—	—	—	—
Ground barley	120	740	—	140	590	—	120	510	—	76	526	—	270	610	550	685
Oats	—	—	—	—	—	—	—	—	—	—	—	—	120	—	—	—
Maize starch	—	—	—	—	200	—	—	250	—	—	136	—	—	—	—	—
Molasses*	—	—	462	—	—	568	—	—	489	—	—	481	—	—	—	—
Soya-bean meal	120	100	180	—	—	—	—	—	—	115	183	192	100	—	150	—
Urea	—	—	—	15	15	35	—	—	—	—	—	—	—	—	—	15
Ground white-fish meal	—	—	—	—	—	—	80	80	140	—	—	—	—	90	—	—
Dried grass	—	—	—	—	—	—	—	—	—	—	—	—	480	—	—	—
Mineral mix†	50	50	50	45	45	45	45	45	45	49	45	45	30	50	50	50
Nutramol‡	—	—	—	—	—	—	—	—	—	—	—	—	—	100	100	100
Daily allowance	1610	1050	1040	1320	1120	920	1160	1170	1040	1100	1300	1300	1000	1270	1230	1310

* Molasses and ground wheat straw were fed together as a molassed ground wheat-straw preparation (60% molasses).

† The mineral mix contained three parts by weight of common salt, steamed bone-flour and limestone and one part of a commercial vitamin and mineral supplement to supply the vitamin and mineral requirements of the sheep as stated by the Agricultural Research Council (1965).

‡ Supplied by Rumenco Ltd, Burton-on-Trent. Contains 50% molasses on a basically moss peat carrier.

Duodenal digesta collections

Sheep H, M and P were acclimatized to the experimental routine by making two collections of 24 h duration before the start of Expt 1. They were given a diet of dried, pelleted grass at that time. Expt 1 began immediately thereafter. Collections of digesta were made from each of the other sheep before the experiments described here, but these were of less than 8 h duration.

Total manual collections of duodenal digesta were made as follows. Flexible tubing (Visking Dialysis tubing) was attached to each cannula. Digesta leaving the cannula drained into a polythene measuring cylinder fixed level with the floor of the metabolism crate, and each 100 ml collected was sampled (5%) after vigorous manual agitation. The sample was replaced with donor digesta collected the week before each collection and stored frozen until the day of collection. Digesta was returned to the sheep via flexible tubing attached to a plastic funnel clamped to the metabolism crate above the sheep.

The sheep could lie or stand without hindrance to themselves or to the collection apparatus.

Experimental programme

Expt 1. Three sheep (H, M and P) were offered each of diets 1–9 in a randomized block design experiment. For each treatment with each sheep, three 24 h duodenal digesta collections were made. On ten occasions when diets 6–9 were being fed two of the 24 h collections were made consecutively so that digesta flow was measured over a continuous 48 h period.

Expt 2. Three sheep were used (M, Q and S). Diets 10–12 were offered in a Latin-square experimental design. Sheep S was withdrawn from the experiment after completing only two of the three experimental periods because it refused the diet offered. For each treatment with each sheep, one continuous 72 h duodenal digesta collection was made.

Expt 3. Three sheep were used (Q, W and K). Diets 14–16 were offered to each sheep in a Latin-square experimental design and one 72 h duodenal digesta collection was made from each sheep on each treatment. In addition three separate 72 h collections were made from sheep Q fed on diet 13.

Treatment of samples. A 5% sample of digesta was collected and freeze-dried to determine DM.

Statistical analysis. Analysis of variance was used throughout according to Snedecor & Cochran (1967). Two missing values were calculated for Expt 1, one each for sheep H and P who refused diets 2 and 7 respectively. For Expt 2 one missing value was calculated for sheep S who was withdrawn after receiving two of the three experimental diets.

RESULTS

Expt 1

Table 2 shows the flow of DM and digesta through the duodenum. DM flow was significantly greater ($P < 0.05$) for diets 1, 4 and 7 than for other diets. These diets

Table 2. *Expt* 1. *Daily intake of dry matter* (DM) (*g*) *and passage of* DM (*g*) *and digesta* (*ml*) *through the duodenum of sheep given experimental diets* 1–9*

Diet ...	1	2	3	4	5	6	7	8	9	SE of mean
Intake ...	1610	1050	1040	1320	1120	920	1160	1170	1040	—
Duodenal flow										
DM	904	413	363	942	459	396	714	461	452	76·1
Digesta	16017	7654	8696	18132	8856	8618	13680	9435	10496	1259·0

* For details of diets, see Table 1

Table 3. *Expt* 1. *Duodenal dry matter* (DM) *flow in hours* 0–24 *of a* 48 *h collection represented as a percentage of* DM *flow in hours* 24–48 *for sheep given diets* 6–9*

Diet ...		6	7	8	9
Sheep	H	107·1	90·4	108·8	102·8
	M	—	101·6	118·5	111·3
	P	107·6	55·6	119·3	—

Mean adjusted for missing values = 103·2 SE = 8·94.
* For details of diets, see Table 1.

Table 4. *Dry matter* (DM) *flow through the duodenum of sheep given diets* 10–12 (*Expt* 2) *and* 14–16 (*Expt* 3). *Results are presented as mean* DM *flow* (*g*) *in each* 24 *h period of the* 72 *h collection and as daily* DM *flow represented as a percentage of mean* DM *flow estimated over the entire* 72 *h collection period*

Hours of collection ...	0–24	24–48	48–72	SE of mean
Mean DM flow through the duodenum (g/24 h)				
Expt 2	504	493	473	28·8
Expt 3	518	539	550	33·0
Daily DM flow through the duodenum as a percentage of mean flow measured over 72 h				
Expt 2	100·4	102·6	96·9	5·82
Expt 3	95·8	101·2	103·0	6·70

contained a large proportion of barley straw. Digesta flow followed a similar pattern, with flow for diets 1, 4 and 7 greater ($P < 0·05$) than for all others, with the exception that the difference between diets 7 and 9 was non-significant at the 5% level.

Coefficients of variation (CV) associated with DM flow and digesta flow were 22·1 and 18·3% respectively.

When flow was measured continuously over 48 h the results shown in Table 3 were produced. In this table, flow of DM during the first 24 h of the 48 h collection is represented as a percentage of flow during the second 24 h. These results were subjected to analysis of variance for a randomized block experiment with two missing values. Mean DM flow during the first 24 h of collection was 103·2% (SE = 8·94) of DM flow during the subsequent 24 h. There were no significant differences between sheep or between diets.

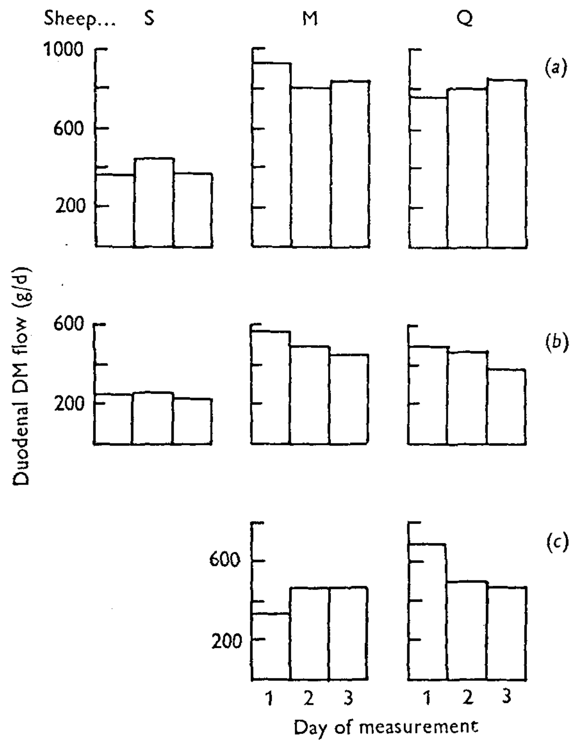

Fig. 1. The flow of dry matter (DM), measured on three consecutive days in digesta passing through the duodenum of sheep S, M and Q given (a) diet 10, (b) diet 11 and (c) diet 12 (Expt 2). For details of diets, see Table 1.

Expts 2 and 3

Duodenal DM flow for diets 10, 11 and 12 (Expt 2) was 686, 403 and 381 g/24 h (SE of mean = 10·5 g) respectively. DM flow for diet 10 was significantly greater ($P < 0{\cdot}05$) than for diets 11 and 12. Duodenal DM flow for diets 14, 15 and 16 (Expt 3) was 522, 523 and 562 g/24 h (SE of mean = 68·2 g) respectively. Differences in DM flow between these diets were not significant.

The results of each 24 h period of the 72 h collections carried out in Expts 2 and 3 are compared in Table 4. The pattern of DM flow for individual measurements is shown in Figs 1 (Expt 2) and 2 (Expt 3). There was no significant difference between the collection means for 0–24 h, 24–48 h and 48–72 h in either experiment. When each period of 24 h was represented as a percentage of mean daily flow over 72 h there was, again, no significant difference between days in either experiment. Thus DM flow measured during the first 24 h of collection did not differ significantly from mean flow measured over 72 h in either experiment.

The results of three 72 h collections of digesta from one sheep (Q) given diet 13 are shown in Fig. 3. Mean DM flow was 331 g/d (SE of mean = 40·5 g) and did not differ significantly ($P > 0{\cdot}05$) between replicate 72 h collections. Flow measured on day 1 of the collections was not significantly different from flow measured on days 2 or 3.

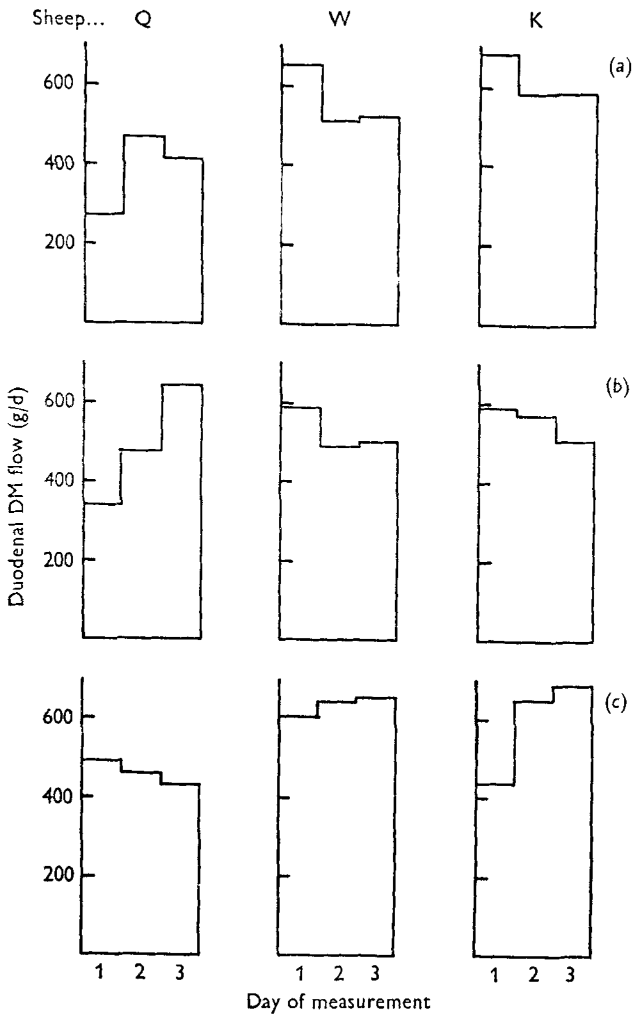

Fig. 2. The flow of dry matter (DM), measured on three consecutive days, in digesta passing through the duodenum of sheep Q, W and K (*a*) diet 14, (*b*) diet 15 and (*c*) diet 16 (Expt 3). For details of diets, see Table 1.

DISCUSSION

These results have been gathered together from several investigations in which measurements of duodenal digesta flow were made. They contain all of the 72 h digesta flow measurements made by the authors over a 2 year period, a total of twenty-one collections from sheep fed on a wide variety of diets. In addition there are results from fifty-three 24 h collections and ten 48 h collections. These data do not demonstrate any consistent difference in duodenal DM flow between days during 72 h or 48 h collections.

There are few reports of digesta collections of longer than 24 h duration with which to compare these results. All of the available published findings are presented in Table 5. To simplify comparisons, values for Cr_2O_3 recovery or DM or digesta

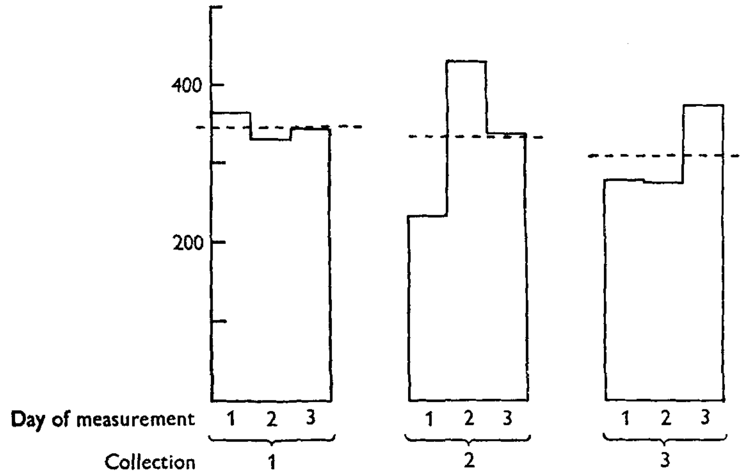

Fig. 3. The flow of dry matter (DM), measured over three consecutive days, on three separate occasions, in digesta passing through the duodenum of one sheep (sheep Q) fed on diet 13 (Expt 3). Dotted lines represent mean DM flow on each occasion. For details of diet, see Table 1.

Table 5. *A survey of published values for measurements over an extended period of chromic oxide (Cr_2O_3), dry matter* (DM) *or whole digesta (digesta) passage in the intestines of ruminants*

(Mean values over all animals and treatments represented for each day of collection as a percentage of the mean value for the entire collection)

Reference	No. of animals	No. of collections	Parameter	Day of collection 1	2	3	4	5	6	7
Van 't Klooster, Rogers & Sharma (1969)	2 (sheep)	2	Cr_2O_3	92	105	101				
Van 't Klooster, Kemp, Geurinck & Rogers (1972)	2 (cows)	4	Cr_2O_3	91	99	96	112	102		
Osbourn (1975)	(sheep)	17	Cr_2O_3	86	94	107	98	103	107	105
Goodall & Kay (1965)	2 (sheep)	6	DM	89	104	107				
Thompson & Lamming (1972)	2 (sheep)	6	digesta	99	100	101				
Johnson & Armstrong (quoted by MacRae, 1975)	1 (sheep)	1	DM	100	98	98	106	102	99	98
			Cr_2O_3	99	98	99	107	97	100	99
Sutton, Youssef & Oldham (1976)	4 (cows)	8	DM	92	104	103	99	102		
			Cr_2O_3	93	100	99	104	104		
Present results										
Expt 1 (Table 3)	3 (sheep)	10	DM	100	100					
Expt 2 (Table 4)	3 (sheep)	8	DM	100	103	97				
Expt 3 (Table 4)	3 (sheep)	9	DM	96	101	103				
Expt 3 (Figure 1)	1 (sheep)	3	DM	89	104	107				

flow on each day of collection are presented as a percentage of the mean for the whole collection period. The results of van 't Klooster *et al.* (1969, 1972) and Osbourn (1975) give support to the concept, first demonstrated by Goodall & Kay (1965), that digesta flow is depressed during the first 24 h of collection. However, there is no consistent evidence for a compensatory increase in flow on day 2 of collection from the values in

Table 5. In 5 d collections of duodenal digesta from adult cattle with re-entrant cannulas, Sutton *et al.* (1976) found that DM flow and Cr_2O_3 recovery was lowest on day 1 of collection, but that correction for Cr_2O_3 recovery increased the standard error associated with mean DM flow and only partially reduced day-to-day variation in mean flow. They concluded that it was preferable to avoid adjustment for Cr_2O_3 recovery and that 72 h collections, uncorrected for Cr_2O_3 recovery, could be used to measure DM flow at the duodenum. From Table 5 it appears that on those occasions when a reduction in flow was observed on day 1 of an extended term measurement, the loss was about 10%.

No significant differences between days of collection were found in Expt 3 when three separate collections of 72 h duration were made from one sheep, but the mean results (Table 5) for this sheep show a 10% reduction in DM flow on day 1. This was largely the result of a big depression in flow on day 1 of collection 2, followed by an elevation in flow on day 2 (Fig. 3). The pattern of flow in collection 2 thus typified the scheme of depression (day 1) and compensatory elevation (day 2), suggested by Goodall & Kay (1965) but this was not found in collections 1 and 3, and it appeared to be a chance occurrence. This pattern was rarely seen in the other 72 h collections (Figs 1 and 2).

No difference in DM flow between day 1 and subsequent days of collection was found in Expt 1, 2 or 3 in agreement with the findings of Thompson & Lamming (1972). Nevertheless there is the possibility that in this and in the work of Thompson & Lamming (1972), flow was uniformly depressed throughout the entire 72 h period. This is unlikely in the light of the other reports cited in Table 5. Recovery of Cr_2O_3 over 7 d was only 93·3% in Osbourn's (1975) experiments, but recovery increased daily during the first 3 d of collection. Recovery on day 4 was surprisingly low. In eight duodenal digesta collections from cattle, D. A. Corse (unpublished results) found mean recoveries of Cr_2O_3 on days 1, 2 and 3 to be 79, 90 and 92% respectively. Represented in the terms of Table 5, recoveries on days 1, 2 and 3 were 90, 103 and 106% respectively. Thus, in relation to the entire collection, Cr_2O_3 recovery on day 1 was reduced by 10%, in agreement with the other reports of a reduction in flow. Though Cr_2O_3 recovery was depressed throughout the 72 h period, the depression on day 1 was greater than it was on other days. There is, therefore, no supporting evidence for a uniform depression in flow over 72 h from any of these studies. So it can be concluded that digesta flow was not reduced during the first 24 h of duodenal digesta collection from our sheep.

This is quite different from the conclusion which must be drawn from those studies in which mean Cr_2O_3 recovery in 24 h duodenal digesta collections has been found to be in the range 82–87·1% (Topps *et al.* 1968; Bruce *et al.* 1966; MacRae & Armstrong, 1969; MacRae *et al.* 1972; Nicholson & Sutton, 1969). The approximate 15% depression in Cr_2O_3 recovery found by these workers is similar to the 10% depression apparent from some sources in Table 5 and, taken together, these various reports strongly suggest that a reduction in digesta flow during the first 24 h of collection does often occur.

The reason why a depression in flow was not found in the present work is not clear.

We feel that our sheep were adequately trained to the experimental procedure. No differences between experimental periods were found in Expts 2 and 3 which might have been expected if adaptation to the sampling procedure was a major factor influencing the sheep's response to experimentation. The collection procedure we used was not markedly different from that described by other workers quoted here. As discussed above there is no reason to believe that DM flow was depressed for 72 h in these experiments as no other workers have observed such an effect. There was no increase in DM flow from day to day which would be likely if flow was grossly depressed at the start of measurement. We therefore conclude that in these experiments DM flow measured over 24 h, without a marker correction, gave a valid estimate of DM flow through the duodenum.

The CV associated with repeated 24 h measurements of DM flow in Expt 1 was 22·1%. In Expts 2 and 3 CV of DM flow was 3·7% in both instances. These CVs were surprisingly low, and it appeared that variability in measurements of DM flow was reduced in 72 h measurements in comparison with three separate 24 h measurements per sheep (Expt 1).

MacRae (1975) has reviewed the variability of Cr_2O_3-corrected 24 h digesta DM flow data and found CV for duodenal flow varying from 5–20%. Our values, therefore, were high, but not dissimilar to those obtained by Cr_2O_3 correction. Sutton *et al.* (1976) found that correcting DM flow for 100% recovery of Cr_2O_3 was of doubtful value for reducing the variability of measurements. Offer, Axford & Evans (1972) have administered Cr_2O_3 to sheep in several ways and used automated sampling equipment (Axford *et al.* 1971) to measure recovery over several weeks. The variation in Cr_2O_3 recovery which they found was a measure of day-to-day variation in Cr_2O_3 flow along the duodenum. A direct comparison between the present work and that of the above group is not possible as they gave values for mean recovery with standard errors and the CV cannot be calculated. However, it is of interest that the variability they found was substantial (93·7 ± 9·6% for Cr_2O_3 in diet and 99·3 ± 10·2% for Cr_2O_3 paper administered twice daily). Unless the standard errors derived were from a very small number of replicates, CV for that work would have been at least as great as CV found here for mean DM flow derived from several measurements on a number of sheep. It is concluded, therefore, that our measurements of duodenal DM flow were reproducible within day-to-day variation. In addition our experiments have demonstrated that duodenal DM flow is not always depressed during the first 24 h of measurement as other workers have shown. Leibholz & Hartmann (1972) have suggested that 24 h flow measurements, uncorrected, yield valid estimates of flow. Their conclusion was based on daily recoveries of the marker polyethylene glycol (PEG) which were consistently close to 100% at the duodenum. PEG has, however, been shown by Corse & Sutton (1971) to be less satisfactory than Cr_2O_3 as a duodenal digesta marker, so the findings of Leibholz & Hartmann (1972) should be viewed with some caution.

The source of the difference between these studies and most others cannot be identified. The method of collection used here was similar in all major respects to those employed elsewhere. Also, in common with those of most other workers, our sheep were familiarized with the experimental procedure before experimentation began. It is regrettable that it is still necessary to rely on such relatively short collection periods to determine nutrient digestibility in the ruminant forestomachs. It is possible that the apparent contradictions between the results reported here and those from elsewhere will be resolved when long-term digesta collections are made practicable. The duration of collection required may then be found to be similar to that for a normal digestibility trial.

The authors wish to thank Dr R. Mawson and Mr G. Neale for excellent surgical preparations, Mrs J. Simpson and Mr J. B. Soar for invaluable assistance during the digesta collections and Mrs P. Buckingham and Mr D. R. Westgarth for a great deal of statistical advice. J.D.O. acknowledges receipt of a Research Assistantship from the Agricultural Research Council. J.R.L. acknowledges receipt of a Post-graduate Agricultural Studentship from the Ministry of Agriculture, Fisheries & Food.

REFERENCES

Agricultural Research Council (1965). *Nutrient Requirements of Farm Livestock*, No. 3 *Ruminants*, London: Her Majesty's Stationery Office.
Axford, R. F. E., Evans, R. A. & Offer, N. W. (1971). *Res. vet. Sci.* **12**, 128.
Bruce, J., Goodall, E. D., Kay, R. N. B., Phillipson, A. T. & Vowles, L. E. (1966). *Proc. R. Soc. B.* **166**, 46.
Corse, D. A. (1974). *Proc. Nutr. Soc.* **33**, 141.
Corse, D. A. & Sutton, J. D. (1971). *Proc. Nutr. Soc.* **30**, 18A.
Drennan, M. J., Holmes, J. H. G. & Garrett, W. N. (1970). *Br. J. Nutr.* **24**, 961.
Faichney, G. J. (1972). *J. agric. Sci., Camb.* **79**, 493.
Goodall, E. D. & Kay, R. N. B. (1965). *J. Physiol., Lond.* **176**, 12.
Leibholz, J. & Hartmann, P. E. (1972). *Aust. J. agric. Res.* **23**, 1059.
MacRae, J. C. (1974). *Proc. Nutr. Soc.* **33**, 147.
MacRae, J. C. (1975). In *Physiology of Digestion in the Ruminant*, p. 261. [I. W. McDonald and A. C. I. Warner, editors]. Armidale: University of New England.
MacRae, J. C. & Armstrong, D. G. (1969). *Br. J. Nutr.* **23**, 15.
MacRae, J. C., Ulyatt, M. J., Pearce, P. D. & Hendtlass, J. (1972). *Br. J. Nutr.* **27**, 39.
Nicholson, J. W. G. & Sutton, J. D. (1969). *Br. J. Nutr.* **23**, 585.
Offer, N. W., Axford, R. F. E. & Evans, R. A. (1972). *Proc. Nutr. Soc.* **31**, 39A.
Offer, N. W., Evans, R. A. & Axford, R. F. E. (1972). *Proc. Nutr. Soc.* **31**, 104A.
Osbourn, D. F. (1975). *Rep. Grassld Res. Inst.* 1974, p. 66.
Snedecor, G. W. & Cochran, W. G. (1967). *Statistical Methods* 6th edn. Ames, Iowa: Iowa State University Press.
Sutton, J. D., Youssef, F. G. & Oldham, J. D. (1976). *Proc. Nutr. Soc.* **35**, 100A.
Tas, M. V., Offer, N. W., Evans, R. A. & Axford, R. F. E. (1974). *Proc. Nutr. Soc.* **33**, 69A.
Thompson, F. (1973). *Br. J. Nutr.* **30**, 87.
Thompson, F. & Lamming, G. E. (1972). *Br. J. Nutr.* **28**, 391.
Thomson, D. J., Beever, D. E., Coelho da Silva, J. F. & Armstrong, D. G. (1972). *Br. J. Nutr.* **28**, 31.
Topps, J. H., Kay, R. N. B. & Goodall, E. D. (1968). *Br. J. Nutr.* **22**, 261.
Ulyatt, M. J. & MacRae, J. C. (1974). *J. agric. Sci., Camb.* **82**, 295.
van 't Klooster, A. Th., Kemp, A., Geurinck, J. H. & Rogers, P. A. M. (1972). *Neth. J. agric. Sci.*, **20**, 314.
van 't Klooster, A. Th., Rogers, P. A. M. & Sharma, H. R. (1969). *Neth. J. agric. Sci.* **17**, 60.

Printed in Great Britain