

Automatic sampling of digesta

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Discussion in this paper is confined to consideration of methods that have been developed for collecting and sampling digesta from ruminants fitted with re-entrant intestinal cannulas. The considerable volume of literature relating to animals fitted with simple cannulas is not included; marker dilution techniques are used to calculate flow through the intestines in these preparations and they are reviewed in this symposium (MacRae, 1974). The use of indigestible markers to estimate depression in flow of digesta during short-term periods of total collection from re-entrant cannulas is, however, examined; an advantage of automated systems is the ability to continue collections for extended periods of time and hence to provide a basis for evaluation of the accuracy of marker-corrected flow in short-term manual collections.

Development of methods for measuring flow through re-entrant cannulas

Phillipson (1952) recognised that the extent of digestion of food in different segments of the ruminant gastrointestinal tract could be determined by measurement of the flow of digesta from one section to another combined with analysis of samples of the intestinal contents. From this work using sheep fitted with re-entrant duodenal cannulas and abomasal-duodenal re-entrant cannulas, it was apparent that the pattern and rate of flow of digesta, particularly from the abomasum, was markedly affected by the procedure adopted to collect digesta from, and to return them to, the animal. Failure to return contents to the distal cannula increased the output of digesta from the abomasum; pouring digesta into the distal limb brought about temporary cessation of flow from the abomasum. Hogan & Phillipson (1960) confirmed this phenomenon in the proximal duodenum but were unable to demonstrate the effect clearly at the terminal ileum due to the intermittent nature of flow in the latter section. They concluded that, in order to measure flow accurately, total collection was required of small portions of digesta which should be returned immediately to the animal following sampling.

The procedure was further refined by Harris & Phillipson (1962) by the addition of donor digesta to replace those removed in the sample and hence to equalize the volume returned with that collected.

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Harris & Phillipson (1962) noted also that when an indigestible marker, chromium sesquioxide (Cr_2O_3) impregnated in paper, was placed in the rumen, it was not fully recovered during 72 h collections of abomasal contents. The marker was quantitatively recovered in the faeces. The observation suggested that the manipulations to the animal associated with the collection technique depressed flow from the abomasum, perhaps by affecting gut motility. Work by Goodall & Kay (1965), though a marker was not employed, demonstrated the effect also at the terminal ileum. Flow was reduced on the 1st day of a 72 h collection period but compensation in flow occurred on the 2nd and 3rd days. Failure to recover quantitatively a marker in 24 h collections at the terminal ileum was later reported by Bruce, Goodall, Kay, Phillipson & Vowles (1966) and by MacRae & Armstrong (1969). The practice of correcting flow for 100% recovery of a marker has since been generally adopted by most groups who have conducted short-term collections.

The markers most commonly used are Cr_2O_3 , Cr EDTA, polyethylene glycol (PEG), polyethylene particles, lignin and ^{103}Ru phenanthroline. Based on the assumption that flow of the different phases of digesta from the rumen (i.e. large and small particles, fluids and marker) is depressed to an equal extent, only one marker is usually administered. Proof of the validity of the assumption was suggested by the observation of MacRae, Ulyatt, Pearce & Hendtlass (1972) that the flow of Cr_2O_3 reflected the flows of dry matter, organic matter, nitrogen, gross energy, hemicellulose, and cellulose. MacRae & Ulyatt (1972), on the other hand, demonstrated that Cr_2O_3 did not closely associate itself with the solid phase of digesta. Nicholson & Sutton (1969) noted that the ratio of the mean recovery of Cr_2O_3 to that of PEG was close to unity in 24 h collections. Whilst this was so also in 12 h periods of collection (Corse & Sutton, 1971), the extent of recovery of each marker from individual sheep was frequently different, and the higher recovery of Cr_2O_3 than of PEG occurring in some collections was reversed in others. A conclusion from this observation was that either the depression of flow of solid and fluid phases is not the same or that the markers do not associate with the respective phases. A similar conclusion was reached from experiments with cows when collections were continued at the duodenum for 72 h (D. A. Corse, unpublished results). Though mean recoveries of the two markers were in quite close agreement over the whole period, the recovery of Cr_2O_3 often differed from that of PEG on any one day. This confirms the data of van't Klooster, Kemp, Guerinck & Rogers (1972) who quoted recoveries from duodenal digesta of cows at $100.3 \pm 1.5\%$ and $98.3 \pm 6.0\%$ for PEG and Cr_2O_3 respectively after 120 h of collection. As well as obtaining considerable day-to-day variation in recovery, the Dutch workers calculated that mean recoveries of PEG and Cr_2O_3 during the 1st 24 h were 95% and 85% of the respective values for 120 h. The authors concluded that flow rates adjusted for 100% recovery of markers in 24 h 'may deviate considerably from mean flow rate, measured directly over the longer (120 h) period'.

A conclusion cannot be derived from the data presently available as to whether flow of digesta in short-term collections should be corrected for recovery of one marker, for the mean recovery of two or more markers, or for the recovery of

markers in different, separated phases of digesta (Neudoerffer, Leadbeater, Horney & Bayley, 1971). Thompson & Lamming (1972) concluded, indeed, because measured flow did not vary significantly from day to day over 3 d, that actual flow could be used. Whilst lack of statistically significant day-to-day variation was shown also in the work with cows (D. A. Corse, unpublished results), the results demonstrated that corrected flow was significantly different from measured flow, an obvious conclusion when recovery of the markers was not 100%. It is clear that collection techniques used by different groups of workers are not uniform, and therefore comparisons of data among groups are not always possible. In the opinion of this author the accuracy of measurement in the short term can be gauged only by continuing collections through the stage of depressed flow into the equilibrium stage, and this can be accomplished effectively only by long-term automated collections.

Development of semi-automated equipment

Before fully automated systems for collecting and sampling digesta were developed, certain equipment was designed to reduce the tedium associated with, and the considerable amount of labour required for, manual collections. Attempts were made either to simplify the measurement of flow through cannulas by avoiding total collection, or to partly automate the return of digesta to the distal cannula in order that a greater number of animals could be supervised by one operator. Ridges & Singleton (1962) combined the use of an electromagnetic flowmeter, to automatically record flow through re-entrant duodenal cannulas, with manual collection of large samples (200 ml) at 3 h intervals to obtain an estimate of the composition of abomasal contents. Disadvantages associated with the use of the flowmeter, especially the need to maintain the animal in a standing position (Singleton, 1961), have ruled this out as a practical technique at present. The possible development, nonetheless, of a less cumbersome flowmeter warrants further examination, although the main application would appear to lie in studying physiology of flow through the intestine, rather than in measurement of extent of digestion in different segments.

A second piece of labour-saving equipment was later described by the group at Liverpool (Porter & Singleton, 1971). It is made up of two plastic bottles fitted with air-tight screw caps and connected by an air pipe. As digesta flow from the proximal cannula into the first bottle, air is displaced into the second, forcing an equivalent amount of donor digesta from this bottle into the distal cannula. Once the collecting bottle is full the contents are aspirated manually from the bottle, the volume is measured, a sample is removed, donor digesta are added and they are returned to the second bottle. The equipment is light in weight and is suspended on the side of the animal in a canvas saddle, permitting the animal to stand and lie down. An operator is required to remove and sample the digesta at approximately 30 min intervals.

Nicholson & Sutton (1969) described apparatus for closely relating return of digesta to output in an otherwise manual system of collection. The flow of digesta, by gravity, from a reservoir through rubber tubing is regulated by a solenoid-

operated pinch clamp. This, in turn, is controlled by a remotely-operated simmerstat regulator and hot-wire relay which varies the opening and closing of the clamp over a range from 2 s every 4 min to being opened continuously. Whilst adjustment of the simmerstat regulator is made manually, one operator alone is able to supervise collection from three sheep as well as maintaining a supply of digesta from donor animals.

Kaufmann, Pfeffer & Dirksen (1972) described a combined pumping system and two balances to closely relate return of digesta to outflow from cows. As contents flow into a bucket on the first balance, an equivalent amount of donor material is returned from a warmed container on the second, by operating the pump until the scale readings coincide.

Development of automated equipment

Fully automated equipment is defined in this paper as equipment which can be used for long periods of time to measure the flow of digesta through intestinal cannulas, to collect representative samples of the digesta and, since consideration is being given only to re-entrant cannulas, to return the digesta to the proximal cannula in amounts closely related to the output.

(a) Apparatus for sheep

Apparatus fulfilling the above criteria was first developed for use with sheep by Axford, Evans & Offer (1971). Detailed description of the apparatus is given in the original publication. It consists basically of a collecting beaker and a pumping system to deliver the digesta to a sampling device. The sampling sequence becomes operational when the rising level of digesta in the beaker causes a glass float to make contact with a micro-switch, locking in a relay which in turn controls two solenoids. These divert the digesta either to a sample bottle, or to a funnel, from which the contents are returned by gravity into the distal cannula. Since the pumping system is activated by a timer every 3 min, it delivers digesta back to the collecting vessel when the float has not made contact with the micro-switch. Blockage in the tubing is prevented by reversing the pump once every 3 min and by a system of reciprocating coils in the tubing returning digesta to the animal. A recent further development by the Bangor group was modification of the equipment to measure flow of digesta simultaneously at two sites in the intestines (Tas, Offer, Evans & Axford, 1974).

Apparatus for sheep designed on an entirely different principle was described by Taylor, Weller & Reid (1971). A small reservoir and sampling unit is attached to the cannulas by flexible elbows. Digesta in the reservoir are stirred and at frequent intervals small samples (1.5 g) are forced through an outlet tube by a syringe sampler. The sampler is driven by a motor which makes one revolution following an impulse from a timer. This is a discontinuous sampler with total flow calculated by the marker dilution technique in accumulated samples collected during long periods of collection.

(b) Apparatus for cattle

By scaling up and incorporating various modifications to the design of the apparatus of Axford *et al.* (1971), equipment has been constructed for automated sampling from cows (Corse, Budd, Austen & Haggett, unpublished results). The apparatus is built into a frame which is located on two parallel bars directly above the cow. The cannulas are connected to the apparatus by light, flexible tubing that does not kink or block when the cow is standing or lying down. Digesta flow from the proximal cannula into a small plastic bottle strapped to the animal. They are pumped immediately by a continuously operating peristaltic pump into a Perspex collecting vessel where they are stirred. Further mixing, for efficient sampling, is effected by continuously circulating the contents at a constant rate through the first outlet of a three-way sampling device back to the collecting vessel by a bellows-type pump (Black, Jones & Melcher, 1971). When the digesta make contact with a level probe in the vessel a sampling sequence begins, the stream of circulating contents being diverted by a solenoid to the second outlet of the sampling device from where they flow to a sample bottle, held in solid CO₂. A second solenoid then diverts flow through the third outlet connected by a flexible tube to the distal cannula.

Transistorized timers control the duration of the sampling and return sequence; they are adjustable in the ranges 0.2–5.0 s and 0.2–20.0 s respectively. During a 'sampling cycle' the proportion taken as sample is determined by the relative settings of the two timers. Settings, for example, of 1 s and 19 s, mean that 5% and 95% of flow are diverted to the sample and return positions. An event recorder gives a continuous record of the number and frequency of sampling cycles during any period of collection, and total flow is measured by the product of the number of sampling cycles and the combined mass of sample and return digesta for each cycle. This mass, the calibration factor, is established *in vitro* prior to a collection and is checked at intervals during the collection. In repeated tests the calibration factor has been shown not to vary over periods as long as 10 d. Further, the proportion of digesta sampled (usually 5%) remains constant. By weighing the accumulated samples, flow can be calculated from the knowledge that this mass is 5% of the total flow of digesta. The two methods have been checked and have been shown to agree closely.

Another criterion of an automated system, the ability to obtain samples representative of the digesta collected, has been tested by analysing the sampled and returned digesta obtained in series of sampling cycles. These have been shown to be identical in their respective contents of dry matter, PEG and Cr₂O₃.

Conclusions

Following the development of apparatus for automated sampling of digesta from sheep and cattle it is likely that the equipment will be used increasingly to conduct long-term collections at the duodenum and ileum. The data from these collections will aid in elucidating questions on the reliability of short-term, marker-corrected

measurements and will eliminate the need to use markers if these measurements are still called into question. It will be possible to train animals to the collection procedure, will permit greater numbers of animals to be included in experiments and will reduce considerably the amount of labour required.

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