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AUTOMATED TOTAL COLLECTION AND INDICATOR METHODS FOR ESTIMATING DIGESTA FLOW IN STEERS FED ROUGHAGE OR CONCENTRATE DIETS

by

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As members of the Final Examination Committee, we certify that we have read the dissertation prepared by Ruy da Carvalheira Wanderley

entitled Automated Total Collection and Indicator Methods for Estimating Digesta Flow in Steers Fed Roughage or Concentrate Diets

and recommend that it be accepted as fulfilling the dissertation requirement for the Degree of Doctor of Philosophy.

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Final approval and acceptance of this dissertation is contingent upon the candidate's submission of the final copy of the dissertation to the Graduate College.

I hereby certify that I have read this dissertation prepared under my direction and recommend that it be accepted as fulfilling the dissertation requirement.

C. Brent Theurer
Dissertation Director

Date 1 February 1982

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SIGNED: ______________________

To my wife Graça, my daughter
Patricia and sons, Ruy, Jr.,
Marcelo and Romero

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ABSTRACT

Automated total collection (ATC) of digesta from four steers fitted with duodenal re-entrant cannulas was compared with flow estimations based on Cr_2O_3 and acid detergent lignin concentrations of ATC samples. In two successive periods, each steer was fed about 4 kg daily of an all-roughage or an 80% sorghum grain diet. Digesta samples were automatically taken and pooled every 2 hr, during 3- to 6-day collections. Sample aliquots, representing 4% of the digesta measured in each 2 hr were pooled to represent 24-hr digesta flow. Data of 2-hr samples were used to study diurnal flow patterns. Duodenal digesta and dry matter flow rates estimated by Cr_2O_3 and lignin were greater by ~ 15% than flow rates measured by ATC. Mean recoveries of Cr_2O_3 and lignin at the duodenum were 93 and 89%, respectively. Animals fed the roughage diet had about 55% greater digesta flow rates than when fed the concentrate diet (42 versus 65 l daily average by ATC). There appeared to be an interaction between diets and markers. Apparent ruminal dry matter digestibility calculated from direct measurements by ATC was 44% for the concentrate diet and 37% for the roughage diet. Rumen digestibility values based on Cr_2O_3 and lignin were about 25% lower than the ATC values. Average coefficients of diurnal variation for digesta flow were 29% in the concentrate diet and 34% in the roughage diet. Estimations from Cr_2O_3 and lignin based on 2-hr samples could either under- or overestimate digesta flow by 31 to 350% of the 24-hr flow based on ATC.

Thus, special emphasis should be given to the problem of sampling procedures when using indicators and spot-sampling technique to estimate digesta flow. There was evidence of a 24-hr cycle for the whole digesta flow in the grain diet, with a period of low flow before the onset of the light hours. No consistent flow pattern could be identified in the roughage diet. Lignin concentration patterns in digesta were somewhat similar for both diets; however, Cr_2O_3 patterns were different between diets. Digesta dry matter concentration followed a similar pattern to that of Cr_2O_3 in the grain diet and to that of lignin in the roughage diet, suggesting that the flow pattern of solid phase components of digesta may also be differentially affected by the dietary concentrate: roughage ratio.

CHAPTER 1

INTRODUCTION

In the last few years many attempts have been made to quantify the extent of ruminal and intestinal digestion, particularly in sheep. Ruminants fitted with gastrointestinal cannulas are widely used to study the flow of digesta and the changes that occur in the food passing through the alimentary tract, including measurement of absorption of digesta constituents at various gastrointestinal sites. Partitioning of the digestion of nutrients depends on the accurate measurement of digesta flow to quantitate the amount of a nutrient at a particular site in the gastrointestinal tract. There are several types of cannulas and many methods for measuring digesta flow. All methods have some disadvantages and none have proven to be entirely satisfactory.

Based on the use of markers, contradictory data have been obtained concerning the flow of digesta or quantitating nutrients entering the small intestine in ruminants, particularly in the bovine. Several studies have been conducted using total collection of digesta, but most have utilized periods of less than 24 hr of continuous measurement. The development of automated methods for total collection of digesta have made longer term studies (exceeding 24 hr) possible. Comparisons between the use of markers and total collection methods for several continuous days are limited, especially in the bovine. Little consideration has been given to possible interactions among dietary regimens and flow marker estimations.

Possible effects of long-term total collection on intestinal motility and digesta passage have not been reported. Daily variation in digesta flow pattern and behavior of markers entering or leaving the various sections of the alimentary tract of ruminants has received only limited attention in long-term studies.

Compared to indicator methods, total collections are difficult and laborious. Automation has improved the total collection approach, but problems associated with animal management still remain. The limited numbers of animals used in these type of studies and the often apparent depressed flow rate noted with total collection methods continue to be problems, even with the automation of the collection process. Nevertheless, indicator methods have been associated with greater variability as compared with total collection. Therefore, studies must be conducted to develop more practical and suitable techniques for accurately estimating rumen and intestinal digesta flow for partitioning digestibility.

The objectives of this study were to: (1) compare automated total collection with indicator methods (chromium oxide and lignin) for estimating the flow of digesta into the duodenum of the bovine; (2) determine daily variation in digesta flow patterns in the proximal duodenum; and (3) determine marker-diet interactions by estimating duodenal flow rates in steers fed an all-roughage versus an 80% grain diet.

CHAPTER 2

LITERATURE REVIEW

Techniques for Measuring Digesta Flow

Flow of digesta in the intestine of ruminants has been measured using two general approaches: (1) estimated from marker concentrations of digesta sampled intermittently from T-type or re-entrant cannulas or (2) total collection where digesta is diverted outside the animal via re-entrant cannulas. A combination of both approaches has often been used by correcting short-term total collection measurements according to marker recovery. Poncet et al. (1976) used the technique proposed by Singleton (1967) in which the flow of intestinal contents in sheep was measured by an electromagnetic flow-meter. Sheep were fitted with re-entrant duodenal cannulas and an electromagnetic apparatus for measuring blood flow was adapted to measure the digesta flowing from the proximal cannula. After crossing the probe, the duodenal digesta was taken into a measuring cylinder to allow comparisons between this new technique and total collection procedure. The digesta was reintroduced manually. Correlation was high ($r = .99$) between the two methods. A valve was placed just before the probe to prevent the digesta from flowing back into the abomasum.

Corse (1974) reviewed automatic sampling of digesta in ruminants and made several interesting remarks. He pointed out that the manner in which digesta is returned to the duodenum in total collections could affect the flow from the abomasum (either by increasing the output flow when contents are not returned to distal cannulas immediately after recovery, or causing temporary cessation of flow from the abomasum when returning digesta to the distal cannula). Therefore, Corse (1974) concluded that digesta contents should be returned in small portions and immediately after recovery. Conclusions could not be derived from the data reviewed as to whether flow of digesta in short-term total collections should be corrected for recovery of one marker, for mean recovery of two markers, or for recovery of multiple markers of different phases of digesta. It was pointed out that the development of automated equipment for measuring and sampling digesta flow in long-term total collections would aid in elucidating questions on the reliability of short-term, marker-corrected measurements and on other important questions relating to the digestion process itself.

Corse (1974) also described an automated apparatus for measuring and sampling digesta flow in cattle. Digesta recovered from the proximal cannula into a collecting vessel was continuously circulating at a constant rate through a first outlet of a three-way sampling device back to the collection vessel by a bellows-type pump. When enough digesta was recovered to make contact with a level probe in the vessel, the stream of circulating digesta was diverted by a solenoid through a second outlet to a sample bottle and then by a second solenoid through a third outlet to the distal cannula. Two transistorized timers were used for controlling the two solenoids and consequently the duration of sampling and return sequence. The timers were adjusted for sampling 5% of digesta. Therefore, knowing the amount of sampled digesta, the total flow could be estimated. A recorder registered number and frequency of sampling cycle during any period of collection.

Several other systems for measuring intestinal contents in sheep or cattle are reported in the literature (Axford, Evans and Offer, 1971; Tas et al., 1974; Tamminga, Dikstaal and Van der Koelen, 1973; Tamminga, 1975; Zinn et al., 1980). All proposed systems consist basically of a collecting container and a pumping system to deliver the digesta either to a sample collector or back to the animal. The main difference among systems is in those component parts used for measurements, sampling, and to control the succession of events. Effect of automated collection procedure on flow of digesta was evaluated by Zinn et al. (1980) on the basis of chromium oxide recovery during a period of 36 hr of collections. Average recovery of chromium oxide was 79% (ranging from 71 to 97%). It was not possible to hold the animals long enough in the collection crate to adequately evaluate time versus normalization of flow. Total collection adjusted to 100% chromium oxide recovery was compared with spot sampling (500 ml samples taken at 6-hr intervals for 48 hr). No significant differences were detected. It was concluded that the real value of automated total collection would lie in the ability it gives one to study patterns and fluctuations of digesta flow, rather than in direct advantages in flow measurements.

Cannulation

Studies have been conducted to provide an efficient surgery technique and an adequate type of cannula for digestion trials with ruminant animals. Numerous cannula designs are reported in the literature. MacRae and Wilson (1977) considered that these cannula designs basically form two categories: (1) simple cannulas, which provide a permanent fistula in the tract; and (2) re-entrant cannulas, which divert the flow of the whole digesta exterior to the animal.

Duodenal re-entry cannulas have been made mostly from hard materials (PVC, polypropylene, etc.). Nearly all cannulations still follow the general procedure proposed by Ash (1962). The Ash procedure involves transection of the intestine. However, Wenham and Wyburn (1980) recommended a technique in which the intervening section of the intestine between the two cannulas is not transected. This section would be occluded by a loop of a 5 mm diameter PVC catheter passed around it.

New approaches have been tried to provide uninterrupted flow of digesta through the small intestine except when collection is required (Ivan, 1974, 1977; Haaland et al., 1977; Ivan and Johnston, 1979, 1981; Komarek, 1981). In addition, very little damage occurrs in the blood and nervous systems in contrast to the Ash procedure because the intestinal transection and mesenteric incision are not required.

K. L. Mizwicki (1979, personal communication) recommended a cannula made from a flexible plastic tygon tubing molded into an "L" shape, with a ring-shaped flange at the intestinal end instead of the usual gutter-shaped flanges of the T-shaped cannulas. The surgical procedure involves transection of the intestine.

Typical problems associated with cannulation include post-surgical recovery, blockage and leakage of digesta, maintenance of the cannulated animals for an extended period of time, reduction in flow of digesta and temporary lack of apetite. However, very little consideration has been given to the effect of re-entrant cannulations on normal functioning of the animals digestive system. The animal is often assumed to be completely normal when recovered from the surgery and after body weight and feed intake have stabilized. Insufficient studies have reported the effect of re-entrant cannulation on changes in digestibility, voluntary feed intake, transport of markers, intestinal motility and flow of digesta, particularly with the bovines.

Harris and Phillipson (1962) compared losses of organic matter, ash and nitrogen in the whole alimentary tract of sheep fed hay with low nitrogen content, when all animals were intact, about three weeks after implantation of re-entry duodenal cannulas, and also during periods of digesta flow measurements. No evidence was found from food intake and water consumed, from body weight changes or from comparisons of organic matter, nitrogen and ash contents in food and feces that cannulation or short-term (12 hr periods) manual total collection of digesta had caused any major disturbance to digestion or permanent damage to the animal's digestive system. Hays, Little and Mitchel (1964) evaluated the effects of ruminal, abomasal and intestinal fistulation on digestion in steers and found no significant effect on apparent digestion coefficients. One set of dizygous and three sets of monozygous twin steers were used and comparisons were made between steers fistulated in different sites of the gastrointestinal tract and between pre- and post-fistulation performance of individuals.

MacRae and Wilson (1977) did not find significant differences in digestive or blood measurements between intact sheep and sheep prepared with various forms of gastrointestinal cannulas. Digestive comparisons were based on voluntary food intake, dry matter digestibility, nitrogen balance, and rates of passage of particulate- and liquid-phase digesta markers (Ru-phenantroline and Cr-EDTA, respectively). Stress conditions imposed by cannulations were evaluated based on venous concentrations of corticosteroids, serum aspartate, aminotransferase, protein bound iodine, urea and glucose. No changes were detected in these paramaters which could be associated with cannulations. The only difference was in wool growth rate. Sheep fitted with re-entrant cannulas had lower ($P < .01$) wool growtn rates than those with rumen and T-shaped single cannulas.

Phillips, Webb and Fontenot (1978) reported massive adhesions of the small intestine as a serious problem in sheep with re-entrant cannulas. Animals with this problem died after progressive reduction in feed intake and flow of digesta. Correct positioning of cannulas in the jejunum and illeum was considered of great importance. Cannulas positioned to allow downward flow of digesta facilitated digesta passage through the cannulas. Blockages occurred in cannulas positioned to allow the flow of digesta in an upward direction.

Wenham and Wyburn (1980), by means of radiological examinations, investigated the effects of cannulations on intestinal motility and digesta flow in sheep. After radiological examinations to establish individual patterns of normal gut motility and digesta flow, each sheep was fitted either with a single T-shaped cannula, the Ash re-entrant cannulas, or with the Wenham and Wyburn (1980) cannulas. The cannulas were placed either in the ascending duodenum, transverse duodenum, transverse jejunum, or terminal illeum. All cannulations caused some disruption of the normal flow of digesta, including retention of digesta and distention of the intestine around the intraluminal flanges of the cannulas. Another difficulty was the resistance of the rigid cannula barrels which appeared to be a deterrent on the effectiveness and force of contractions. When re-entrant cannulas were placed in the ascending duodenum, it was necessary that the duodenal segment between the pylorus and the proximal cannula was long enough to contain sufficient digesta to initiate a contraction and peristaltic rush when propelled through the cannulas. Minor disturbance of intestinal activity occurred, however, in sheep with re-entrant cannulas in the ascending duodenum as compared to those cannulated in other intestinal segments.

Markers

The use of markers in nutrition has been extensively reviewed by Kotb and Luckey (1972). They considered that an effective marker for nutritional studies should be inert with no toxic, physiological or psychological effect; be neither absorbed nor metabolized within the gastrointestinal tract; have no appreciable bulk; mix intimately with and remain uniformly distributed in the digesta; have no influence on gastrointestinal secretion, digestion, absorption, or normal motility; have no influence on the gastrointestinal microflora; have physio-chemical properties to allow ready, precise, quantitative measurement. Unfortunately, none of the substances employed as markers to date have completely satisfied all of the aforementioned conditions. The selection of one particular marker, therefore, depends upon the conditions and requirements of the experiment.

MacRae (1974) reviewed the use of intestinal markers to measure digestive function in ruminants and suggested that dual-phase marker systems are likely to give the most meaningful results for the majority of studies and would provide a feasible alternative to the long-term automated total collection technique.

The practice of correcting flow for 100% marker recovery using short-term total collection is widely used and, according to Corse (1974), this practice is based on the assumption drawn from the observation of MacRae and Evans (1972) that the flow of the different phases of digesta and markers would be depressed to an equal extent, when depression occurs for any reason. The markers most commonly selected for correcting and estimating flow of digesta in ruminant nutrition studies are also those considered as fecal markers. They are assumed to be not absorbed from the alimentary tract and are almost completely recovered in the feces.

The following are common markers used in ruminant digestion studies: chromium: the sesquioxide (Cr_2O_3) as single or particulate marker, ethylenediaminetetracetic acid complex (Cr-EDTA) as a water-soluble marker (used in radioactive or non-radioactive form); lignin: as internal marker (indicator which occurs naturally in diet components); polyethylene gylcol (PEG): as a water-soluble marker; ruthenium: as a radionuclide ^{103}Ru-phenanthroline complex, particulate or multiple marker; lanthanum, cerium, samarium: used a stable isotopes or in radioactive form (^{104}La, ^{141}Ce, ^{153}Sm), particulate or multiple markers; dysprosium: as a stable isotope ($DyCl_3$), particulate marker; cobalt and europium: recently introduced (Co-EDTA and Eu-EDTA) as liquid phase markers.

According to the review on markers by Kotb and Luckey (1972), there is a great variability in reports on digestibility and recovery of lignin. This may be attributed to the analytical methods for lignin. Apparent digestibilities ranged from -7 to 42% for lignin in ruminants, and it was reported that fecal and dietary lignin differ from each other in their chemical composition. The apparent digestibility for lignin was reported to occur mainly after feed had left the rumen. The rate of flow of lignin leaving the rumen was somewhat less variable than that of other widely used markers such as chromium oxide.

Elam et al. (1962) compared lignin and chromium oxide ratio methods versus total collection of feces to determine dry matter digestibility in sheep. The mean coefficient of digestibility (61.4%) determined by the lignin ratio was significantly less ($P < .01$) than the mean coefficient determined by total collection (65.4%) and the mean coefficient (65.6%) estimated by chromium oxide. However, Drennan, Holmes and Garrett (1970) found ruminal dry matter digestibilities estimated by lignin ratio (57 to 68%) to be more consistent than those estimated from chromium oxide (-7 to 36%). These results were obtained from experiments conducted with sheep and cattle receiving high concentrate diet (80% milo) and samples were taken from abomasal cannulas. The amount of starch digested in the rumen estimated from chromium oxide was more than the estimated amount of organic matter digested, while results from lignin were more consistent.

From several points of view, lignin should be an ideal particulate marker. However, according to MacRae (1974), the empirical nature of lignin determination, the reported variation in lignin digestibility, and the apparent change in its composition during the passage through the gastrointestinal tract, have imposed limitations on its use as a marker for digestion studies in ruminants.

The acid detergent lignin (ADL) method introduced by Van Soest and Wine (1967) represents a real improvement in analytical determination of lignin. The knowledge about effects of heating and drying on lignin analytical determination (Van Soest, 1965) and the acetone drying procedure suggested by Goering and Van Soest (1970) as an alternate technique for handling wet samples, has created new possibilities for the use of lignin as a marker for estimating flow of duodenal contents in studies partitioning digestion in ruminants. However, in spite of these improvements in lignin determination, metal atoms are still more easily identified and quantitated than complex organic compounds such as lignin. Muntifering, Tucker and Mitchell (1981) compared acetyl bromide soluble lignin (ABSL) with ADL, $KMnO_4$ lignin and CR_2O_3 as digesta flow markers in abomasal passage studies in lambs and concluded that the ABSL did not offer any advantage over the other compared marker techniques.

Chromium sesquioxide (Cr_2O_3), according to MacRae (1974), is the most commonly used marker in nutrition studies. Despite the increasing use of rare earths, chromium oxide is still the most commonly used marker for correcting and estimating digesta flow in ruminant digestion studies. However, the adequacy of its use depends to a large extent on the purpose for which it is being employed, because of several characteristics of Cr_2O_3 behavior. For example, Cr_2O_3 does not appear to associate with any particular component of digesta flowing somewhat independently of both solid and liquid phases, although Cr_2O_3 flow rates appear closer to the particulate than to the liquid phase.

Uden, Colucci and Van Soest (1980) proposed a new approach using the chromium mordanted plant cell walls. This is an attempt to combine the advantages of a widely used metal oxide marker with the intrinsic advantage of an internal marker. Based on several experiments (*in vitro* and *in vivo*) it was concluded that Cr-mordant fulfilled most of the criteria for a particulate marker to be used in digestion studies. Pond et al. (1981) found that Cr-mordanted fiber and the rate earth $^{177}Lu_2(NO_3)_3$, adsorbed on the same fiber fraction, responded similarly as particulate flow markers in cows fed coastal bermuda hay twice daily.

Events occurring in the rumen have great influence on the supply of nutrients to the ruminant animal. It is important to know the rate of removal of the particular components of digesta from the rumen and flow to the small intestine, to better understand the process of digestion in ruminants. The development and use of the dual phase (liquid/particulate) marker techniques such as PEG/Cr_2O_3 (Corbett et al., 1958; Van't Klooster, Rogers and Sharma, 1969); Cr-EDTA/Ru-phenanthroline (Tan, Weston and Hogan, 1971; Faichney and Griffiths, 1978), Co-EDTA/Cr-mordanted cell wall (Uden et al., 1980) and the multiple marker technique proposed by Hartnell and Satter (1979) have been used to obtain this information. Faichney and Griffiths (1978) suggested that such information could be obtained from the analysis of marker concentration patterns. In this aspect, the utilization of indicators would have another connotation by being used in combination with total collection or an automated continuous sampling technique to allow identification of patterns of flow of any particular component of the digesta through the gastrointestinal tract.

The utilization of rare earth elements as markers is based on the strong adsorptive properties that those elements have for particulate matter and the development of the EDTA complexes for the liquid phase. Absorption of the EDTA-complex liquid markers is small according to Downes and McDonald (1964), Weston and Hogan (1969), Goodall and Kay (1972), Uden et al. (1980) and Teeter and Owens (1981). This use has generated the potential of studying simultaneously more than one diet or digesta component, although Hartnell and Satter (1979) and Crooker, Clark and Shanks (1981) found some marker movement between particles.

Flow of Digesta

As digesta flows through the gastrointestinal tract, volume and composition change considerably as a result of digestion and absorption processes, secretions, and recycling of minerals, water and other nutrients. Quantitation of digesta flow represents an important tool for improving basic knowledge and possible manipulating of digestion processes in ruminant animals.

Harris and Phillipson (1962) used manual total collection of digesta in the proximal duodenum of sheep in two 12-hr periods (10 A.M. - 10 P.M. and 10 P.M. - 10 A.M.). These workers found that digesta flow tended to increase throughout the night, peaking by early morning at feeding time. Two principal factors, feeding and rumination, influenced flow rate. Sheep were fed twice a day (7:15 A.M. and 4:15 P.M.). Flow rate was high at feeding periods and for non-feeding periods, a positive linear relationship was found between rumination and flow rate. Van't Klooster et al. (1969) found appreciable diurnal and day-to-day variations in digesta flow rates in the duodenum of sheep. Digesta flow was measured for 72 to 120 hr either by manual total collection or estimated by indicator (PEG and CR_2O_3) concentrations in digesta samples. Direct measurements of digesta flow agreed with indirect estimations of flow rates based on concentrations of PEG and Cr_2O_3 in representative samples of total digesta flow. Estimations based on marker concentrations in spot samples showed discrepancies as compared with estimation from representative samples (pooled sample aliquots of each total collection), probably due to the great diurnal variation.

With manual total collection of duodenal contents for 24 hr periods in sheep fed on an hourly basis, Leibholz and Hartmann (1972) found great diurnal variation. Measurements and samples were polled for each 2 hr and varied as much as 10-fold within-day, but no consistent pattern was detected. Flow measured at the first 2-hr collection was significantly greater than at subsequent 2-hr collections. Variations in flow rates were greater in sheep receiving a low-nitrogen diet. Flow of organic matter in the duodenum varied in a similar manner to the flow of total digesta and average dry matter digestibility was 52%.

Hevelplund et al. (1976) used semi-automatic equipment to measure digesta flow for 72 hr in the duodenum of non-lactating cows fed either two or twelve times per day (all roughage diet or a mixed grass silage and concentrate). Feeding frequencies had only a minor effect on flow rate and pH of duodenal digesta. There was no relationship between time of feeding and digesta flow into the duodenum. A close positive relationship (r = .86) between percent dry matter in the digesta (2 to 4%) and dry matter intake (3.5 to 12 kg/day) was detected. Average pH in digesta samples was 3.6 and average digesta flow was 78 kg per day per animal. Sutton, Youssef and Oldham (1976), using automatic equipment to measure flow of digesta in the proximal duodenum of Friesian cows, found no effect of frequency of feeding on the flow of digesta or flow of chromium oxide. Flow of dry matter and chromium oxide were lowest on day 1 of the 5-day collection period. Mean dry matter flow was 4 ± .16 kg/day with an average of 7.6 kg of daily dry matter intake. Mean recovery of chromium oxide in duodenal digesta was 95 ± 2.7%. Higher standard errors were associated with dry matter flow adjusted for marker recovery than for unadjusted flow, due to the variability in the flow of chromium oxide.

Oldham and Ling (1977), performing manual total collections of duodenal digesta for continuous periods of 24 to 72 hr in sheep fed on a variety of diets, found no depression in digesta flow during the first 24 hr of collection. Mean coefficient of variation of measurements was 22%. The authors concluded that the variability of repeated 24 hr measurements of flow was within day-to-day variation and that flow measured over 24 hr without marker correction gave a valid estimation of dry matter flow through the duodenum. Digesta flow rates increased (from 8 to 18 l/day) when roughage increased from 10 to 80% in the diet.

Teeter and Owens (1981) found 80% greater ruminal liquid dilution rate and 23% larger liquid rumen volume in steers fed a 90% chopped alfalfa hay diet than in steers receiving an 80% whole corn ration. Huntington, Britton and Prior (1981) reported a decrease in rumen fluid turnover rate and rumen fluid volume when concentrate/roughate ratio increased in the diet of wethers.

In a study of behavior of solute and particle markers (^{51}Cr-EDTA and ^{103}Ru-phenantroline) in the stomach of sheep receiving a concentrate diet, Faichney and Griffiths (1978) found cylindrical fluctuations in concentrations of ^{51}Cr-EDTA in the rumen, indicating daily variation

(24-hr cycle) in net water flux in the rumen. The evidence of a circadian rhythm occurred despite conditions of continuous feeding and lightening. Leão, Coelho da Silva and Carneiro (1978) used manual total collection and found greater duodenal digesta flow rates during the day (8 A.M. to 8 P.M.) than during the night (8 P.M. to 8 A.M.), with the lowest period between midnight and 8 A.M. Duodenal recovery of Cr_2O_3 ranged from 78 to 105% in sheep fed an 80% sorghum grain diet.

Zinn et al. (1980) concluded that there was some inhibition of digesta flow at the duodenum of Holstein steers due to the 36 hr automated total collection procedure. The animals were fed 2 kg of a 40% chopped alfalfa hay and 60% ground corn diet twice daily. Average chromium oxide recovery in the duodenum was 79% (ranging from 71 to 97%). Comparisons of digesta flow based on spot-sampling (6 hr intervals) versus flow based on automated total collection adjusted for 100% duodenal recovery of Cr_2O_3 were not significantly different. Average marker corrected flow was 73 l/day and average organic matter passage was 2.3 kg in 24 hr. Zinn, Bull and Heinken (1981) used an automated collecting and sampling device for quantitating digesta flow for 36 hr periods in the duodenum of steers fitted with re-entrant cannulas. They reported average flow rates of 59 l/day in steers fed 3 kg/day and 70 l/day in steers fed 4 kg/day of a 40% chopped alfalfa hay and 60% grain diet. Digesta flow values were corrected for 100% recovery of Cr_2O_3 in the duodenum. Average CV's were 11% and there was a remarkable constancy in crude protein (1%) content in the digesta entering the duodenum, despite a wide range in the total passage of crude protein per day. It was concluded that liquid flow leaving the abomasum might be biologically adjusted to maintain the concentration of crude protein in the flow entering the duodenum.

MacRae et al. (1973) found rates of passage of solid and liquid phase markers (^{103}Ru-phenantroline and ^{51}Cr-EDTA, respectively) were similar in the large intestine of sheep and concluded that both, liquid and solid phases of digesta, moved through the large intestine together. This suggests that differences in flow rates of digesta components in the whole tract occur mainly within the rumen and the small intestine.

Ruminants, like monogastric animals, usually exhibit a resting/activity cycle periodicity and feeding behavior in circadian rhythm, but it is not clear yet if the same diurnal variation would occur with other physiological and metabolical functions. Events concerning digestion and flow of digesta in the gastrointestinal tract of monogastrics occur in an integrated manner with the resting/activity cycle and with the natural feeding behavior pattern.

Armstrong, Clarke and Coleman (1978), studying the light-dark (L-D) variation in the laboratory rat stomach and small intestine, found variation in total stomach content weight but not in small intestine content weight which tended to be relatively constant over a 24 hr period. Laboratory albino rate have the greatest activity during the dark hours. Under conditions of continuous feeding, the L-D variation in stomach content showed a bimodal distribution, with a peak occurring after the onset of the activity period (dark hours) and a second peak at the end of the dark period just before the onset of the resting period.

Also, there were differences in the quality (undigested food and fluid content) of the stomach content. Integrating these findings with the lipogenic-lipolytic cycle, it was suggested that the 24 hr period could be divided into four stages: (1) first half of dark (first peak in stomach content), used for immediate energy requirements and lipogenesis; (2) second half of dark, lipogenesis diminishes and food is stored in stomach which acts as a reservoir (at the end of this period occurred the second and major peak in stomach content); (3) first half of light, the stomach contents sustain the rat's reduced energy requirements during sleep and inactivity; and (4) second half of light, stomach contents diminish and lipolysis increases to a maximum. The absence of L-D variation in the small intestine contents was an interesting finding because it was in contrast to another observation that during the dark period the small intestine appeared much pinker in color, more apparent visceral bleeding, and the jejunum and ileum appeared much more distended than toward the end of the light period, possibly reflecting differences in digestive and absorptive capacities at different times of the day.

Montgomery, Flux and Carr (1978), studying the effect of amino acid deficiency and feeding behavior in pigs, found a circadian feeding pattern (70% of food intake during the light period which is the period of activity of pigs). The feeding pattern was bimodal with the first peak in the beginning of the light period and the second and major one just before the dark period. This feeding pattern agreed with the digesta content pattern found in the variation of stomach content of albino rats by Armstrong et al. (1978).

Summary

As digesta flows through the gastrointestinal tract, volume and composition change considerably due to digestion and absorption processes. Events occurring in the rumen have great influence on the supply of nutrients to the ruminant animal. Therefore, it is important to know the rate of removal of the ingesta components from the rumen and flow through the different parts of the gastrointestinal tract.

The flow of digesta components has been measured by total collection or estimated by marker concentrations in digesta. Inherent problems in both approaches have been reported such as sampling technique for marker estimates, and the effect of total collection on digesta flow.

Several types of cannulas and surgery techniques have been used in an attempt to measure digesta flow; however, none have proven to be entirely satisfactory. The fistulated animal has been assumed to be normal when recovered from the surgery and after body weight and feed intake have stabilized. Few studies and almost no conclusions have been reported concerning the effect of cannulations on intestinal motility and digestion and absorption functions.

Large diurnal and day-to-day variation in flow of digesta and markers leaving the rumen and entering the duodenum have been reported and regimens altering feeding frequencies do not appear to markedly reduce this variation. Some studies indicate a characteristic pattern in the diurnal variation of digesta flow in the gastrointestinal tract of ruminants. There is some evidence for interactions between diet and flow of digesta.

Additional studies which combine the advantages of the automated continuous sampling (total collection) and measurement of marker concentrations in digesta need to be conducted to more adequately define any characteristic patterns of the flow of digesta components, rather than quantitating flow on an average daily basis. The effect of diet and potential diet-marker interactions on flow patterns also need to be clarified.

CHAPTER 3

AUTOMATED LONG-TERM TOTAL COLLECTION VERSUS INDICATOR METHODS TO ESTIMATE DUODENAL DIGESTA FLOW IN CATTLE

Summary

Automated total collection (ATC) of digesta from four steers fitted with re-entrant duodenal cannulas was compared with flow estimations based on Cr_2O_3 and acid detergent lignin concentrations in representative samples of 24 hr digesta flow. During successive collection periods, each steer (average weight, 300 kg) was fed about 4 kg daily of an all-roughage diet (83% alfalfa hay, 17% wheat straw) or an 88% concentrate diet. The steers were adjusted to the collection crate and apparatus for 3 to 6 days before each collection period (24 hr total collection for 3 to 6 continuous days). Digesta samples (about 50 ml) were automatically taken during each respective measuring cycle event and pooled every 2 hr. Sample aliquots, representing 4% of the total digesta measured in each 2 hr, were pooled to represent 24 hr digesta flow. For animals adapted to the collection procedures, there was no consistent evidence of flow inhibition in the first 24 hr of a long-term total collection and no evidence of a compensatory increase in flow rate during subsequent collection days. Flow rates for digesta, dry matter, Cr_2O_3 and lignin in the first day of collection averaged 88 to 108% of the entire 3 to 6 day collection period. Individual differences were

within the day-to-day variation. Estimations of digesta and dry matter flow rates in the proximal duodenum based on Cr_2O_3 and lignin markers tended to be more variable and were about 15% greater as compared with ATC measurements. This was consistent with the overall duodenal mean recoveries of Cr_2O_3 and lignin for the entire collection period (93 and 89%, respectively). Animals fed the roughage diet had greater digesta flow rates (about 55% more) than when fed the concentrate diet, indicating a greater ruminal liquid turnover rate. Interactions between diets (concentrate/roughage) and markers were detected and suggest that flow rate pattern for several components of the solid phase of digesta may also be affected by diet, although the major effect in this study appeared to be with the liquid phase. Apparent ruminal dry matter digestibility calculated from direct measurements by ATC was 44% for the concentrate diet and 37% for the roughage diet. Rumen digestibility values based on Cr_2O_3 and lignin concentrations were about 25% lower than the ATC values. Standard deviations for flow of dry matter and rumen dry matter digestibility based on the lignin method with the concentrate diet and on the Cr_2O_3 method with the roughage diet were about twice as large as the standard deviations for the other two methods in each diet. This data suggest caution in the use of lignin as a marker in concentrate diets and, that of Cr_2O_3 as a marker in roughage diets for partitioning digestion in the duodenum of ruminants. The use of other forms of chromium may be satisfactory with roughage diets. The data suggest that duodenal flow rates adjusted for 100% recovery of a single marker in 24 hr may deviate considerably from the mean of long-term total collections, and that short-term total collections should be replicated to minimize day-to-day variation.

Introduction

Events occurring in the rumen have great influence on the supply of nutrients to the animal. To more clearly understand the process of digestion, it is important to know the rate of removal of the digesta components from the rumen and the amounts entering the small intestine.

Two general approaches have been used for quantitating duodenal digesta flow in ruminants: the marker dilution technique (MacRae, 1974) and direct measurements by total collection (Corse, 1974). Both approaches have several disadvantages as compared to each other. Sampling procedure difficulties have been the major problems related to marker estimations. Automation has improved the total collection procedure, but due to the difficulties with animal management during long-term total collections, short-term total collections (less than 24 hr) with flow estimations adjusted for 100% marker recovery (usually Cr_2O_3) have been largely used. This practice is based on the assumption drawn from MacRae and Evans (1972) that flow of different phases of digesta and markers would be depressed at an equal extent if depression had occurred. However, some inadequacies of this technique have been reported (Van't Kloster et al., 1972; Corse, 1974; Sutton et al., 1976).

Studies of long-term automated total collection (ATC), using simultaneously more than one marker to aid in elucidating questions concerning the techniques for quantitating digesta flow are limited, especially in the bovine. Therefore, this study was conducted to compare ATC with indicator methods (Cr_2O_3 and lignin) for quantifying daily flow rates of digesta in the duodenum of steers fed different diets, and to determine possible diet (concentrate versus roughage) interactions with these markers.

Materials and Methods

Four steers fitted with re-entrant duodenal cannulas were used to compare digesta flow measured by ATC with flow estimations based on chromium oxide and lignin concentrations of representative samples of 24 hr collections of digesta. During two successive periods, each steer (avg. weight, 300 kg) was fed about 4 kg daily of either an all-roughage diet or an 80% sorghum grain diet (Table 1). Diets were offered twice daily at 7:30 am and 3:30 pm. Water and salt were available to the animals at all times.

Table 1. Diet composition.

Ingredient	IRN	Concentrate Diet	Roughage Diet
Chopped alfalfa hay (w/4% cane molasses)	1-00-063 (4-04-696)	5.0	83.0
Chopped wheat straw	1-05-175	7.0	17.0
Sorghum grain (steam processed and flaked)	4-04-444	80.0	--
Cottonseed meal	5-01-621	8.0	--
Dry Matter Basis			
Protein		12.59	14.92
Neutral detergent fiber		7.20	40.33
Acid detergent lignin		3.30	10.73

Animals received their respective diet (and indicators) for at least 15 consecutive days before initiating the collection period. In addition, each steer was adjusted to the collection crate and apparatus for 3 to 5 days before each collection period. Chromium oxide (Cr_2O_3) was given by bolus in a gelatin capsule twice faily about 30 minutes after feeding. Each gelatin capsule contained 4 g of Cr_2O_3. This corresponded to 8 g of Cr_2O_3 per animal per day.

Two permanent fistulas were prepared in the proximal duodenum between the pyloric sphincter and the site where biliary and pancreatic ducts enter the duodenum, using a modification of the surgical technique of Wenham and Wyburn (1980). The intestine was tied between the two fistulas to allow digesta flow only through two tygon plastic re-entrant cannulas. Two types of cannulas were used (Figure 1): first, two T-shaped cannulas with the usual gutter-shaped flange were inserted during the surgery; second, a cannula with a ring-shaped flange on the intestinal end was used to replace the proximal T-cannula after the fistulas had healed (about 20 days after the surgery) and prior to collection. This second type of cannula was used to facilitate the flow of digesta and to avoid possible retention of digesta and distention of the intestine around the intraluminal flange of the proximal T-cannula, as noted by Wenham and Wyburn (1980).

The cannulas were made from 15.9 mm I.D. Tygon plastic tubing, curve-molded to facilitate the flow of digesta, according to the procedure of K. L. Mizwicki, 1979, personal communication). The curve was by inserting a flexible copper tubing into the plastic tubing while

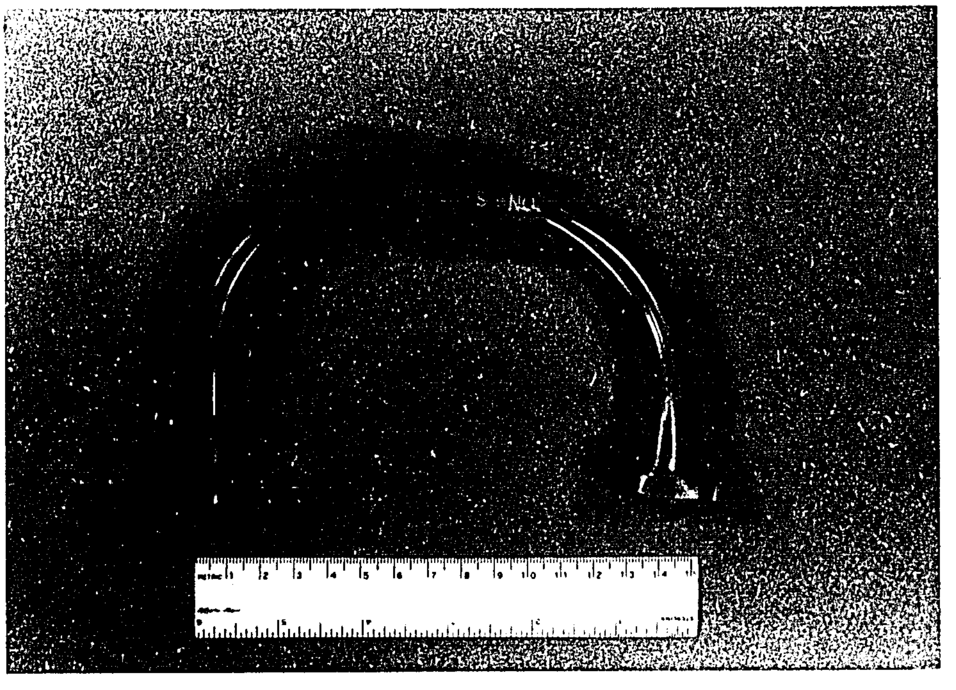

Figure 1. Re-entrant duodenal cannulas.

heating for 20 min. at 100°C. The flange and the ring were made on one end of the cannulas from two different Tygon plastic tubings (25.4 mm and 19 mm I.D.) glued with cyclohexanone solvent. After placement in the animal, the two cannulas were connected outside the body by a small piece (about 5 cm length) of a 19 mm I.D. plastic tubing forming a duodenal plastic bypass (Figure 2).

A special apparatus for measuring and sampling digesta entering in the proximal duodenum was built (Figure 3), based on the apparatus described by Zinn et al. (1980). The apparatus is described in Appendix A. The metabolism crate was adapted to facilitate the automated total collection of digesta. Horizontal bars were removed on the right side of the crate and two vertical adjustable bars were fitted in their place. The floor of the crate was slightly elevated on the left side (~ 3 cm) to encourage the animals to lie on their left side and to assure a free flow of digesta from the proximal cannula to the collection container.

Samples were automatically pooled every 2 hr and sample aliquots of each 2 hr ATC were proportionally pooled for each 24 hr collection period. Samples taken were 4 to 8% of the volume of digesta being pumped in each respective cycle event. However, only 4% of the total digesta measured was kept as sample aliquots for further laboratory analysis. Amounts sampled in excess were returned to the animal. A regression equation was established to estimate digesta flow (ml) from pumping time periods (minutes) recorded during collection phases. This equation was derived from measurement of recorded pumping time for known volumes of digesta.

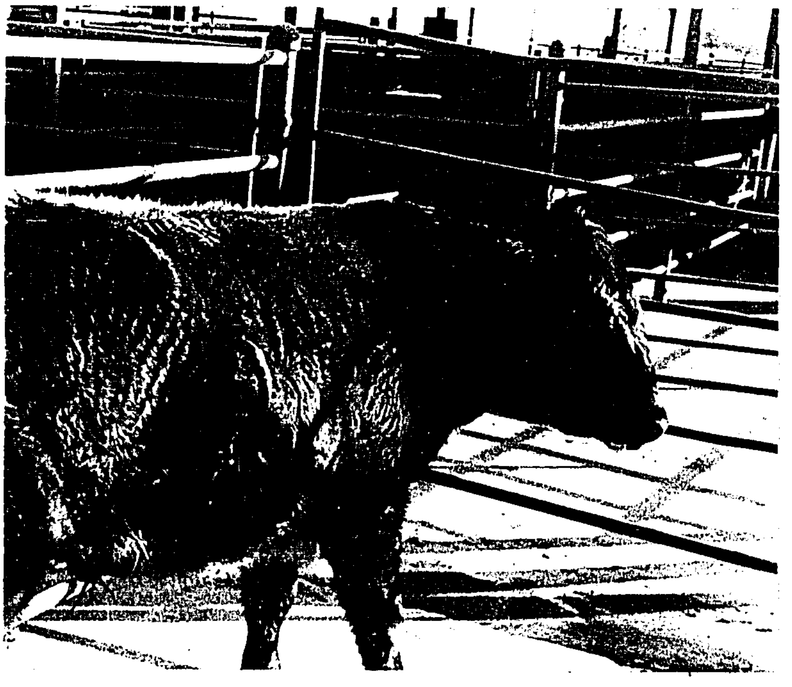

Figure 2. Steer fitted with duodenal re-entrant cannula.

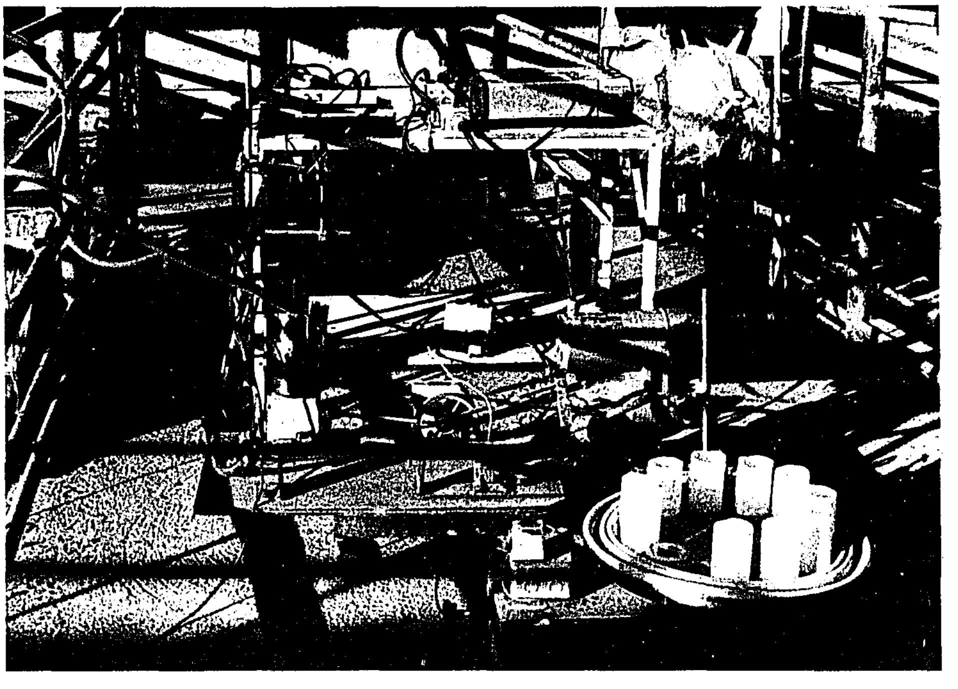

Figure 3. Apparatus for automatically measuring and sampling duodenal digesta flow.

Duration of collection periods were 6 to 8 days, from which 3 to 6 days of continuous 24 hr automated total collection of digesta were compared with flow estimations based on indicator concentrations of ATC samples.

Chromium oxide was determined by the perchloric acid method of Kimura and Miller (1957). Analyses of duodenal digesta were performed on wet sample aliquots (as is basis).

To avoid heat damage of digesta samples and the laborious process of freeze drying, determinations of lignin in duodenal digesta sample aliquots were performed on acetone-insoluble dry matter (Goering and Van Soest, 1970), as suggested by P. J. Van Soest (1980, personal communication). Measured amounts of digesta were placed in Gooch-type crucibles and washed with acetone (about 4 times with volume of digesta); the remaining acetone dry sample residues were then transferred to fiber beakers following the usual routine for acid detergent lignin (Goering and Van Soest, 1970).

Results and Discussion

Cannulation and Automatic Sampling

Animals were considered recovered from the surgery after body weight and feed intake had stabilized. Each animal was used within 6 months after cannulation and no major problems related to the cannulation and cannulas were observed during this period. One of four animals died about 10 months after the surgical preparation and 4 months after he had been used for collection. Necropsy showed massive adhesions of the small intestine similar to those described by Phillips et al. (1978). Blockages in cannulas positioned to allow the flow in a vertical direction were also reported by Phillips et al. (1978) in sheep fitted with intestinal re-entrant cannulas. In the present study, when the steers were not under collection, blockages occurred several times in the cannulas. With increased intestinal pressure the cannulas separated (on the outside of the animal), resulting in loss of digesta contents. There was no clear evidence of a connection between this problem and the type of diet. However, this problem diminished considerably when the animals were on low feed intake (about 1.5% of the body weight), suggesting a possibility of some minor disruption of the normal intestinal motility by cannulation as noted by Wenhan and Wyburn (1980). Harris and Phillipson (1962), Hays et al. (1964) and MacRae and Wilson (1977) concluded that re-entrant cannulation had no major negative effect on digestive functions of sheep and cattle recovered from the surgery. Considering the relative lack of problems with cannulation and the animal performance during the collection periods, the cannulation technique and type of cannula utilized in the present study were considered satisfactory.

The automatic apparatus for sampling and measuring continuously the duodenal digesta was also considered satisfactory for long-term collection. Including training and collection periods, the apparatus was used for about 3,500 hours with no major problems. When collections were conducted in animals receiving roughage diet, blockages in the outlet of the collection container occurred several times and special attention was required, primarily during the first hours of collection.

Effect of Collection Procedure on Duodenal Digesta and Dry Matter Flow Rates

To estimate the effect of the ATC procedures on the rate of flow of digesta contents and the extent of this effect during the first hours of collection, average flow rates of digesta, dry matter, Cr_2O_3 and lignin in the first 24 hr were compared with the average of the subsequent days of collection. Table 2 summarizes these comparisons for digesta and dry matter flow.

Table 2. Hourly digesta and dry method flow: first day versus subsequent days of collection.*

	First Day		Subsequent Days	
Diet	Mean	SD	Mean	SD
Digesta flow (l/hr)				
Concentrate	1.83[a]	.26	1.72[a]	.26
Roughage	2.83[b]	.82	2.62[b]	.49
Dry matter flow (g/hr)				
Concentrate	84.8	20.4	86.9	8.6
Roughage	95.6	12.9	91.7	13.8

* Means with unlike superscripts within columns are different ($P < .05$). Means of four animals and 3 to 6 days of collection.

There was no evidence of flow inhibition due to collection procedure in the first day of collection as compared to the average of the subsequent days. Steers were well trained to the collection procedures, and this may account for the similar values for flow rates in the first 24 hr of collection as compared to the average in the subsequent days of collection. Individual differences were within the day-to-day variation and if collection procedures had some inhibitory effect it was on the entire period. It is unknown if the latter occurred, but any flow inhibition for multiple-day collections should be minimal.

To verify the pattern of day-to-day variation in digesta flow through the proximal duodenum, hourly average flow rates of digesta, dry matter, Cr_2O_3 and lignin were studied based on each daily value as a percentage of the mean for the entire period (Table 3).

Table 3. Digesta and marker flow: each day as a percentage of the mean for the entire period.

Diet	Number of Animals	Parameter	Days				
			1	2	3	4	5
Concentrate	4	Digesta	104	109	95	98	95
		Dry Matter	97	107	97	103	97
		Cr_2O_3	94	82	113	112	99
		Lignin	88	108	92	110	102
Roughage	4	Digesta	104	93	95	115	
		Dry Matter	104	95	94	114	
		Cr_2O_3	100	92	88	138	
		Lignin	108	97	95	98	

No consistent pattern was detected. Any apparent differences were within day-to-day variation. There was also no consistent evidence of a depression on the first day of collection and of subsequent compensatory increases of digesta flow contents.

Leibholz and Hartmann (1972) found greater flow rate in the first 2 hr of total collection than in subsequent 2 hr collections of digesta. It has been widely accepted that for continuous digesta collections, the flow of digesta is depressed as a result of collection procedures in the first 24 hr with a possible compensatory increase in subsequent collection days. This conclusion has been largely based on incomplete recovery of a marker (usually Cr_2O_3). Based on incomplete Cr_2O_3 recovery (79%), Zinn et al. (1980) concluded that there was an inhibition of flow due to automated total collection procedures during a period of 36 hr of continuous collection of duodenal digesta. Tamminga (1975) reported that Cr_2O_3 recovery in duodenal flow of dairy cows increased from 83% in the first day up to 99% in the day 4 of a long-term automated total collection. On the other hand, with long-term total collections, Thompson and Lamming (1972) and Oldham and Ling (1977) found no consistent evidence of flow inhibition on the first day of collection compared to flow on subsequent days of collections. (See Appendix Table B-1 for published comparisons of flow by day of collection.) The latter authors concluded that the variability of repeated 24 hr measurements of flow during long-term collections was within day-to-day variation. This agrees with the data of the present study which suggests that any differences in duodenal digesta flow during the first 24 hr of the long-term ATC and daily flow for subsequent days was within day-to-day variation.

Digesta Flow Estimations

Estimations of duodenal digesta flow based on Cr_2O_3 and lignin concentrations in representative samples of 24 hr flow tended to be 15% higher than measurements made by automated total collection (Table 4).

Table 4. Average daily digesta flow (1/24 hr) for methods and diets.[a]

	Concentrate		Roughage	
Methods	Mean	SD	Mean	SD
ATC	41.6^{b}	6.1	64.7	13.9
Cr_2O_3	46.4^{bc}	9.8	76.1	21.2
Lignin	49.8^{c}	12.8	72.3	17.7
Average	45.8^{c}		71.1^{e}	

a. Means of 4 animals and 3 to 6 days of collection.
b,c. Means within columns with unlike superscripts are different (P<.05).
d,e. Means between columns with unlike superscripts are different (P<.01).

Overall means of digesta flow (1/24 hr) based on ATC, Cr_2O_3 and lignin were 53.2, 61.1, 61.0, respectively. With the concentrate diet, digesta flow based on lignin was greater ($P < .05$) than flow determined by ATC. The same tendency for greater flow was observed with Cr_2O_3 in the concentrate diet and both markers in the roughage diet. Standard deviations also tended to be greater for both marker methods than for ATC. Coefficients of variation for day-to-day variation in digesta flow from ATC were 15% in the concentrate diet and 22% in the roughage diet. Oldham and Ling (1977) reported an average CV of 22% in repeated 24 hr measurements of digesta flow in sheep fed a variety of diets, varying in roughage-to-concentrate levels.

Average daily flow (l) based on the three methods for the concentrate and roughage diets were 45.8 and 71.1, respectively (Table 4). Individual values and statistical analyses are given in Appendix C. Daily flow of digesta estimated from Cr_2O_3 and lignin in the roughage diet agrees with the results of Zinn et al. (1980, 1981) based on marker corrected flow using ATC, with cattle fed a diet containing 60% grain.

With similar dry matter intakes, digesta flow rates in the proximal duodenum of steers fed the roughage diet were 55% greater ($P < .01$) than for steers fed an 88% concentrate diet. These data agree with the results of Grovum and Williams (1973a) and Oldham and Ling (1977) and is probably due to differences in dilution rate and consequently greater ruminal liquid turnover rate with the roughage diet. Teeter and Owens (1981) found 80% greater ruminal liquid dilution rate and 23% larger liquid rumen volume in steers fed 90% chopped alfalfa hay than in steers fed a 90% corn diet. Similarly, Huntington et al. (1981) found a decrease in rumen fluid volume by about 25% and a linear reduction in ruminal fluid turnover rate when the proportion of concentrate was increased in the diet from 0 to 85%.

Dry Matter Flow Estimations

As expected from the digesta flow data, the amount of dry matter entering the duodenum also tended to be greater (9-22%) based on Cr_2O_3 and lignin as compared with ATC measurements (Table 5). Individual values and statistical analyses are given in Appendix C. Average values for ATC, Cr_2O_3 and lignin for both diets were 2143, 2427 and 2505 g/24 hr.

Table 5. Average dry matter flow (g/24 hr) for methods and diets.*

	Concentrate		Roughage	
Methods	Mean	SD	Mean	SD
ATC	2101[a]	380	2219	388
Cr_2O_3	2299[ab]	399	2583	755
Lignin	2566[b]	747	2509	384
Average	2294		2422	

* Mean values of four animals and 3 to 6 days collection.
a,b. Means within columns with unlike superscripts are different ($P<.05$).

Differences among methods for daily passage of dry matter were similar to that for digesta flow data. The standard deviations for the lignin method in the concentrate diet and for Cr_2O_3 method in the roughage diet were about twice as large as the standard deviations for the other two methods in each diet. This indicates a different variability in flow rates of those markers within diets and thus suggests a diet/marker interaction. The high variability of lignin-based values with the concentrate diet could be due in part to analytical analysis of low concentrations of lignin in feed and digesta compared to analysis of samples from the roughage diet.

Average daily dry matter flow of steers receiving the roughage diet was not different from that of the same steers fed the concentrate diet (2294 to 2422 g), which is in contrast to the digesta flow data.

Results of the digesta and dry matter flow rates suggest differential flow and ruminal turnover rates for liquid and solid phases of digesta between these two diets (although water intake was not monitored and differential intake between diets could have occurred). These findings agree with those of Grovum and Williams (1973b), Corse (1974) and MacRae (1974). These results also indicate that diet composition (roughage/concentrate) may have a major effect on the flow and turnover rates of the liquid phase of digesta. This suggests a differential interaction effect between diet and markers and between diets and each different component of the digesta, as is discussed in more detail in Chapter 4. Therefore, it can be concluded that diet composition and ruminal dilution rate may also have effects on flow and turnover rate patterns of several components of the solid phase of digesta. Data from Huntington et al. (1981) support this conclusion.

Marker Recovery

Overall mean duodenal recoveries of Cr_2O_3 and lignin were similar (92.7 versus 88.6%) based on the concentrations of these markers in representative samples of 24 hr flow of digesta. These recovered did not vary appreciably between diets (Table 6). The incomplete duodenal recovery of Cr_2O_3 agrees with results reported by Tamminga (1975), Sutton et al. (1976) and Zinn et al. (1980), who obtained values of 93, 95 and 79%, with cattle based on total collection methods.

Incomplete recovery of lignin has often been reported (Kotb and Luckey, 1972; MacRae, 1974; Muntifering et al., 1981). Drennan et al.

Table 6. Duodenal marker recoveries (%).[a]

Markers	Concentrate		Roughage	
	Mean	SD	Mean	SD
Cr_2O_3	92.60	17.95	91.64	33.20
Lignin	86.88	19.30	90.68	13.05

a. Means of four animals and 3 to 6 days of collection.

(1970) found more consistent results estimating fed concentrate diets ruminal digestibility using lignin as a marker in sheep and cattle than using Cr_2O_3. Less variability in the rate of flow of lignin leaving the rumen than of other markers such as Cr_2O_3 was also reported in the review by Kotb and Luckey (1970). The high standard deviations reflect the great day-to-day variations in recovery for both markers. Thus, flow rates adjusted for 100% recovery of a single marker in a short-term total collection may deviate considerably from the mean measured or estimated over long-term total collection. Inadequacies of marker corrections for flow rates based on short-term collections have also been reported by Van't Klooster et al. (1972), Corse (1974) and Sutton et al. (1976). Theurer et al. (1981), however, found no differences in abomasal digestibilities estimated from 2 versus 6 day collections of abomasal samples when careful attention was given to sample collections. It appears that careful attention to the sampling process over more than one day is necessary to represent the mean for long-term collection.

Ruminal Digestibility of Dry Matter

As expected from the marker recovery data, apparent ruminal dry matter digestibility was about 25% higher for ATC compared to the marker methods (Table 7). For more detail, see Appendix C. Apparent ruminal dry matter digestibility was more consistent when calculated from direct measurements of ATC (smaller standard deviation) than from estimations based on the lignin method with the concentrate diet and the Cr_2O_3 method with the roughage diet. Marker standard deviations suggest a diet/marker interaction. The high standard deviation of lignin-based estimations in the concentrate diet and of Cr_2O_3-based estimations in the roughage diet, makes the use of these two indicators questionable as intestinal markers in those respective diets for quantitating duodenal digesta flow.

Table 7. Apparent ruminal dry matter digestibility (%).[c]

	Concentrate		Roughage	
Methods	Mean	SD	Mean	SD
ATC	43.9[a]	11.3	37.1	9.4
Cr_2O_3	38.8[ab]	11.5	26.2	22.8
Lignin	31.2[b]	22.3	28.8	9.5
Average	39.4		30.9	

a,b. Means within columns with unlike superscripts are different ($P<.05$).
c. Means of four animals and 3 to 6 days of collection.

The 44% apparent ruminal dry matter digestibility based on ATC for the concentrate diet agrees with the result (47%) reported for ATC in cattle by Sutton et al. (1976). The 37% digestibility value based on

ATC for the roughage diet agrees with the results of Tamminga (1975) who found an apparent ruminal dry matter digestibility of 36.5% in dairy cows and with the results of Oldham and Ling (1977) for rumen digestibility (37%), from manual total collection, of roughage diets fed to sheep. Average apparent ruminal dry matter digestibility determined by total collection of duodenal digesta in sheep for a variety of diets (concentrate, mixed and roughage diets) from the data of Liebholz and Hartmann (1972) and Oldham and Ling (1977) was about 52%. However, Van't Klooster et al. (1969) reported lower results (average 17%) and explained this low apparent digestibility by assuming that a substantial part of the measured duodenal digesta dry matter was of endogenous nature.

Discrepancies in results found in the literature concerning ruminal dry matter digestibility estimated from marker concentration of digesta samples (Drennan et al., 1970) are probably related to sampling difficulties due to the great diurnal and day-to-day variation of digesta and marker flow rates (Van't Klooster et al., 1969; Liebholz and Hartmann, 1972; Corse, 1972; Sutton et al., 1976; and Oldham and Ling, 1977).

Conclusions

It is not possible to discern whether the ATC method or the marker methods most accurately estimated flow of digesta and apparent ruminal digestibility in this study; however, the ATC method was generally less variable. Markers were ingested for 13 to 20 days before collection and showed no consistent changes in day-to-day recovery during the collection period. The long-term total collections in this study and others (Tamminga, 1975; Sutton et al., 1976) suggest that Cr_2O_3 recovery will often be incomplete and that long-term ATC does not necessarily inhibit digesta flow. The present ATC studies indicate that with well trained animals, a depression in first day flow is unlikely versus flow on subsequent days. Animals had a greater digesta flow rate on the roughage diet than on the concentrate diet, apparently due to differences in ruminal liquid dilution rate.

This study suggests a difference between ATC and the marker methods in estimating flow and partitioning digestibility, and further suggests a possible diet/marker interaction. It appears that the use of the lignin marker method with concentrate diets and the Cr_2O_3 marker method with roughage diets should be cautiously considered. Flow rates of duodenal digesta contents adjusted for 100% recovery of a single marker in short-term total collection may deviate considerably from the mean measured or estimated over a longer period due to the great diurnal and day-to-day variability in digesta and marker flow. Short-term total collection, however, may give a valid estimation of duodenal digesta flow, if replicated over days.

CHAPTER 4

TRENDS AND PATTERNS OF DIGESTA CONTENT FLOW IN THE GASTROINTESTINAL TRACT OF RUMINANTS

Summary

Magnitude of variation in digesta flow was studied in four steers with duodenal re-entrant cannulas by long-term automated total collection (ATC) of duodenal digesta. Data from three steers were used to study diurnal flow patterns and behavior of markers (Cr_2O_3 and acid detergent lignin) in duodenal digesta. Each steer received an all-roughage diet, and an 88% concentrate diet in each of two ATC periods of 3 to 6 days. Diurnal variation was large, with a magnitude greater than the day-to-day variation. Average coefficients of diurnal variation were 29% with the concentrate diet and 34% with the roughage diet. Estimations based on Cr_2O_3 or lignin concentrations of 2 hr samples could either under- or overestimate digesta flow by 31 to 350% of the 24 hr ATC flow. Thus, due to the great diurnal variation, spot-sampling techniques must be considered very carefully, since inadequate sampling could easily misrepresent total collection samples and flow estimations. Digesta flow rates showed evidence of a cyclic pattern with the concentrate diet, with a period of low flow before the onset of the light hours. No clear flow patterns were detected for duodenal digesta with the roughage diet. The 24 hr pattern of Cr_2O_3 concentrations in duodenal

digesta was different from that of lignin concentrations and similar to the pattern of the concentrations of whole digesta with the concentrate diet, but not with the roughage diet. The 24 hr pattern of digesta dry matter concentrations with the concentrate diet was somewhat closer to the concentration pattern of Cr_2O_3 than that of lignin, while with the roughage diet lignin and dry matter concentration patterns were closely related. Thus, the data suggests that flow estimations from a marker closely associated with a particular component of the solid phase of digesta (such as lignin with fiber), may not necessarily represent flow of the whole particulate phase or of the flow of other major components of the digesta dry matter (i.e., starch or protein) depending upon the diet. Also, the flow of the whole particulate phase may not represent the flow of one particular component of the solid phase of digesta. Therefore, for quantitating flow rates, markers and/or labeled components of food or digesta must be chosen carefully.

Introduction

In an effort to better understand the process of digestion in ruminants, studies have been conducted to estimate and measure flow of digesta in the intestine of sheep and cattle. Markers (reviewed by Kotb and Luckey, 1972; and MacRae, 1974) and total collection of digesta (Corse, 1974) have been used in these studies. Diurnal variation of digesta flow has been reported (Van't Klooster et al., 1969; Faichney and Griffiths, 1978). Zinn et al. (1980) suggested the use of automated total collection for studying patterns and fluctuations of digesta flow rather than for quantitating daily digesta flow rates. Faichney and Griffiths (1978) suggested that such information could be obtained from the analysis of marker concentration patterns. The present study used both approaches in elucidating patterns of digesta flow since only minimal data are found in the literature.

Materials and Methods

The experimental procedures have been described in detail in Chapter 3. In brief, four steers were fitted with duodenal re-entrant cannulas by a modification of the surgery procedure used by Wenham and Wyburn (1980). A special apparatus based on that described by Zinn et al. (1980) was used for automatically sampling and measuring continuously (3 to 6 days) the digesta flow in the proximal duodenum of steers fed two different diets: all-roughate and an 80% sorghum grain diet. Feeding times were 1730 and 1530 hr. Digesta samples were automatically pooled every 2 hr. Magnitude of variation of digesta flow utilized data from four steers. Data from three of the four steers were used to study diurnal variation patterns of digesta and marker behavior.

Results and Discussion

Diurnal Versus Day-to-Day Variation of Digesta Flow Entering the Duodenum

Day-to-day duodenal digesta flow rates (based on repeated 24 hr measurements, Chapter 3) showed coefficients of variation (CV's) of 15% with the concentrate diet and 22% with the roughage diet. Among-day variations for each respective 2 hr measurement period (Table 8) showed

Table 8. Average CV's (%) for each 2 hr measurement of digesta flow (among days).*

2-hr Period	Diet: Concentrate	Diet: Roughage	Mean
8 - 10	23	35	29
10 - 12	24	26	25
12 - 14	27	29	28
14 - 16	28	35	31
16 - 18	37	33	35
18 - 20	30	48	30
20 - 22	26	21	24
22 - 24	23	44	33
24 - 2	19	31	26
2 - 4	36	36	36
4 - 6	31	27	29
6 - 8	39	37	38
Average	29	34	

* Average of four animals and 3 to 6 days of collection.

greater variation than that observed among 24 hr measurements. See Appendix D for individual steer values. Average CV's were 29% in the concentrate diet and 34% in the roughage diet. No difference (P > .05) between diet was detected for this particular variation. The magnitude of the among-day variation was not different (P > .05) for all twelve 2 hour periods. Thus, the variation among 2 hr periods was large whether the animals were feeding or resting.

Table 9 illustrates the extremely large within-day variation (by 2 hr periods) noted in this study, which agrees with data reported by

Table 9. Average CV's (%) for each 2 hr measurement of digesta flow within days.*

Diet	Days 1	2	3	4	5	Mean
Concentrate	37	22	27	22	36	29
Roughage	32	34	30	40		34
Mean	34	39	28	31	36	

* Average of four animals and twelve (2 hr) collection measurements.

Van't Klooster et al. (1969) and Faichney and Griffiths (1978). Coefficients fo diurnal variation for individual steers varied from 10 to 68% (Appendix Table D-6). In the present study the steers were fed twice daily, but other studies suggest that diurnal variation would not be substantially reduced by manipulating feeding frequencies (Leibholz and Hartmann, 1972; Hevelplund et al., 1976; Sutton et al., 1976). This large diurnal variation in digesta flow may be the most plausible explanation for the discrepancies in results from spot-sample estimations for partitioning digestion in ruminants versus total collection estimations (Van't Klooster et al., 1969).

Although flow estimations based on marker concentrations in representative samples of 24 hr digesta flow (pooled samples per day) were greater by 12 to 20% than daily flow measured by ATC (Chapter 3); estimations based on Cr_2O_3 or lignin concentrations of 2 hr samples could either greatly under- or overestimate digesta flow due to the large diurnal variation of flow of digesta (and marker concentration).

Estimations of digesta flow by marker concentration varied from 31 to 350% of the 24 hr flow based on ATC (Table 10). Estimates of daily flow based on marker concentrations of 2 hr samples varied two- to five-fold for individual steers (Appendix Table D-7).

Table 10. Range of digesta flow estimations based on Cr_2O_3 or lignin concentrations of each within-day 2 hr sample (expressed as a percentage of 24 hr ATC flow).

Diet	Animal	Cr_2O_3	Lignin
Concentrate		Range	Range
	1	59 - 223	31 - 128
	2	87 - 170	55 - 178
	3	91 - 202	40 - 142
Roughage			
	1	60 - 240	52 - 228
	2	122 - 350	48 - 287
	3	38 - 130	47 - 264

Few reports are found in the literature comparing estimations from spot-samples and estimations from representative samples (samples from total collection) of 24 hr digesta flow. Van't Klooster et al. (1969) found that direct measurements agreed with indirect estimations based on concentrations of PEG and Cr_2O_3 in representative samples of digesta flow; however, estimations based on concentrations of those markers in spot-samples showed great discrepancies. Later, Van't Klooster et al. (1972) concluded that frequent spot sampling for several days might be a valid technique. Zinn et al. (1980) found no significant differences between estimations based on spot-sampling (500 ml of duodenal digesta at 6 hr intervals for 48 hr) and that from 36 hr total collection in steers. Total collection estimations were corrected for 100% duodenal recovery of Cr_2O_3. Faichney (1972) found a relative excess of Cr_2O_3 in abomasal spot-samples of wethers so that calculations based on Cr_2O_3 concentrations underestimated digesta flow from the abomasum and overestimated ruminal digestibility, despite the fact that fecal recovery ranged from 91 to 101%. These findings support the conclusion of the present study that independent of marker recovery and due to the great diurnal variation, spot-sampling techniques must be considered very carefully since inadequate sampling could easily misrepresent total collection samples and lead to either under- or overestimations of digesta content flow from the abomasum to the duodenum.

Diurnal Variation Patterns for Markers and Digesta

Average CV's of about 30% (Tables 8 and 9) indicate the large diurnal variation for digesta flow rates. Similar or even greater variations (including diurnal, among-day and animal variations) were observed in flow rates and concentrations of Cr_2O_3, lignin and dry matter (Table 11).

Although the variations are of great magnitude, regression analysis for diurnal variation showed significant ($P < .04$ and $P < .005$) patterns for digesta and Cr_2O_3 24 hr flow rates with the concentrate diet, with a bimodal distribution (Figure 4). Commencing about the time

Table 11. Average CV's (%) for dry matter, Cr_2O_3 and lignin for each 2 hr collection (within-day).*

	Concentrate Diet		Roughage Diet	
Parameter	Flow	Concentration	Flow	Concentration
Dry matter	38	31	40	27
Cr_2O_3	43	25	58	39
Lignin	46	39	45	40

* Average of three steers.

of the morning feeding, digesta flow increased to a peak flow just before the afternoon feeding. A second peak was noted about midnight, followed by a period of low flow before the onset of the light period. Chromium oxide showed a rather similar pattern; however, flow rate increased more rapidly in the morning hours and decreased more rapidly following the midafternoon peak. The regression equations are:

$$\text{Digesta flow } y' = 3231.87-163.96H+43.77H^2-2.86H^3+5.71 \times 10^{-2}H^4$$

$$Cr_2O_3 \quad y' = 660.53-159.58H+31.95H^2-1.97H^3+3.78 \times 10^{-2}H^4$$

where H = hour at beginning of 2 hr sampling period.

No significant ($P > .05$) patterns were detected for dry matter and lignin flow rates in the concentrate diet nor for any of the flow parameters in the roughage diet. Correlation analysis showed a positive relationship ($P < .01$; $r \sim .6$) between respective pairs of all marker and digesta flow parameters (based on 2 hr collections) (Appendix 9 gives

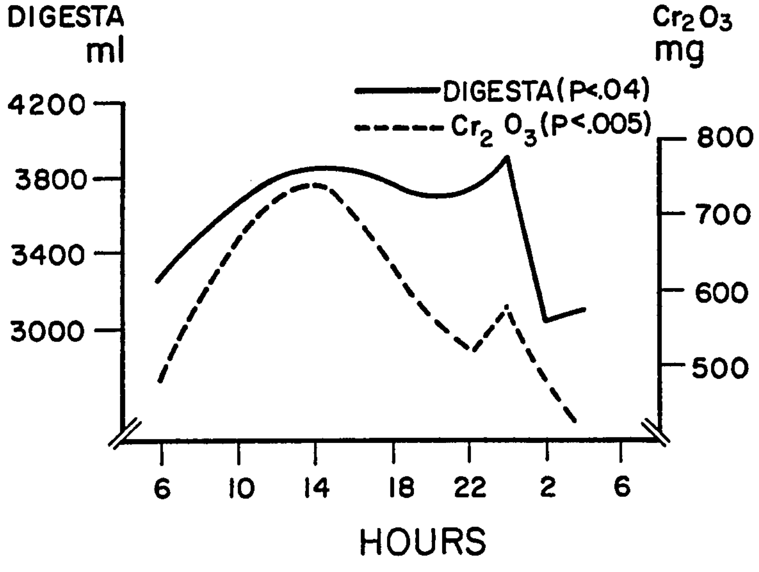

Figure 4. Flow pattern of digesta and Cr_2O_3 based on regression analysis of 2-hr measurements of duodenal digesta from three steers fed the concentrate diet. -- Feeding times were 0730 and 1530 hr. See text for regression equations.

the respective correlation values), suggesting that lignin and dry matter flow rates may also have somewhat similar patterns to that observed for digesta and Cr_2O_3 in the concentrate diet.

Concentrations of Cr_2O_3 in the digesta showed a significant ($P < .04$) bimodal pattern in both diets. However, the shape of the distribution curve was different for each diet (Figure 5 and Figure 6), suggesting an interaction between marker and diet. It was not determined whether this interaction also occurred with other components of digesta (fiber, starch, etc.). Regression equations for the concentrate and roughage diet, respectively, are:

$$y' = .115+8.732x10^{-3}H+1.892x10^{-4}H^2-5.812x10^{-5}H^3+1.567x10^{-6}H^4$$

$$y' = .156-2.043xk0^{-3}H-1.121x10^{-3}H^2+9.299x10^{-5}H^3-1.900x10^{-6}H^4$$

Lignin concentrations in the digesta showed a significant ($P < .05$) pattern in the roughage diet, but not in the concentrate diet. The regression equaton for the roughage diet is:

$$y' = 2.21 + 2.51H - .41H^2 + 2.81 \times 10^{-2}H^3 - 4.21 \times 10^{-4}H^4$$

Although not significant in the concentrate diet, a regression equation of the same exponential order was used to draw a tendency curve for lignin concentrations in the digesta (Figure 5). From this curve, it appears that lignin flows at a different rate from Cr_2O_3 in concentrate diets. The lignin concentration patterns appear somewhat similar for the two diets; however, it is not possible to draw any clear

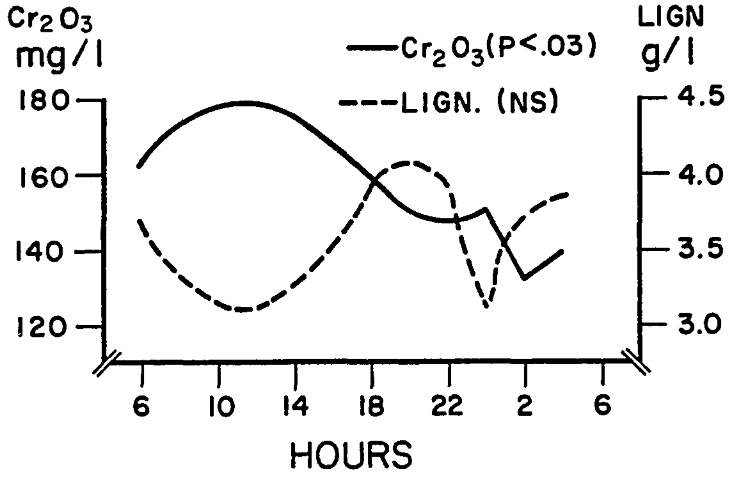

Figure 5. Flow pattern of Cr_2O_3 and lignin concentrations based on regression analysis of 2-hr measurements of duodenal digesta from three steers fed the concentrate diet. -- Feeding times were 0730 and 1530 hr. See text for regression equations.

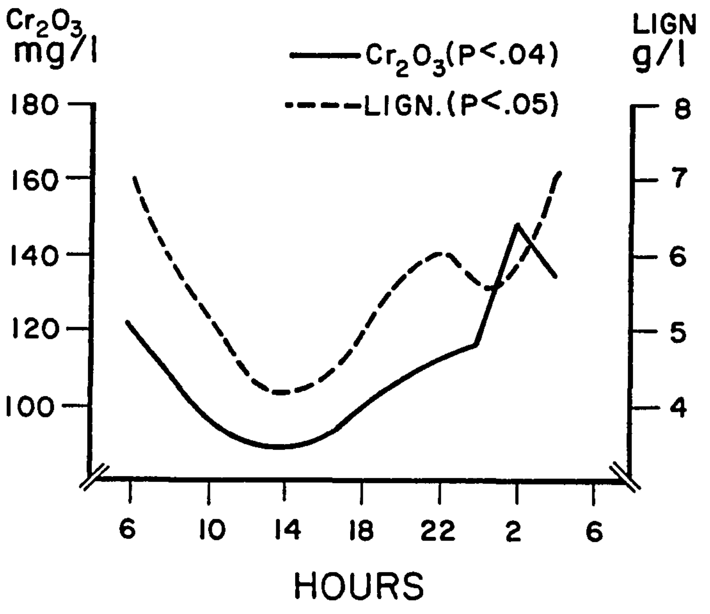

Figure 6. Flow pattern of Cr_2O_3 and lignin concentrations based on regression analysis of 2-hour measurements of duodenal digesta from three steers fed the roughage diet. -- Feeding times were 0730 and 1530 hr. See text for regression equations.

conclusion due to the non-significance of the lignin concentration curve for the concentrate diet.

With the roughage diet, concentrations of Cr_2O_3 and lignin in the digesta tended to decrease in the morning and increase throughout the afternoon and night, in contrast to the Cr_2O_3 pattern in the concentrate diet.

Predictability of all regression equations was low (R^2 = .11 to .22), probably due to the large among-day and animal variations. It is not clear if the total collection procedure would be cause for a more erratic variation and less consistent pattern than in the intact animal.

From short-term collections, Harris and Phillipson (1962) found that the duodenal digesta flow in sheep fed low-quality hay tended to increase throughout the night and reach a peak by early morning at feeding time and that the period following the afternoon feeding was always a period of low flow. Contrary to this, the data of Leão et al. (1978), with sheep fed sorghum grain diet, indicated that greater duodenal contents flow occurred during the day (from 8 A.M. to 8 P.M.) rather than during the night. The period from midnight to 8 A.M. was always a period of low flow. The data of the present study for the concentrate diet agrees with that of Leão et al. (1978). The reduction in digesta flow rates following the afternoon feeding observed by Harris and Phillipson (1962) was also detected in the present study. Liebholz and Hartmann (1972) did not find any consistent pattern from an up to 10-fold variation among successive periods of 2 hr total collection measurements of duodenal digesta in sheep fed a variety of diets.

Faichey and Griffiths (1978) found cyclical fluctuation (24 hr cycle) in the net water flux in the rumen of sheep fed a concentrate diet, despite the use of continuous feeding and lighting conditions. Thus, his data suggest that the cyclical fluctuations found in the present study may not necessarily be due to frequency of feeding. Hevelplund et al. (1976) and Sutton et al. (1976) found no major effect of feeding frequencies on digesta flow rates in the proximal duodenum of cows. It is possible that the diurnal variation of the digesta content flow to the duodenum is an intrinsic phenomenon regardless of feeding frequencies and lighting conditions.

According to Armstrong et al. (1978), monogastric animals have a characteristic biological cycle for energy that is coupled with the metabolic and digestive phenomena as well as with the feeding behavior and resting/activity periodicity. The cyclical fluctuation of flow patterns found in the present study suggest that a similar model may also be true for ruminants. However, further studies are necessary to substantiate this suggestion.

Average concentrations of dry matter in the duodenal digesta were 50 g/l in the concentrate diet and 34 g/l in the roughage diet in the present study. This difference in the digesta dry matter concentration between diets suggests that the liquid and the solid phases of digesta had differential flow rates to the duodenum, since dry matter intake was similar for both diets. Data from daily flow rates of digesta and dry matter support this suggestion (Chapter 3). Water intake was not monitored, however, and differential intake between diets could have some influence on concentrations of dry matter in digesta.

Faichey and Griffiths (1978) suggested that mean retention time in the rumen and the rate of removal of a particular component of the diet could be estimated from the analysis of marker concentration patterns. Differential patterns for Cr_2O_3 and lignin concentrations in the digesta (Figure 5 and Figure 6) indicates the variability in the ratio of Cr_2O_3 to lignin concentrations in the digesta and suggests differential behavior not only for the liquid and solid phases, but probably also among other components of the digesta. This and the effect of diet on that behavior is also illustrated by the variability in the ratio of dry matter to Cr_2O_3 or to lignin concentrations in the digesta, shown by the correlation values (Table 12; Appendix Table D-9). This apparent

Table 12. Correlation values for dry matter (DM), lignin and Cr_2O_3 concentrations in the digesta.*

	Concentrate Diet	Roughage Diet
DM with Cr_2O_3	$r = .3$ $P < .05$	$r \leq .2$ NS
DM with lignin	$r = -.001$ NS	$r = .6$ $P < .01$

* Values of three steers for each diet.

variability in marker behavior and possible diet/marker interaction has been noted by those who advocate the use of dual-phase markers or multiple marker techniques (Corbert et al., 1958; Van't Klooster et al., 1969; Tan et al., 1978; MacRae, 1974; Raichney and Griffiths, 1978; Hartnell and Satter, 1980; Uden et al., 1980). The concept of dual-phase markers assumes that the utilization of a particular marker closely associated to a component of the particulate phase (i.e., rare earth elements or metal oxide mordanted fiber), would be preferable to those markers (i.e., Cr_2O_3) not attached or absorbed to any particular component of the digesta (MacRae, 1972 and Uden et al., 1980). However, the data from the present study showed a positive relationship ($P < .01$; $r = .63$) between dry matter and lignin concentrations in the digesta of cattle fed the roughage diet, but not in digesta from cattle fed the concentrate diet ($r = -.001$). Dry matter concentration in the digesta was somewhat more closely related to Cr_2O_3 concentrations ($P < .05$; $r = .3$) than to that of lignin ($r = -.001$) in the concentrate diet. These relationships suggest different behavior among components of the particulate phase of digesta and further suggest that estimations from a marker closely associated with a particular component of the solid phase may not necessarily represent the flow of the whole particulate phase of digesta or of the flow of a major component of the digesta dry matter. In that case, a marker not attached to any particular component of the digesta could more correctly estimate flow of the entire digesta.

For quantitating flow rates of the whole particulate or solid phase of digesta, markers or a labeled component of food or digesta must be chosen carefully. Of course the flow of the whole particulate phase may not represent the flow of one particular component of the solid phase of digesta.

The cyclical flow patterns observed in the present study might be considered as an indication of cyclical fluctuations in metabolic and digestive phenomena in ruminants, coupled with the resting/activity cycle. However, further studies are necessary to substantiate this assumption.

APPENDIX A

APPARATUS FOR AUTOMATED TOTAL COLLECTION OF DUODENAL FLOW OF DIGESTA IN CATTLE

A special apparatus for measuring and sampling digesta entering the proximal duodenum was built, based on the apparatus described by Zinn et al. (1980). The apparatus was basically formed by five units:

1. Collection unit: a 2.5 liter stainless steel container, with a plastic outlet spout, which received the digesta from the proximal cannula; a hotplate, to keep the digesta warm; a laboratory stirrer, to mix the collected digesta in the collection container; an electronic liquid level controller volume sensing device.
2. Pumping unit: a peristaltic tube pump with a variable speed gear electric motor.
3. Divert-flow unit: two hose shut-off clamps which, acting together, close and open the two tubing branches allowing the digesta flow back to the animal or be diverted to the sampling collector unit; a solenoid valve which controlled the shut-off clamps.
4. Sampling collector unit: A turnstyle carousel fraction collector.
5. Recorder and control unit: An omni inscribe recorder, adapted to receive 120 v impulse electric current, measured the time of pumping; four electronic timers and an electric connection integrator, which acting together with the liquid sensing device in the collection unit, control the events (see Figure 3).

Collection Procedure

A metabolism crate was adapted to facilitate the automated total collection of digesta. The floor of the crate was slightly elevated on the left side (~ 3 cm) to encourage the animals to lie on their left side and assure a free flow of the digesta from the proximal cannula to the collection container. When sufficient digesta had accumulated to contact the upper electrode of the electronic level controller in the collection container, a return cycle event was initiated with the peristaltic pump and recorder activated. Approximately 10 seconds after initiating the return cycle event, the solenoid valve of the divert-flow unit was activated and a sample was taken. Two electronic timers controlled the solenoid valve, determining sample size. The digesta was returned to the animal through a 12.7 mm I.D. flexible ambar latex tubing. The return cycle event stopped when the level of digesta in the collection container dropped below the lower electrode of the volume sensing device. Controlled by two timers, the fraction collector moved to position a new sample vial at 2 hr intervals. A regression equation was established to estimate digesta flow (ml) from pumping time periods (minutes) recorded during collection phases. This equation was derived from measurement of recorded pumping time for known volumes of digesta.

APPENDIX B

PUBLISHED COMPARISONS OF FLOW BY DAY OF COLLECTION

Table B.1. Each day as a percentage of the mean for the entire collection period.[a]

Reference	Number (Type) of Animal	Parameter	Days 1	2	3	4	5	6	7
Van't Klooster et al. (1969)	2(sheep)	Cr_2O_3	92	105	101				
Van't Klooster et al. (1972)	2(cows)	Cr_2O_3	91	99	96	112	102		
Osburn (1975)	(sheep)	Cr_2O_3	86	94	107	98	103	107	105
Goodall et al. (1965)	2(sheep)	D.M.	89	104	107				
Thompson et al. (1972)	2(sheep)	Digesta	99	100	101				
Johnston et al. (quoted by MacRae, 1975)	1(sheep)	D.M.	92	104	103	99	102		
		Cr_2O_3	93	100	99	104	104		
Sutton et al. (1976)	4(cows)	D.M.	92	104	103	99	102		
		Cr_2O_3	93	100	99	104	104		
Oldham et al. (1977)	3(sheep)	D.M.	100	100					
	3(sheep)	D.M.	100	103	97				
	3(sheep)	D.M.	96	101	103				
	1(sheep)	D.M.	89	104	107				

a. Adapted from Oldham et al. (1977).

APPENDIX C

TABULAR AND STATISTICAL DATA, CHAPTER 3

Table C.1. Daily digesta flow (1/24 hr).

		Concentrate			Roughage		
Steer	Day	ATC	Cr_2O_3	Lignin	ATC	Cr_2O_3	Lignin
1	1	46.97	53.66		67.57	115.94	57.82
	2	48.79	46.64	40.83	62.82	89.89	68.10
	3	33.90	51.72	59.92	71.03	90.91	96.81
	4	38.80	54.79	42.95	88.47	70.17	105.89
	5	38.96	37.74	40.77			
	Mean = X	41.49	48.91	46.02	72.47	91.73	82.15
2	1	49.18	54.64	66.81	52.08	68.96	64.19
	2	46.96	39.60	50.67	53.17	89.89	53.57
	3	38.72	55.38	70.33	55.01	114.27	70.97
	4	36.71	41.67	39.67			
	5	42.40	39.22	57.20			
	6	37.80	44.45	60.67			
	Mean =	41.96	45.83	57.56	53.42	9.104	64.58
3	1	39.47	58.18	65.35	96.40	65.04	93.54
	2	54.91	59.04	72.01	65.07	50.93	93.55
	3	50.18	52.98	51.61	72.91	50.95	73.41
	4	46.04	61.30	52.99			
	5	41.93	49.67	48.59			
	Mean =	46.50	56.23	58.11	78.13	55.65	89.90
4	1	37.74	30.77	38.83	56.71	65.57	63.62
	2	32.51	38.83	38.09	56.76	66.12	56.77
	3	36.44	42.11	31.25	47.91	72.07	50.44
	4	40.96	36.36	34.58	59.98	54.42	59.01
	5	34.64	38.09	33.99			
	Mean =	36.46	34.63	35.35	55.34	64.54	57.46

Table C.2. Daily digesta flow (two-way analysis).

Source	DR	SS	MS	F
Methods	2	326	163.2	1.085
Diets	1	3860	3859.8	25.666
M x D	2	48	23.8	0.158
Error	18	2707	150.4	
Total	23	6941		

Methods Significance = 0.360
Diets Significance = 0.000
M x D Significance = 0.855

Methods	Means
ATC	53.22
Cr_2O_3	61.07
Lignin	61.02

LSD (0.05) = 12.88

Diets	Means
Concentrate	45.75^b
Roughage	71.12^a

LSD (0.05) = 10.52

Table C.3. Daily digesta flow-concentrate (one-way analysis).

Source	DF	SS	MS	F
Treatments	2	699	349.6	3.553
Error	59	5805	98.4	
Total	61	6504		

Significance = 0.034

Treatment	Mean	SD
ATC	41.62^{a}	6.07
Cr_2O_3	46.37^{ab}	9.82
Lignin	49.84^{b}	12.85

LSD (.05) = 6.17

Table C.4. Daily digesta flow-roughage (one-way analysis).

Source	DF	SS	MS	F
Treatments	2	941	470.6	1.479
Error	39	12408	318.2	
Total	41	13349		

Significance = 0.239

Treatment	Mean	SD
ATC	64.71	13.89
Cr O	76.08	21.15
Lignin	72.34	17.73

LSD (.05) = 13.63

Table C.5. Daily dry matter flow (g/24 hr).

Steer	Day	Concentrate ATC	Concentrate Cr_2O_3	Concentrate Lignin	Roughage ATC	Roughage Cr_2O_3	Roughage Lignin
1	1	2718	2598	1919	2582	3695	2799
	2	1593	2431	2816	2173	2782	2962
	3	1808	2553	2001	2663	2112	3187
	4	2170	2102	2249			
	Mean =	2072	2421	2246	2473	3863	2983
2	1	1997	2218	2712	1859	2462	2291
	2	3109	2621	3354	1733	2930	1909
	3	2273	3251	4128	1722	3725	2221
	4	2408	2734	4023			
	5	2518	2329	3398			
	6	1988	2338	3191			
	Mean =	2382	2582	3468	1771	3039	2140
3	1	1559	2298	2581	2314	1561	2245
	2	2097	2255	2751	1796	1406	2587
	3	2223	2241	2183	2209	1544	2224
	4	2081	2771	2395			
	5	1862	2205	2157			
	Mean =	1964	2354	2413	2106	1504	2352
4	1	1872	1526	1926	2421	2800	2717
	2	2256	1791	2643	2296	2711	2328
	3	1793	2072	1537	2084	3135	2194
	4	1937	1720	1636	2999	2721	2950
	5	1756	1931	1723			
	Mean =	1923	1808	1893	2450	2842	2547

Table C.6. Daily dry matter flow (two-way analysis).

Source	DF	SS	MS	F
Methods	2	582336	291168.0	1.285
Diets	1	99288	99288.0	0.438
M x D	2	73584	36792.0	0.162
Error	18	2078240	226569.0	
Total	13	4833448		

Methods Significance = 0.301
Diets Significance = 0.523
M x D Significance = 0.852

Daily dry matter flow (methods).

Treatment	Means
ATC	2142.62
Cr O	2426.62
Lignin	2505.25

LSD (0.05) = 500.03

Daily dry matter flow (diets).

Treatment	Means
Concentrate	2293.83
Roughage	2422.50

LSD (0.05) = 408.27

Table C.7. Daily dry matter flow-concentrate (one-way analysis).

Source	DF	SS	MS	F
Treatments	2	2180510	1090260.0	3.7894
Error	57	16399500	287710.0	
Total	59	18580000		

Significance = 0.02770

Treatment	Mean	SD
ATC	2100.90[a]	380.46
Cr_2O_3	2299.25[ab]	399.54
Lignin	2566.15[b]	747.50

LSD (.05) = 339.24

Table C.8. Daily dry matter flow-roughage (one-way analysis).

Source	DF	SS	MS	F
Treatments	2	961472	480736.0	1.663
Error	36	10405200	289032.0	
Total	38	11366600		

Significance = 0.202

Treatment	Mean	SD
ATC	2219.31	387.86
Cr_2O_3	2583.38	754.63
Lignin	2508.77	383.65

Table C.9. Duodenal marker recovery (%).

Steer	Day	Concentrate Cr_2O_3	Concentrate Lignin	Roughage Cr_2O_3	Roughage Lignin
1	1	87.5		58.3	116.6
	2	104.6	119.5	69.9	92.1
	3	65.5	56.6	78.1	73.2
	4	70.8	90.3	126.1	83.4
	5	103.2	96.5		
	Mean =	86.3	90.7	83.1	91.3
2	1	90.0	73.5	75.5	81.1
	2	118.6	92.7	59.1	88.1
	3	69.9	55.1	48.1	77.5
	4	88.1	92.5		
	5	108.1	74.1		
	6	85.0	62.3		
	Mean =	93.3	75.0	60.9	82.2
3	1	67.8	60.4	148.1	103.1
	2	93.0	76.3	127.7	69.4
	3	94.7	97.2	143.0	99.3
	4	75.1	86.9		
	5	84.4	86.9		
	Mean =	83.0	81.6	139.6	90.6
4	1	122.6	97.2	86.5	89.1
	2	125.8	85.3	85.8	100.0
	3	86.5	116.6	66.5	95.0
	4	112.6	115.7	110.2	101.6
	5	90.9	101.9		
	Mean =	107.7	103.3	87.3	96.4

Table C.10. Duodenal marker recovery (two-way analysis).

Source	DF	SS	MS	F
Markers	1	68	68.1	0.195
Diets	1	3	3.2	0.009
M x D	1	7	7.5	0.021
Error	12	4189	349.1	
Total	15	4267		

Markers Significance = 0.669
Diets Significance = 0.922
M x D Significance = 0.881

Duodenal marker recovery (markers).

Treatment	Means
Cr_2O_3	92.75
Lignin	88.62

LSD (0.05) = 20.35

Duodenal marker recovery (diets).

Treatment	Means
Concentrate	90.25
Roughage	91.12

LSD (0.05) = 20.35

Table C.11. Duodenal marker recovery-concentrate (one-way analysis).

Source	DF	SS	MS	F
Treatments	1	337	336.5	0.971
Error	39	13519	346.6	
Total	40	13855		

Significance = 0.668

Treatment	Mean	SD
Cr_2O_3	92.60	17.95
Lignin	86.88	19.30

LSD (.05) = 11.75

Table C.12. Duodenal marker recovery-roughage (one-way analysis).

Source	DF	SS	MS	F
Treatments	1	6	6.4	0.010
Error	26	16546	636.4	
Total	27	16552		

Significance = 0.918

Treatment	Mean	SD
Cr_2O_3	91.64	33.20
Lignin	90.68	13.05

LSD (.05) = 19.60

Table C.13. Apparent ruminal dry matter digestibility (%).

Steer	Day	Concentrate ATC	Concentrate Cr_2O_3	Concentrate Lignin	Roughage ATC	Roughage Cr_2O_3	Roughage Lignin
1	1	39.60	42.27	57.36	28.28	.95	22.25
	2	64.60	45.98	37.42	39.64	22.72	17.72
	3	59.82	43.26	55.53	26.03	41.33	11.47
	4	51.78	53.29	50.02			
Mean =		53.95	46.20	50.09	31.31	21.03	17.14
2	1	44.53	38.39	24.67	42.62	24.01	29.29
	2	13.64	27.19	7.83	46.51	9.57	41.08
	3	36.86	9.69	-14.67	46.85	-14.97	31.45
	4	33.11	24.06	-11.75			
	5	30.06	35.31	5.61			
	6	44.78	35.06	11.36			
Mean =		33.83	28.28	3.67			
3	1	56.69	36.17	28.30	35.72	56.65	37.64
	2	41.75	37.36	23.58	50.11	60.94	28.14
	3	38.25	37.75	39.36	38.64	57.11	38.22
	4	42.19	23.03	33.47			
	5	48.28	38.75	40.08			
Mean =		45.53	34.61	32.96	41.50	58.23	34.67
4	1	48.00	57.61	46.50	32.75	22.22	24.53
	2	37.33	50.25	26.58	36.22	24.69	35.33
	3	50.19	42.44	32.96	42.11	12.92	39.06
	4	46.19	52.22	54.56	16.69	24.42	18.06
	5	51.22	46.36	52.14			
Mean =		46.58	49.78	47.42	31.94	21.06	29.24

Table C.14. Apparent ruminal dry matter digestibility (two-way analysis).

Source	DF	SS	MS	F
Methods	2	458	229.1	1.116
Diets	1	432	431.7	2.103
M x D	2	75	37.3	0.182
Error	18	3695	205.3	
Total	23	4659		

Methods Significance = 0.350
Diets Significance = 0.161
M x D Significance = 0.836

Apparent ruminal dry matter digestibility (methods).

Treatment	Means
ATC	41.24
Cr_2O_3	33.10
Lignin	31.14

LSD (0.05) = 15.05

Apparent ruminal dry matter digestibility (diets).

Treatment	Means
Concentrate	39.40
Roughage	30.92

LSD (0.05) = 12.289

Table C.15. Apparent ruminal dry matter digestibility-concentrate (one-way analysis).

Source	DF	SS	MS	F
Treatments	2	1641	820.7	3.255
Error	57	14372	252.1	
Total	29	16013		

Significance = 0.045

Treatment	Mean	SD
ATC	43.84[a]	11.28
Cr_2O_3	38.83[ab]	11.50
Lignin	31.21[b]	22.29

LSD (.05) = 10.04

Table C.16. Apparent ruminal dry matter digestibility-roughage (one-way analysis).

Source	DF	SS	MS	F
Treatments	2	841	420.6	1.809
Error	36	8370	232.5	
Total	38	9211		

Significance = 0.177

Treatment	Mean	SD
ATC	37.09	9.38
Cr_2O_3	26.20	22.81
Lignin	28.79	9.46

LSD (.05) = 12.09

APPENDIX D

TABULAR AND STATISTICAL DATA, CHAPTER 4

Table D.1. Each 2 hr among-day variation (average CV's) of digesta flow.

Period (hour)	Concentrate Diet (Steer Number) 1	2	3	4	Mean	Roughage Diet (Steer Number) 1	2	3	4	Mean
8 - 10	29	20	21	23	23	66	14	36	23	35
10 - 12	26	24	25	20	24	27	18	46	13	26
12 - 14	14	31	42	22	27	29	25	27	34	29
14 - 16	21	29	46	15	28	59	27	42	13	35
16 - 18	66	15	53	16	37	51	31	30	18	33
18 - 20	57	10	29	24	30	75	62	43	11	48
20 - 22	38	26	23	17	26	8	13	38	26	21
22 - 24	27	27	19	18	23	27	87	31	31	44
24 - 2	35	13	7	22	19	31	21	32	50	31
2 - 4	72	25	32	14	36	18	12	30	86	36
4 - 6	80	8	23	13	31	17	43	18	32	27
6 - 8	58	30	39	30	39	35	87	11	14	37
Average					29					34

Table D.2. Each 2 hr among-days variation (two-way analysis).

Source	DF	SS	MS	F
Hours	11	2425	220.4	0.5625
Diets	1	575	575.2	1.4677
Hr x D	11	1771	161.0	0.4108
Error	72	28216	391.9	
Total	95	32987		

Hours Significance = 0.85300
Diets Significance = 0.22760
F1 x F2 Significance = 0.94680

Each 2 hr among-days variation by hours.

Treatment Number	Means
1 (8 - 10)	29.00
2 (10 - 12)	24.875
3 (12 - 14)	28.000
4 (14 - 16)	31.500
5 (16 - 18)	35.000
6 (18 - 20)	38.875
7 (20 - 22)	23.625
8 (22 - 24)	33.375
9 (24 - 2)	25.000
10 (2 - 4)	36.125
11 (4 - 6)	29.250
12 (6 - 8)	38.000

LSD (0.05) = 19.7961

Each 2 hr among-days variation.

Treatment Number	Means
Concentrate	28.604
Roughage	33.500

LSD (0.05) = 8.08172

Table D.3. Hourly digesta and dry matter flow: first day versus subsequent days of collection.

Animal Number	Digesta (l)			
	Concentrate		Roughage	
	First Day	Subsequent Days	First Day	Subsequent Days
1	2.08	1.67	2.81	3.08
2	2.04	1.69	2.17	2.25
3	1.64	2.01	4.00	2.87
4	1.57	1.51	2.36	2.29
	Dry Matter (g)			
1	113.25	86.33	107.58	103.04
2	83.21	99.25	77.46	73.79
3	64.96	81.83	96.42	87.75
4	78.00	80.12	100.87	102.08

Table D.4. Hourly digesta flow (two-way analysis).

Source	DF	SS	MS	F
Periods	1	0	0.1	0.439
Diets	1	4	3.6	15.082
P x D	1	0	0.0	0.041
Error	12	3	0.2	
Total	15	7		

Periods Significance = 0.526
Diets Significance = 0.002
F1 x F2 Significance = 0.836

Hourly digesta flow (periods).

Treatment	Means
1	2.334
2	2.171

LSD (0.05) = .534435

Hourly digesta flow (diets).

Treatment	Means
Concentrate	1.776[b]
Roughage	2.729[a]

LSD (0.05) = .534

Table D.5. Hourly dry matter flow (g) (two-way analysis).

Source	DF	SS	MS	F
Periods	1	4	3.6	0.017
Diets	1	241	240.6	1.132
P x D	1	35	35.2	0.166
Error	12	2550	212.5	
Total	15	2829		

Periods Significance = 0.893
Diets Significance = 0.309
F1 x F2 Significance = 0.692

Dry matter flow (periods).

Treatment Number	Means
1	90.22
2	89.27

LSD (0.05) = 15.88

Dry matter flow (diets).

Treatment	Means
Concentrate	85.87
Roughage	93.62

LSD (0.05) = 15.88

Table 6. Each 2 hr within-day variation average coefficients of variation (%).

		Days					
Diet	Steer	1	2	3	4	5	6
Concentrate	1	68	17	41	24	53	
	2	11	24	20	17	32	15
	3	47	28	22	26	36	
	4	21	18	26	19	23	
			Mean = 29				
Roughage	1	33	33	42	41		
	2	51	39	26			
	3	32	31	23			
	4	10	32	28	38		
			Mean = 34				

Table D.7. Digesta flow (1/24 hr) estimated from each 2 hr sample marker concentration.

	Chromium (Steer Number)						Lignin (Steer Number)					
	1		2		3		1		2		3	
Concentrate Diet												
8 - 10	30	58	45	48	89	50	20	34	35	40	72	25
10 - 12	28	36	59	54	73	47	25	50	60	66	63	--
12 - 14	37	41	41	36	56	48	40	43	37	31	47	33
14 - 16	36	50	48	46	57	65	36	38	41	44	58	18
16 - 18	58	44	47	58	52	77	32	43	39	46	36	23
18 - 20	79	45	56	54	57	83	15	33	35	39	62	50
20 - 22	72	43	53	40	50	68	19	20	26	46	44	44
22 - 24	105	61	56	63	58	52	26	43	47	34	42	17
24 - 2	77	56	63	61	76	45	32	36	45	49	78	23
2 - 4	76	85	45	63	74	59	22	--	59	24	51	30
4 - 6	--	93	47	51	56	47	32	26	42	32	50	19
6 - 8	--	49	57	60	66	52	--	44	42	47	25	29

Table D.7. -- Continued.

	Chromium (Steer Number)						Lignin (Steer Number)					
	1		2		3		4		5		6	
Roughage Diet												
8 - 10	87	53	101	89	58	--	49	60	25	52	60	--
10 - 12	127	62	103	103	75	95	107	71	60	82	148	188
12 - 14	131	110	110	--	93	65	75	160	72	--	193	83
14 - 16	151	71	160	67	53	61	78	68	98	56	142	99
16 - 18	107	160	182	138	65	38	45	254	59	91	99	64
18 - 20	82	--	--	127	77	41	52	--	44	90	110	66
20 - 22	--	--	104	186	83	56	81	--	73	116	116	85
22 - 24	91	55	73	127	83	56	52	46	49	76	88	122
24 - 2	77	56	78	103	69	45	45	62	46	74	103	85
2 - 4	73	50	--	111	40	41	52	52	66	61	100	59
4 - 6	99	69	75	99	37	46	74	81	25	158	73	48
6 - 8	65	75	--	--	47	49	46	141	--	--	69	32

Table D.8. Correlation analysis.*

	Digesta Flow	D.M. Flow	Cr_2O_2 Flow
Concentrate			
Dry matter flow	.52009 N = 71 Sig = .01	--	--
Cr_2O_3 flow	.59949 N = 93 Sig = .01	.66557 N = 71 Sig = .01	--
Lignin flow	.50028 N = 69 Sig = .01	.36997 N = 69 Sig = .01	.60696 N = 69 Sig = .01
Roughage			
Dry matter flow	.66449 N = 66 Sig = .01	--	--
Cr_2O_3 flow	.72091 N = 63 Sig = .01	.62481 N = 63 Sig = .01	--
Lignin flow	.51500 N = 66 Sig = .01	.80770 N = 66 Sig = .01	.53237 N = 63 Sig = .01

* Data from three steers in each diet.

Table D.9. Correlation analysis.*

	Digesta Flow	D.M. Conc.	Cr_2O_3 Conc.
Concentrate Diet			
D.M. Concentration	-.01574 N = 71 Sig = NS	--	--
Cr_2O_3 Concentration	.00947 N = 71 Sig = NS	.27446 N = 71 Sig = NS	--
Lignin Concentration	-.05901 N = 68 Sig = NS	-.00149 N = 68 Sig = NS	.21989 N = 68 Sig = NS
Roughage Diet			
D.M. Concentration	.05302 N = 65 Sig = NS	--	--
Cr_2O_3 Concentration	.15781 N = 63 Sig = NS	.20117 N = 62 Sig = NS	--
Lignin Concentrate	-.28241 N = 66 Sig = .05	.63337 N = 65 Sig = .01	.19989 N = 63 Sig = NS

* Data from three steers in each diet.

Table D.10. Each 2 hr among-day variation for dry matter, Cr_2O_3 and lignin (flow and concentration).*

Diet	Parameter	Hours												Mean
		8	10	12	14	16	18	20	22	24	2	4	6	
Conc.	D.M. Flow	33	16	48	42	46	40	34	33	34	56	54	26	38
	D.M. Conc.	36	23	38	28	36	29	35	33	26	26	38	28	31
Rough	D.M. Flow	54	24	25	54	33	49	30	23	45	31	38	78	40
	D.M. Conc.	36	29	22	24	35	27	39	32	26	10	27	17	27
Conc.	C.R. Flow	42	32	29	39	58	53	33	33	25	61	58	50	43
	C.R. Conc.	31	34	19	21	22	29	24	25	28	27	22	17	25
Rough	C.R. Flow	68	33	42	73	87	71	69	41	29	52	75	57	58
	C.R. Conc.	44	26	29	41	69	50	46	30	30	38	43	22	39
Conc.	Lig Flow	60	50	15	17	45	70	39	58	41	63	61	38	46
	Lig Conc.	41	49	15	48	28	58	42	43	40	40	34	29	39
Rough	Lig Flow	48	38	37	68	42	58	43	43	45	26	42	49	45
	Lig Conc.	38	37	39	30	49	32	45	35	32	23	60	56	40

* Average CV's (%) of three steers in each diet.

Table D.11. Concentrate diet summary table of regression (flow).

Variable	Multiple R	R^2	Overall F	Significance
Digesta Flow				
Hour	.19059	.03632	7.16188	.008
$Hour^2$	.21703	.04710	4.67120	.010
$Hour^3$	.21808	.04756	3.12927	.027
$Hour^4$	.22747	.05174	2.55093	.041
Dry Matter Flow				
Hour	.08946	.00800	.55667	.458
$Hour^2$	.12292	.01511	.52158	.596
$Hour^3$	.12367	.01529	.34688	.792
$Hour^4$	.12985	.01686	.28297	.888
Cr_2O_3 Flow				
Hour	.14963	.02239	2.15280	.146
$Hour^2$	.33436	.11179	5.85270	.004
$Hour^3$	.33436	.1180	3.85996	.012
$Hour^4$	.38863	.15103	4.04733	.005
Lignin Flow				
Hour	.11382	.01296	.89251	.348
$Hour^2$	.14716	.02166	.74156	.480
$Hour^3$	.15843	.02510	.56641	.639
$Hour^4$	.21628	.04678	.79742	.531

Table D.12. Roughage diet summary table of regression (flow).

Variable	Multiple R	R^2	Overall F	Significance
Digesta Flow				
Hour	.09203	.00847	1.00801	.317
$Hour^2$	.09935	.00987	.58316	.560
$Hour^3$	.11387	.01297	.50796	.678
$Hour^4$	.11915	.01420	.41404	.798
Dry Matter Flow				
Hour	.08349	.00697	.45628	.502
$Hour^2$	.09862	.00973	.31431	.731
$Hour^3$	.11745	.01379	.29372	.830
$Hour^4$	.12302	.01513	.23819	.916
Cr_2O_3 Flow				
Hour	.4663	.00217	.13290	.717
$Hour^2$	.12169	.01481	.45091	.639
$Hour^3$	.15923	.02535	.51157	.676
$Hour^4$	.16289	.02653	.39523	.811
Lignin Flow				
Hour	.00836	.00007	.00447	.947
$Hour^2$	.13010	.01693	.54232	.584
$Hour^3$	.17150	.02941	.62629	.601
$Hour^4$	.24385	.05946	.96417	.434

Table D.13. Concentrate diet summary table of regression (concentrations).

Variable	Multiple R	R^2	Overall F	Significance
Cr_2O_3 Concentrations				
Hour	.00895	.00008	.00730	.932
$Hour^2$	.30395	.09239	4.58056	.013
$Hour^3$	.33433	.11178	3.73343	.014
$Hour^4$	.33854	.11461	2.84786	.029
Lignin Concentrations				
Hour	.00880	.00008	.00519	.943
$Hour^2$	.07718	.00596	.19777	.821
$Hour^3$	.16686	.02784	.62052	.604
$Hour^4$	.23855	.05690	.96541	.433
Roughage Diet				
Cr_2O_3 Concentration				
Hour	.19732	.03893	2.47122	.121
$Hour^2$	.39355	.15488	5.49796	.006
$Hour^3$	.39494	.15598	3.63452	.018
$Hour^4$	.39976	.15981	2.75795	.036
Lignin Concentrations				
Hour	.14222	.02023	1.32124	.255
$Hour^2$	.25574	.06540	2.20441	.119
$Hour^3$	.28190	.07947	1.78414	.159
$Hour^4$	.37173	.13818	2.44517	.056

Table D.14. Digesta flow. -- Mean and SD of observed values for each 2 hr collection period.*

Period (hour)	Digesta Flow (ml)			
	Concentrate Diet		Roughage Diet	
	Mean	SD	Mean	SD
8 - 10	3950	995	5703	2363
10 - 12	3428	1056	5026	1412
12 - 14	4213	1297	5182	1434
14 - 16	3719	1152	6274	3024
16 - 18	3632	1769	7172	2883
18 - 20	3567	1312	5236	3325
20 - 22	3887	1176	6077	2052
22 - 24	3943	1015	5586	2464
24 - 2	3759	1005	5946	1774
2 - 4	3194	1432	5459	1081
4 - 6	2904	1112	5181	1476
6 - 8	3034	1203	5611	3246

* Average of three steers in each diet

Table D.15. Dry matter. -- Mean and SD of observed values for each 2 hr collection period.*

Period (hour)	Dry Matter Flow (g)				Dry Matter Conc. (g/l)			
	Conc. Diet		Roughage Diet		Conc. Diet		Roughage Diet	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
8 - 10	206	69	245	133	50	18	50	18
10 - 12	170	28	147	36	47	11	31	9
12 - 14	268	130	161	40	52	20	27	6
14 - 16	188	79	222	119	50	14	33	8
16 - 18	188	87	259	85	53	19	34	12
18 - 20	176	70	146	71	49	14	37	10
20 - 22	237	81	205	61	57	20	33	13
22 - 24	193	64	239	56	52	17	38	12
24 - 2	197	67	230	104	57	15	38	10
2 - 4	151	84	216	67	50	13	38	4
4 - 6	171	92	174	67	58	22	44	12
6 - 8	182	47	243	190	58	16	36	6

* Average of three steers in each diet.

Table D.16. Chromium oxide. -- Mean and SD of observed values for each 2 hr collection period.*

Period (hour)	Cr_2O_3 Flow (mg)				Cr_2O_3 Concentrations (mg/l)			
	Conc. Diet		Roughage Diet		Conc. Diet		Roughage Diet	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
8 - 10	654	272	690	471	170	52	90	40
10 - 12	605	192	431	141	190	64	88	23
12 - 14	924	273	501	212	186	36	83	24
14 - 16	690	268	758	550	170	35	103	42
16 - 18	589	340	823	717	157	35	93	64
18 - 20	557	293	642	457	151	42	112	56
20 - 22	699	230	674	463	169	41	89	41
22 - 24	535	174	706	288	143	36	108	32
24 - 2	549	138	691	199	149	41	120	36
2 - 4	485	294	871	450	132	35	148	56
4 - 6	386	223	647	488	152	34	129	56
6 - 8	404	203	799	459	148	25	140	31

* Average of three steers in each diet

Table D.17. Lignin. -- Mean and SD of observed values for each 2 hr collection period.*

Period (hour)	Lignin Flow (g)				Lignin Concentrations (g/l)			
	Conc. Diet		Roughage Diet		Conc. Diet		Roughage Diet	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
8 - 10	17.0	10.2	47.3	22.8	3.8	1.5	8.1	3.1
10 - 12	9.5	4.8	18.5	7.1	2.6	1.3	3.9	1.4
12 - 14	15.8	2.4	21.9	8.2	3.2	.5	3.8	1.5
14 - 16	11.7	2.0	30.6	20.7	3.5	1.7	4.5	1.4
16 - 18	12.2	5.5	36.1	15.0	3.5	1.0	4.9	2.4
18 - 20	14.0	9.8	22.6	13.2	3.7	2.2	5.7	1.8
20 - 22	17.4	6.8	30.8	13.3	4.2	1.8	4.8	2.2
22 - 24	15.2	8.8	38.5	16.6	3.9	1.7	5.8	2.0
24 - 2	10.8	4.4	34.5	15.7	3.2	1.3	5.8	1.9
2 - 4	11.2	7.1	32.4	8.3	3.7	1.5	6.0	1.4
4 - 6	11.3	6.9	27.2	11.3	3.9	1.3	6.5	4.0
6 - 8	10.9	4.1	58.8	19.2	3.6	1.0	7.2	4.0

* Average of three steers in each diet.

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