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AUTOMATED LONG-TERM TOTAL COLLECTION VERSUS INDICATOR METHOD TO ESTIMATE DUODENAL DIGESTA FLOW IN CATTLE^{1,2}

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ABSTRACT

Four steers, fitted with re-entrant duodenal cannulas, were used to compare digesta flow as measured by automated total collection (ATC) with flow estimations based on chromium oxide (Cr2O1) and lignin concentrations of representative samples of 24-h collection of digesta. In two successive periods, each steer was fed about 4 kg/d of either an all-forage or an 80% sorghum grain diet. Duodenal digesta samples were automatically taken and pooled every 2 h, during the 3- to 6-d total collections. For animals adapted to the collection procedures, there was no consistent evidence of flow inhibition in the first 24 h of a long-term total collection. Flow rates of duodenal digesta and dry matter (DM), estimated by Cr_2O_3 and lignin were 15% greater and more variable than those measured by ATC. Digesta flow rates measured by ATC averaged 65 liters/d in steers fed the forage diet and 42 liters/d in the same steers receiving the grain diet. Apparent ruminal DM disappearance, calculated from direct measurements by ATC was 44% for the grain diet and 37% for the forage diet. Ruminal DM disappearance, based on Cr_2O_3 and lignin, was about 25% lower than the ATC values. The data indicate that the use of total collection procedures should include replication over days. Duodenal flow rates suggested a 24-h cyclic pattern of digesta flow for the grain diet, with a peak about midnight followed by a period of low flow before the onset of the light hours. Chromium oxide showed a similar flow pattern, but flow rate decreased more rapidly following the midafternoon peak. With the forage diet, 24-h patterns were only observed for Cr_2O_3 and lignin concentrations in duodenal digesta, which appeared different from the Cr2O3 concentration pattern with the grain diet. Diurnal variation of digesta flow was greater than day-to-day variation. Because of the diurnal variation, spot-sampling techniques must be considered very carefully, since inadequate sampling could easily misrepresent total collection samples and flow estimates. The data also suggest caution in the use of lignin as a marker in grain diets, and of Cr₂O₃ as a marker in forage diets for partitioning digestion in the duodenum of ruminants.

(Key Words: Collection, Digestibility Markers, Lignin, Chromic Oxide, Beef Cattle.)

Introduction

To understand more clearly the process of digestion, 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. Sampling has been a

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major problem with estimations based on marker techniques. Automation has improved the total collection procedure, but due to difficulties with animal management during long-term total collections, short-term total collections (less than 24 h) with flow estimations adjusted for 100% marker recovery (usually Cr_2O_3) have been 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 equally depressed if depression of flow occurred. However, inadequacies of this technique have been reported (Van't Klooster et al., 1972; Corse, 1974; Sutton et al., 1976).

Studies of long-term automated total collection (ATC), using more than one marker simultaneously to aid in elucidating questions concerning techniques for quantitating digesta flow are limited, especially in the bovine.

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Therefore, this study was conducted to compare ATC with the indicator method (Cr_2O_3 and lignin) in order to: 1) quantify daily flow rates of digesta in the duodenum of steers fed different diets; 2) determine possible diet (concentrate versus forage) effects on marker estimates; 3) elucidate patterns of 24-h digesta flow.

Materials and Methods

Four steers, fitted with re-entrant duodenal cannulas, were used to compare digesta flow as measured by ATC with estimates based on Cr₂O₃ and lignin concentrations of representative samples of 24-h collections of digesta. During two successive periods, each steer (avg wt 300 kg) was fed about 4 kg/d of either an all-forage or an 80% sorghum grain diet (table 1). Two animals received the grain and two the forage diet in the first period, and the diets were switched in the second period. Animals received their respective diet for at least 21 consecutive d before collection. Diets were offered twice daily at 0730 and 1530 h. Chromium oxide (4 g) was given by bolus in a gelatin capsule about 30 min after each feeding for at least 21 d before and during each collection period. Water and salt were available to animals at all times.

Two permanent fistulas were prepared in the proximal duodenum of the steers (between the pyloric sphincter and the site where biliary and pancreatic ducts enter) using a modification of the surgical technique of Wenham and Wyburn (1980). The intestine was ligated between the two fistulas to force digesta flow through the two Tygon plastic, re-entrant cannulas. Two types of cannulas made of Tygon plastic tubing (15.9 mm id), curve-molded to facilitate the flow of digesta were used (according to the procedure of K. L. Mizwicki, personal communication). Two T-shaped cannulas, with the usual gutter-shaped flange, were inserted in each fistula during surgery. After fistulas had healed (about 20 d), a cannula with a ringshaped flange on the intestinal end replaced the proximal T-cannula. This latter cannula was used to facilitate digesta flow 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). Animals were considered recovered from surgery after body weight and feed intake had stabilized.

An apparatus was built, based on that described by Zinn et al. (1980), for automatically measuring and sampling digesta entering the proximal duodenum. A metabolism crate was adapted to facilitate the automated total collection of digesta. The floor of the crate was slightly elevated (3 cm) on the left side to encourage animals to lie on this side and to facilitate a free flow of digesta from the proximal cannula to the collection container. Each steer was adapted to the collection crate and automatic sampling apparatus for 3 to 5 d before each collection period. Digesta flow in the proximal duodenum was measured and sampled continuously. Samples were automatically pooled for 2-h periods by using a turnstyle carousel fraction collector. Aliquots

Ingredient	Grain diet	Forage diet
Chopped alfalfa hay (IFN 1-00-063)		
with 4% cane molasses (IFN 4-04-696)	5.0	83.0
Chopped wheat straw (IFN 1-05-175)	7.0	17.0
Sorghum grain, steam processed		
and flaked (IFN 4-04-444)	80.0	
Cottonseed meal (IFN 5-01-621)	8.0	
Analysis		
Protein	12.59	14.92
Neutral detergent fiber	7.20	40.33
Acid detergent lignin	3.30	10.73

TABLE 1. DIET COMPOSITION^a

^aDry-matter basis.

of each 2-h ATC pooled sample were then proportionally pooled to represent each 24-h collection period. Days with missing periods of recorded measurements were deleted. Thus, periods of 3 to 6 d of continuous 24-h automated measurement of digesta were compared with flow estimations based on indicator concentrations of ATC samples. Numbers of days of continuous flow measurements for animals consuming the grain or forage diets were, respectively: 4, 6, 5, 5 and 3, 3, 3, 4.

Chromium oxide was determined by the perchloric acid method of Kimura and Miller (1957). Chromium analyses were performed on wet duodenal digesta aliquots. Determinations of lignin in duodenal digesta aliquots were performed on acetone-insoluble dry matter (Goering and Van Soest, 1970), as suggested by P. J. Van Soest (personal communication) by placing measured amounts of wet digesta in Gooch-type crucibles and washing with 4 to 5 volumes of acetone. The remaining acetone-dry residues were then transferred to fiber beakers and acid detergent lignin was determined by the method of Goering and Van Soest (1970). Aliquots of digesta and feed samples were oven-dried at 100 C for dry matter (DM, AOAC, 1975).

Data from 24-h pooled samples were analyzed statistically by analysis of variance, as a factorial arrangement, with diet and flow measurement method as main effects (Steel and Torrie, 1960). Interaction of these two effects was tested. Polynomial regression, based on data of 2-h samples and measurement periods, was used to study the within-day variation and diurnal flow patterns. Polynomial terms were introduced in steps to obtain the simplest possible equation, up to a 4th-degree equation. Since several 2-h samples from one steer were accidentally lost before chemical analysis, only data from three of the four steers were used to determine diurnal DM and marker flow patterns.

Reusits and Discussion

Cannulation and Automatic Sampling. Considering the relative lack of problems with cannulation and animal performance during the collection periods, the cannulation technique and type of cannula used in the present study were considered satisfactory. Digesta collections from each animal were completed within 6 mo after cannulation and no major problems related to the cannulation and cannulas were observed during this period. However, the value of this cannulation technique for extended periods (1 yr or longer) is not clear because necropsy of all animals about 1 yr after surgery showed massive adhesions of the small intestine similar to those described by Phillips et al. (1978) in sheep.

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 problems 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). 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 surgery.

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 3,000 h with no major problems. When collections were conducted with animals receiving the forage diet, blockages in the outlet of the collection container occurred several times and special attention was required. These blockages were the cause for most missing periods during the 6-d collection periods.

Digesta Flow for First Day vs 3- to 6-Day Collection Period. There was no evidence that collection procedure inhibited digesta flow in the first day of collection. Mean flow rates of digesta for grain and roughage diets in the first day were 104 and 105%, respectively, of the means for the 3- to 6-d periods (table 2). Values for DM flow and recovery of Cr_2O_3 were also similar for first day and the 3- to 6-d period and lignin recovery for the first day was within normal day-to-day variation.

In this experiment, steers were well trained in the collection procedures, and this may account for the similar values for digesta flow in the first 24 h as compared with the mean of the 3- to 6-d period. If collection procedures had an inhibitory effect on flow, it was on the entire period. It has been generally accepted that flow of digesta is depressed as a result of continuous collection procedures in the first 24 h, with a possible compensatory increase during subsequent days. This conclusion has been

Item	Grain diet			Forage diet		
	First day	Mean	SDC	First day	Mean	SDc
Digesta flow, liters/d	43.3	41.6	6.5	68.2	64.7	13.9
Dry matter, kg/d	2.0	2.1	.4	2.3	2.2	.4
Cr, O, recovery, %	91.9	92.6	18.0	92 .1	91.6	33.2
Lignin recovery, %	77.0	86.9	19.3	97.5	90.7	13.0

 TABLE 2. DIGESTA FLOW AND MARKER RECOVERY FOR FIRST DAY VERSUS

 THE MEAN OF THE 3- TO 6-DAY COLLECTION PERIOD^{a,b}

^aAverage of four animals.

^bBased on automated total collection.

^cStandard deviation of observations.

based on incomplete recovery of a marker, usually Cr_2O_3 (Tamminga, 1975; Zinn et al., 1980). However, 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 subsequent days.

Flow Estimates: Method and Diet Effects. Mean duodenal recoveries of Cr_2O_3 and lignin were similar (92 and 89%) and did not vary appreciably between diets (table 3). Incomplete duodenal recovery of Cr_2O_3 agrees with results reported by Tamminga (1975), Sutton et al. (1976) and Zinn et al. (1980), and incomplete recovery of lignin has often been indicated (Kotb and Luckey, 1972; MacRae, 1974; Muntifering et al., 1981).

Estimates of duodenal digesta flow based on Cr₂O₃ and lignin concentrations averaged 15% higher than measurements made by ATC (table 3). Overall means across diets (liters/d) based on ATC, Cr₂O₃ and lignin were 53, 61 and 61, respectively. Standard deviations (SD) also tended to be greater for both markers than for ATC. Differences in daily DM flow were similar to those of whole digesta flow. The SD for the lignin method with the grain diet and for the Cr_2O_3 method with the forage diet were about twice as large as the SD for the other two methods in each diet. These data indicate a different variability in flow rates of these markers within diets, although a diet \times marker interaction effect was not detected in the analysis of variance. The high variability of

		Grain diet		Forage diet	
Item	Methods	Mean	SDb	Mean	SDb
Marker recovery (%)	Cr, 0,	92.6	18.0	91.6	33.2
	Lignin	86.9	19.3	90.7	13.0
Digesta flow (liters/d)	AŤC ^c	41.6 ^d	6.1	64.7	13.9
	Cr. O.	46.4de	9.8	76.1	21.2
	Lignin	49.8 ^e	12.8	72.3	17.7
	Average	45.8 ^f		71.1 ^g	
Drv matter flow (g/d)	ATCC	2,101d	380	2,219	388
	Cr, O,	2,299de	399	2,583	755
	Lignin	2,566 ^e	747	2,509	384
	Average	2,294		2,422	

TABLE 3. AVERAGE DAILY FLOW RATES AND MARKER RECOVERIES^a

^aMeans of four animals and 3 to 6 d of collection.

^bStandard deviation of observations.

^cAutomated total collection.

^{d,e}Means within columns that do not have a common superscript differ (P<.05).

 f,g Averages within rows with unlike superscripts differ (P<.01).

estimates based on lignin for the grain diet could be due in part to low concentrations of lignin in grain and digesta compared with high concentrations in forage.

With similar DM intakes, digesta flow rates (average of three estimates) in the proximal duodenum of steers fed the forage diet were 55% greater (P<.01) than for steers fed an 80% grain diet (71 vs 46 liters/d; table 3). However, average daily DM flow of steers receiving the forage diet was not different from that of the same steers fed grain (2.2 vs 2.1 kg/d based on ATC estimations). Greater rates of digesta flow with forage diets, agrees with data of Grovum and Williams (1973) and Oldham and Ling (1977), and might be attributed to a faster dilution rate, and consequently greater ruminal liquid-turnover-rate on roughage diets. Teeter and Owens (1981) found 80% greater ruminal liquid-dilution-rate and 23% larger ruminal liquid volume in steers fed 90% chopped alfalfa hay compared with 90% corn. Similarly, Huntington et al. (1981) found a 25% decrease in ruminal fluid volume and a linear reduction in ruminal fluid-turnover-rate when the proportion of concentrate in the diet increased from 0 to 85%.

Ruminal Disappearance of Dry Matter. Apparent ruminal DM disappearance estimated by Cr_2O_3 and lignin ratio techniques ranged from 12 to 29% lower than did those determined by ATC (table 4). Apparent ruminal DM disappearance was less variable when calculated from direct measurements of ATC (smaller SD) than from estimates based on lignin with the grain diet and Cr_2O_3 with the roughage diet. The high SD of lignin estimates on the grain diet and of Cr_2O_3 estimates on the roughage diet, suggests caution when using these indicators as intestinal markers for quantitating duodenal digesta flow.

The 44% apparent disappearance of ruminal DM based on ATC for the grain diet agrees with 47% reported for ATC in cattle by Sutton et al. (1976). The 37% ruminal disappearance based on ATC for the roughage diet agrees with Tamminga (1975), who reported a 36.5% disappearance in dairy cows fed forage diets using total collection. These results also agree with those of Oldham and Ling (1977) for ruminal DM disappearance (37%) in sheep on roughage diets, using manual total collection. Apparent ruminal DM disappearance determined by total collection of duodenal digesta in sheep for a variety of diets (concentrate to roughage diets) from the data of Leibholz and Hartmann (1972) and Oldham and Ling (1977) averaged about 52%. However, Van't Klooster et al. (1969) reported ruminal DM disappearance in sheep as low as 17% when total collection was used. They explained this low apparent disappearance by assuming that a substantial part of the measured duodenal digesta DM was of endogenous sources.

Discrepancies in results found in the literature concerning ruminal DM digestibility estimated from marker concentration in digesta samples (Drennan et al., 1970; Theurer, 1979) are probably related to difficulties with spotsampling due to the large diurnal and day-today variation of digesta and marker-flow-rates (Van't Klooster et al., 1969; Leibholz and Hartmann, 1972; Corse, 1974; Sutton et al., 1976 and Oldham and Ling, 1977). In the present study, it is not possible to discern whether the ATC method or the marker meth-

Method	Grain	diet	Forage diet	ge diet
	Mean	SDb	Mean	SDb
ATC ^c	43.9d	11.3	37.1	9.4
Cr. 0,	38.8de	11.5	26.2	22.8
Lignin	31.2 ^e	22.3	28.8	9.5
Average	39.4		30.9	

TABLE 4. APPARENT PERCENTAGE OF RUMINAL DRY MATTER DISAPPEARANCE^a

^aMeans of four animals and 3 to 6 d of collection.

^bStandard deviation of observations.

^CAutomated total collection.

 d,e Means within columns that do not have a common superscript differ (P<.05).

		,	Variation
Digesta flow, liters		Diurnal	Day-to-day
Steer	Daily mean	SDb	SDp
Grain diet			
1	41.5	17.5	6.2
2	42.0	9.5	5.2
3	46.5	15.4	6.2
4	36.5	8.2	3.2
Forage diet			
1	72.5	30.0	11.2
2	53.4	20.3	1.5
3	78.1	25.3	16.3
4	55.3	16.0	5.2

TABLE 5. MEAN DUODENAL DIGESTA FLOW RATES AND DIURNAL AND DAY-TO-DAY VARIATIONS FOR STEERS FED GRAIN AND FORAGE DIETS^a

^aBased on automated total collection for 3 to 6 d for each steer per diet.

^bStandard deviation of observations.

ods most accurately estimated flow of digesta and apparent ruminal DM disappearance; however, the ATC method was less variable.

Diurnal Patterns of Digesta Flow. Diurnal variation of digesta flow was greater than day-to-day variation, as indicated by SD values (table 5). Coefficients of variation (CV) for diurnal values ranged from 22 to 41% for individual steers fed grain and forage diets, while CV for day-to-day values ranged from 3 to 21%. The within-day CV for DM flow, and concentrations of Cr_2O_3 and lignin in duodenal digesta averaged 33, 25 and 39%, respectively,

for steers fed the grain diet. Similar CV for the forage diet were 40, 39 and 40%. Means for duodenal digesta-flow-rate for each 2-h ATC period (table 6) also reflect this large diurnal variation. Flow estimates based on Cr_2O_3 and lignin concentrations of individual 2-h samples ranged from 31 to 350% of the 24-h flow measured by ATC (table 7), demonstrating that frequency of sampling is critical when using spot-sampling techniques.

Large diurnal variation of duodenal digesta flow in sheep was also reported by Van't Klooster et al. (1969) and Faichney and

2-h period	Grain	n diet	For	age diet
	Mean	SDp	Mean	SDb
8 - 10	3.8	1.0	5.4	2.0
10 - 12	3.2	1.1	4.8	1.3
12 - 14	3.9	1.3	5.0	1.3
4 – 16	3.7	1.1	5.8	2.8
6 – 18	3.5	1.6	6.7	2.6
8 - 20	3.3	1.3	5.2	2.8
20 - 22	3.7	1.2	6.0	1.7
22 - 24	3.7	1.0	5.4	2.2
24 - 2	3.5	1.0	5.5	1.8
2 - 4	3.2	1.3	5.1	1.5
4 - 6	3.0	1.0	4.7	2.0
6 - 8	3.2	1.2	5.2	2.9

TABLE 6. MEAN DUODENAL DIGESTA FLOW RATES FOR EACH 2-HOUR AUTOMATED TOTAL COLLECTION PERIOD (LITERS)^a

^aAverage of four steers and 3 to 6 d of collection for each diet.

^bStandard deviation of observations.

Diet	Animal	Cr ₂ O ₃	Lignin
Grain	1 2 3	$59 - 223^{a} \\ 87 - 170 \\ 91 - 202$	31 - 128 55 - 178 40 - 142
Forage	1 2 3	60 - 240 122 - 350 38 - 130	52 - 228 48 - 287 47 - 264

TABLE 7. RANGE OF DIGESTA FLOW-RATE ESTIMATES BASED ON Cr₂O₃ OR LIGNIN CONCENTRATIONS OF INDIVIDUAL 2-HOUR SAMPLES (EXPRESSED AS A PERCENTAGE OF 24-HOUR AUTOMATED TOTAL COLLECTION FLOW)

^aRange.

Griffiths (1978). In our study, steers were fed twice daily, but other studies suggest that diurnal variation would not be substantially reduced by changing feeding frequency (Leibholz and Hartmann, 1972; Hevelplund et al., 1976; Sutton et al., 1976). Large diurnal variation in digesta flow may be the most plausible explanation for discrepancies in results of partitioning digestion in ruminants using spot-sampling estimates compared to total collections (Van't Klooster et al., 1969).

Few data are found in the literature comparing spot-sampling and total collection methods to estimate digesta flow. Van't Klooster et al. (1969) found that direct measurements agreed with indirect estimates based on PEG and Cr₂O₃ in sample aliquots of digesta collected by total collection; however, estimates based on marker concentrations in spot-samples showed large 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 estimates based on spot-sampling (500 ml of duodenal digesta at 6-h intervals for 48 h) and that from 36-h total collection in steers (total collection estimations were corrected for 100% duodenal recovery of Cr_2O_3).

Regression analysis showed diurnal patterns for digesta and Cr_2O_3 flow rates (P<.04; P<.005) with the grain diet (figure 1). Commencing at about the morning feeding, digesta flow increased to a peak just before the afternoon feeding. A second peak was noted about midnight, followed by a low flow before daylight. Chromium oxide showed a similar pattern; however, flow rate decreased more rapidly following the midafternoon peak. Regressions were not significant for DM and lignin flow rates on the grain diet, nor for any of the flow parameters on the forage diet.

Faichney 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. Concentrations of Cr_2O_3 in the digesta showed a bimodal pattern (P<.04) for both diets. However, the shape of the curve was different for each diet (figures 2 and 3), suggesting an interaction between marker and diet. Lignin concentrations in the digesta showed a regression with the forage diet (P<.05), but not with the grain



Figure 1. Flow pattern of duodenal digesta and Cr_2O_3 based on regression analysis of 2-h measurements of duodenal digesta from three steers fed a grain diet. Feeding times were 0730 and 1530 h. Digesta: Y = 3,231.87 - 163.96 H + 43.77 H² - 2.86 H³ - 5.71 H⁴ × 10⁻², RSD = 1,233 ml. Cr_2O_3 : Y = 660.53 - 159.60 H + 31.95 H² - 1.97 H³ + 3.78 H⁴ × 10⁻², RSD = 255 mg.



Figure 2. Flow pattern of Cr_2O_3 and lignin (LIGN) concentrations based on regression analysis of 2-h measurements of duodenal digesta from three steers fed a grain diet. Feeding times were 0730 and 1530 h. Cr_2O_3 : Y = 115 + 8.731 H + .189 H² - .058 H³ + .157 H⁴ × 10⁻², RSD = 40 mg/liter. LIGN: Y = 3.09 + .536 H - .115 H² + .008 H³ - .167 H⁴ × 10⁻³, RSD = 1.4 g/liter, P = .43.

diet. With the forage diet, concentrations of Cr_2O_3 and lignin in the diet tended to decrease in the morning and increase throughout the later afternoon and night, in contrast to Cr_2O_3 on the grain diet. A similar pattern was described by Corbett et al. (1979) for organic matter flow rates in grazing sheep.

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. In contrast, the data of Leao et al. (1978), with sheep fed a sorghum grain diet, indicated greater flow of duodenal contents during the day (from 0800 to 2000) than at night. The period from midnight to 0800 was always a period of low flow.

No consistent pattern was found for DM flow rates. However, there was a high positive relationship (P<.001; r = .7) between DM and lignin concentrations in digesta of steers fed the forage diet. When the steers were on the grain diet, DM was somewhat more positively correlated with Cr_2O_3 (P<.01; r = .3) than with lignin (r = -.004).

It is not clear if the total collection procedures would be the cause for more erratic variation and less consistent flow patterns than in intact animals. However, the patterns observed might be considered as indicative of cyclical fluctuations in metabolic and digestive phenomena in ruminants, coupled with the resting/



Figure 3. Flow pattern of $Cr_2 O_3$ and lignin (LIGN) concentrations based on regression analysis of 2-h measurements of duodenal digesta from three steers fed a forage diet. Feeding times were 0730 and 1530 h. $Cr_2 O_3$: Y = 156 - 2.043 H + 1.121 H² + .093 H³ - .19 H⁴ × 10⁻², RSD = 41 mg/liter. LIGN: Y = 2.213 + 2.513 H - .41 H² + .023 H³ - .421 H⁴ × 10⁻³, RSD = 2.38 g/liter.

activity cycle, but further study is necessary to substantiate this assumption. The ratios of DM to Cr_2O_3 or lignin suggest that flow of the entire particulate phase may be different from that of a particular component of this solid phase, and that a marker not attached to any component could more correctly estimate flow of entire digesta.

These data suggest that duodenal flow estimates will be somewhat greater, and ruminal DM disappearance somewhat lower, using lignin or Cr_2O_3 ratios rather than total collections because of incomplete marker recovery in the duodenum. Although not conclusive, a possible diet \times marker interaction may occur with lignin, Cr_2O_3 and the type of diets fed in this study. Due to day-to-day variation, total collection must be long enough to minimize this variation.

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