INDIGESTIBLE MARKERS: METHODOLOGY AND APPLICATIONS IN RUMINANT NUTRITION

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CHAPTER I

INTRODUCTION

Digestion of feedstuffs by the ruminant animal may be estimated by relating feed dry matter consumed to fecal dry matter excreted. This procedure does not permit one to evaluate the contribution of each separate segment of the ruminant's gastrointestinal tract to total digestion. Maximal utilization of feedstuffs by ruminant animals will occur only when each portion of the ruminant's digestive system functions in concert so that maximal quantities of energy and essential nutrients are absorbed. This does not mean that each digestive compartment of the ruminant must function at its maximum capacity. Indeed, feed fractions such as starch and protein of high biological value can be utilized with greater energetic and biological efficiency when digestion is delayed to the intestine and these nutrients escape fermentation in the rumen. Measurement of the dynamic attributes of the ruminant's digestive processes require knowledge of digesta flow. Digesta flow markers are used in such studies.

The research of this dissertation was conducted to evaluate the accuracy of: (1) liquid, and (2) particulate matter digesta flow markers, (3) to monitor rates of passage of individual feed components in the tract of cattle fed roughage or concentrate ration, (4) to develop and apply techniques to reduce the number of samples required to estimate ruminal volume and dilution rate, (5) develop and apply a marker technique to quantitate direct ruminal escape, and (6) to use

digesta flow markers for study of the reason for interactions between roughage and concentrate fractions of a ration.

These six objectives are addressed in individual chapters. Chapters are prepared as manuscripts in the style required by specific journals to facilitate publication of experimental results.

CHAPTER II

THE USE OF WATER SOLUBLE MARKERS TO MEASURE RUMEN LIQUID VOLUME AND DILUTION RATE

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Summary

The potential utility of five water soluble markers (WSM) for measuring rumen liquid volume and dilution rate was examined in nine *in vitro* and *in vivo* experiments. WSM tested included polyethylene glycol (PEG) and ethylenediaminetetraacetic acid (EDTA) complexes of Cr, Co, Fe, and Yb. Feedstuffs absorbed variable amounts of distilled H_2O and autoclaved rumen fluid with roughage imbibing greater ($P<.05$) amounts of fluid than feeds high in starch. Liquids of most feeds reached equilibrium with WSM in about 20 min. Adsorbed liquid comprised from 20 to 80% of the total ruminal liquid. Whole shelled corn grain, ground corn, and soybean meal appeared to exclude 1 to 14% of WSM while imbibing water. Binding of EDTA complexes was measurable only with cottonseed hulls. Cottonseed hulls also reduced the fluid concentration of PEG due to some water soluble substance present. WSM did not influence ($P>.10$) digestibility of dry matter *in vivo* with steers fed a 90% roughage or a 90% concentrate ration. Fecal recovery of WSM ranged from 96.8 to 99.7%. EDTA complexes were detected in urine at levels equal to 1.2 to 3.4% of

the EDTA complex fed. PEG was not detected in the urine. WSM did not produce different dilution rate (P=.65) and volume (P=.29) estimates when ruminally administered to steers fed rations containing 80% whole shelled corn, 90% chopped alfalfa hay, or 90% prairie hay. Ruminal liquid measured by hand removal was about 4% less than volumes estimated by WSM. Compared with steers fed an 80% whole shelled corn ration, steers fed equal dry matter of a 90% chopped alfalfa hay diet had 29% more dry matter in the rumen, 80% greater ruminal liquid dilution rate and 23% larger liquid volume for 22% greater rate of liquid flow from the rumen. With both diets, approximately half of the total fluid in the rumen did not drain readily from solids but the percentage of total ruminal water associated with solids varied from 27 to 86% among steers.

Dilution rate of ruminal liquid during a feeding cycle was examined with six steers fed once daily a 90% chopped alfalfa hay diet at 1.8% of body weight. Dilution rate estimated with Co EDTA increased by 166% during the four hours after feeding. All WSM examined appeared suitable for estimating rumen liquid volume and dilution rate for the diets tested.

Introduction

Water soluble markers (WSM) have been used extensively to estimate rumen fluid volume and liquid dilution rate since Sperber et al. (1953) first demonstrated the potential for such measurements with polyethylene glycol (PEG). Precise estimation of these two rumen parameters is vital for several reasons. They are needed to estimate metabolite production (Bauman et al., 1971). These parameters influence the length of time available for ruminal fermentation, feed intake, and digestibility, as well as efficiency of bacterial growth (Isaacson et al., 1975). That

such measurements are needed is indisputable.

Liquid markers must be water soluble, indigestible, non-absorbable, and innocuous *in vivo*. These factors, however, do not ensure that the flow or marker dilution rate will mimic omasal outflow of ruminal liquid. Ruminal liquid exists in several pools. Some liquid is free, whereas other liquid is closely associated with particulate matter. Associated liquid may reside on the surface, be intracellular, or bound to cell components. Association of liquid with particulate matter complicates interpretation of outflow estimates. Particulates flow out of the rumen at a rate substantially slower than fluid. Association of liquids with solid material creates multiple pools as free fluid moves separately from liquid associated with particulate matter. Failure of WSM to rapidly equilibrate with the total ruminal liquid pool or adsorption of the WSM by particulate matter would make liquid flow rate and volume estimates erroneous indicators of total ruminal liquid.

Problems with various markers have been pointed out. Czerkawski and Breckenbridge (1969) and Alexander *et al.* (1969) have suggested that polyethylene glycol (PEG), due to its large molecular weight, was excluded from the intratissue water of feedstuffs. PEG adsorption by particulate matter also has been reported (Sutherland *et al.*, 1963). In contrast to PEG exclusion, the chromium chelate of ethylenediaminetetraacetic acid (Cr-EDTA), due to its low molecular weight, appeared to be distributed more thoroughly with intratissue water of feedstuffs (99%; Goodal and Kay, 1973). Cr EDTA also has been suggested to bind to ruminal particulate matter (Warner, 1969). Unreasonable flow rate estimates in sheep (Czerkawski and Patterson, 1968; Czerkawski and Breckenbridge, 1969; Sperber *et al.*, 1953) and volume estimates in sheep (Goodal and Kay,

1973) and cattle (Poppi, 1980) have prompted criticism of water soluble markers.

The following experiments were conducted to study certain ruminal parameters of five WSM. These parameters included dispersion kinetics, pool distribution, perturbation equilibrium kinetics, absorption and adsorption by solids, dilution rate similarity, and volume prediction accuracy. In addition, the influence of these WSM on dry matter disappearance was evaluated in vitro and in vivo.

Materials and Methods

Preparation and analysis of WSM

Disodiumethylenediaminetetraacetic acid (EDTA) complexes of cobalt (Co) and chromium (Cr) and iron (Fe) were prepared as specified by Dwyer et al. (1955) and Binnerts et al. (1968), respectively. Ytterbium (Yb) was complexed with EDTA by boiling a water solution containing 0.18 molar Yb ($YbCl_3$) and 0.24 molar EDTA (Na_2H_2EDTA) for six hours. Non-complexed Yb was removed by addition of 50 g cottonseed hulls per liter. Excess EDTA was neutralized with $CaCl_3$ one hour prior to administration of the dose; 20,000 molecular weight polyethyleneglycol (PEG) was utilized.

In vitro experiments

Particulate matter liquid space. Triplicate fifty gram samples of chopped prairie hay, chopped alfalfa hay, cottonseed hulls, whole corn, ground corn, and soybean meal were placed in 250 ml Erlenmeyer flasks of either autoclaved rumen fluid or distilled water. After incubating at 38 C for 24 hours, flasks were covered with two layers of cheesecloth and flasks were inverted and the liquid allowed to drain for thirty minutes.

Retained samples were analyzed for dry matter to estimate the amount of liquid entrapped.

WSM liquid space. Feed samples used in the liquid space study were added to 250 ml erlenmeyer flask containing 150 ml of each of the WSM in distilled water. Amounts of feed added were sufficient to absorb approximately 75% of the initial liquid volume. Samples were incubated at 39 C for 36 hours. Residual liquid was then sampled and analyzed for WSM concentration. Amounts of absorbed liquid were calculated by covering flasks with two layers of cheesecloth, inverting for thirty minutes to allow drainage, and calculating retained liquid by difference.

To further examine WSM feedstuff equilibria, samples (8 g) of chopped alfalfa, chopped prairie hay, cottonseed hulls, cellulose, whole corn, ground corn, and soybean meal were placed in triplicate 50 ml centrifuge tubes and 40 ml of *in vitro* solution (15 ml of autoclaved rumen inoculum and 25 ml artificial saliva McDougalls 1948) added. Rumen fluid used for inoculation was obtained from two steers--one 517 kg fistulated steer fed 6 kg of an 80% concentrate diet twice daily, and one 450 kg fistulated steer fed 4.5 kg alfalfa hay twice daily. Fluid was strained through cheesecloth and equal volumes of filtrate from each steer was mixed and autoclaved. Incubation solutions were spiked with WSM and contained either Cr EDTA (1.1 ppm), Co EDTA (0.97 ppm), Yb EDTA (1.03 ppm) or PEG molecular weight 20,000 (2000 ppm). Following incubation for 96 hours at 39 C, the supernatent was decanted, centrifuged, and analyzed for WSM.

WSM perturbation kinetics. Feed samples used above were placed in 1-L Griffin beakers containing 350 ml of each WSM in distilled water. Amounts of samples used were sufficient to absorb approximately 75% of

the initial liquid. Samples were allowed to equilibrate for 24 hr at 39 C. After equilibration, the liquid pool was sampled and the system was perturbed by addition of 350 ml distilled water. Beaker contents were stirred continuously. Samples of the supernatant liquid were obtained at 0.5, 1.0, 2.0, 4.0, 8.0, 16.0, and 32.0 minutes and 1.0, 2.0, 6.0, 12.0, and 24.0 hours after perturbation and analyzed for WSM.

Fermentation and WSM. Samples (2 g) of glucose, corn starch, and cellulose were placed in 50 ml centrifuge tubes containing the inoculum used above to further examine WSM feedstuff equilibria. Non-autoclaved rumen fluid was substituted for autoclaved rumen fluid. The incubation solutions were spiked with WSM and contained either Cr EDTA (1.2 ppm), Co EDTA (1.1 ppm), and Yb EDTA (1.0 ppm), or PEG with a molecular weight 20,000 (1000 ppm). Following 96 hour incubation at 39 C, the samples were centrifuged (20,000 g, 25 min) and the supernatant fluid analyzed for WSM.

In vivo experiments

Experiment 1. Six mature ruminally cannulated steers (581 kg) were fed a supplemented (table 1) prairie hay ration (table 2) at a level of intake of approximately 1.6% of body weight. Steers were confined in metabolism stalls during the 21-day preliminary and 2-day collection periods. Rations were fed twice daily on days 1-14 and at four equally spaced time intervals per day during the remaining 7 days. Water was available to the animals at all times. WSM solutions were composited and the steers ruminally dosed on day 22 at a time equally spaced between feedings. Dosing was via ruminal cannula. The WSM solution was allowed to flow in through a tygon tube inserted in the middle strata of the dorsal sac of the rumen. The procedure was completed within two minutes.

TABLE 1. RATION SUPPLEMENT FOR EXPERIMENTS 1-4.

Ingredient	%
Soybean meal	68.6
Alfalfa dehy	14.0
Ground corn	6.0
Dicalcium phosphate	3.1
Calcium carbonate	2.0
Salt	2.7
Potassium chloride	3.5
Trace mineral	0.1
Vitamin A, 30,000/g	0.01
Vitamin D, 15,000/g	0.01

TABLE 2. ANALYSIS OF RATION INGREDIENTS USED IN EXPERIMENTS 1-4.

Experiment	Feedstuff	D.M.	C.P.	NDF[a]	ADF[b]
			%		
1	Prairie hay		6.4	59.3	44.3
2,3,4[c]	Whole corn	89.7	10.4	14.7	7.3
	Alfalfa hay	90.8	17.4	51.2	33.9

[a]Neutral Detergent Fiber (Goering and Van Soest, 1970).
[b]Acid Detergent Fiber (Goering and Van Soest, 1970).
[c]In trials 2, 3 and 4, rations were comprised of either 80% whole corn, 10% alfalfa hay, plus 10% supplement or 90% alfalfa hay plus 10% supplement.

Samples were obtained at .0, .2, .4, .6, 1, 2, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, and 23 hours after dosing from a site just dorsal to the infusion site. Immediately following collection, samples were placed in sealed polyethylene containers and frozen for subsequent analysis of WSM. Twenty-four hours after dosing, total rumen contents were manually evacuated. Coarse solids plus contained liquids were placed in one container while fine solids plus absorbed liquids and free liquids were dipped out with a glass beaker and placed in a separate container. These two fractions were sampled and dry matter of samples determined.

Experiment 2. The six steers utilized in experiment 1 were allotted to two rations--80% whole corn, 10% alfalfa, and 10% supplement; or 90% alfalfa and 10% supplement--(table 2) in a switchback design. Housing, adaptation to rations, feeding, ruminal pulse dosing, sampling and evacuation schedules were described for trial 1.

Experiment 3. Six growing ruminally cannulated steers (mean initial weight 296 kg) were fed the 90% alfalfa hay and 10% supplement ration at a level equal to 1.8% of body weight once daily. The ration was consumed within 2 hours after feeding with little waste. Steers were individually housed in metabolism stalls for the 15 day experiment. The first 12 days were used for adaptation to the ration. On day 13, 12 hours before feeding the steers were dosed with Co EDTA as described previously. Rumen samples were obtained at hourly intervals for 10 hours before feeding and at 30 min intervals for 4 hours after feeding and at hourly intervals for the subsequent 6 hours. Samples were stored in polyethylene bottles at -15 C until analyzed for Co.

Experiment 4. Four growing steers (332 kg) were allotted to an 80% whole corn, 10% alfalfa, 10% supplement ration; or a 90% alfalfa hay, 10%

supplement ration with or without WSM in a 4 X 4 latin square. Steers were confined in metabolism stalls during successive 14-day preliminary and 5-day collection periods. Total fecal and urine collections were made daily, mixed, and aliquots saved for analysis. Each steer was fed 5.6 kg of the appropriate experimental diet in two equal portions twice daily. Water was available to the animals at all times.

Results and Discussion

In vitro experiments

Particulate matter liquid space. The capacity to hold distilled water and autoclaved rumen fluid differed ($P<.05$) among feeds (table 3). Although feeds had the identical ranking in liquid holding capacity with distilled water and autoclaved rumen fluid, the samples retained a mean of 6% more rumen fluid. Roughages held more fluid than the concentrates tested. The data indicate that partitioning of ruminal liquid between the free and solids liquid phase would be influenced by the quantity of solids and liquid present in the rumen as well as the type of solids present.

Solids-liquid space available to WSM. Absorption and/or adsorption of 75% of the free liquid phase by solids suspended in distilled water had little effect upon WSM concentration in the free liquid (table 4). This indicates that WSM and liquid were imbibed proportionately by these feeds. If the particulate matter had excluded WSM from the liquid space, then WSM concentration would have increased by approximately 400% in the residual free liquid phase. These conclusions are based on the assumption that WSM binding by the particulate matter did not occur. Any decrease in the supernatant WSM concentration is evidence for such

TABLE 3. IN VITRO LIQUID SPACE[a].

Liquid	Prairie hay	Alfalfa hay	Cottonseed hulls	Whole corn	Ground corn	SEM
			Uptake, g fluid/g sample DM			
H_2O	3.21[b]	4.53[c]	2.64[d]	1.16[e]	1.26[f]	0.09
Autoclaved rumen, fluid	3.47[b]	4.91[c]	2.83[d]	1.01[e]	1.32[e]	0.10

[a]Values represent the mean of three samples.

[b,c,d,e,f]Means in a row with different superscripts differ ($P<.05$).

TABLE 4. LIQUID SPACE IN VARIOUS FEEDS AVAILABLE TO WSM.

Feedstuff	% Liquid absorbed by solids[a]	% Change in WSM concentration in free liquid				
		Cr	Co	Fe	Yb	PEG
None	0.0[b]	0.1[b]	0.0[b]	0.0[b]	0.0[b]	- 0.1[b]
Prairie hay	75.4[c]	0.0[b]	0.1[b]	-0.1[b]	0.0[b]	0.2[b]
Alfalfa hay	76.2[c]	0.1[b]	0.2[b]	0.0[b]	0.3[b]	0.1[b]
Cottonseed hulls	75.1[c]	-0.3[b]	-0.2[b]	0.1[b]	-0.2[b]	-82.4[c]
Soybean meal	73.6[c]	1.2[b]	0.9[b]	1.4[b]	1.1[b]	2.0[c]
Whole corn	73.1[c]	12.7[b]	15.6[b]	12.8[b]	13.7[b]	14.6[b]
Ground corn	74.7	3.7[b]	4.4[bc]	5.1[c]	4.3[bc]	5.6[c]
Pooled SEM	5.9	2.4	3.7	3.8	1.9	4.7

[a]All values are the mean of three observations.

[b,c]Means in a row having different superscripts differ (P<.05).

binding. Indeed, binding of PEG by cottonseed hulls is suggested. In a subsequent trial, incubation of PEG with only the water soluble extract of cottonseed hulls had a similar effect on chemically determined PEG. This indicates that some water soluble factor in cottonseed hulls interferes with the turbidometric assay for PEG. PEG has been observed to be precipitated by certain feeds high in tannins (Kay, 1969) and to bind to particulate matter (Sutherland et al., 1963). Whether the cottonseed hull effect observed here is the result of precipitation of PEG or formation of a water soluble but undetectable form of PEG has not been determined.

Recoveries of WSM incubated with feedstuffs and innocula containing autoclaved rumen fluid are shown in table 5. The feedstuffs in this study provided 20% dry matter to the mixture. That dry matter exceeds most estimates of dry matter of rumen contents. This quantity of solids should reduce WSM concentration in the free liquid phase if binding by solids occurs and increase its concentration if WSM are excluded while H_2O is absorbed. In general, feedstuffs did not significantly alter WSM concentration in the liquid pool following the 96 hr incubation and centrifugation (20,000 g, 20 min). The data indicate that for most feeds binding or exclusion of WSM, if it occurs, is small. Exceptions to this include cottonseed hulls which appeared to have affinity for Cr-EDTA ($P<.05$) and Yb-EDTA ($P<.15$) and also whole shelled corn grain which quantitatively excluded ($P<.05$) WSM from its absorbed liquids. Cottonseed hulls, as shown previously, interfere with the PEG analysis and lowered detectable PEG in the supernatant. Simultaneous exclusion and binding of marker by feedstuffs could negate any change in supernatant marker concentration, however.

Ruminal feedstuff WSM binding and/or exclusion effects would be dependent upon: 1) the net quantity of WSM bound by or excluded from feedstuffs, 2) quantity of feedstuffs present in the rumen, 3) feed consumption post dosing, and 4) relative rates of feedstuff and liquid passage out of the rumen. At low concentrations of WSM in the supernatant, the overall effect of WSM binding or exclusion by feedstuffs appear minimal. Increasing the concentration of WSM would not be expected to influence exclusion. However, increasing WSM concentration could increase the quantity of marker bound to feedstuffs until such binding sites are saturated. Whether the proportion of WSM bound to feedstuffs is altered at higher WSM concentrations is not known. If feedstuff binding sites were saturated in this study, the quantity of marker bound by feedstuffs would be negligible even for cottonseed hulls and Cr-EDTA. If 10 g Cr as Cr-EDTA were dosed and binding capacity of Cr-EDTA by cottonseed hulls is 0.02 ppm, as calculated from table 5, then the quantity of ruminal cottonseed hulls needed to reduce the ruminal dose by 1% would far exceed rumen capacity.

Fermentation and WSM

The effect of incubating WSM with feedstuffs and innocula containing non-autoclaved rumen fluid is presented in table 6. EDTA-WSM complexes recovered in the free liquid, for the feeds which did not bind or exclude WSM in table 5 (including glucose and ground corn), was negatively correlated (R^2=.95) with digestibility. For each percentage increase in feedstuff digestibility, marker recovery was reduced by .15%. Similarly, PEG recovery in the free liquid was correlated (R^2=.76) with feedstuff digestibility. For each percentage increase in feedstuff digestibility, PEG recovery in the free liquid was reduced by .07%. These data indicate

TABLE 5. FEEDSTUFF--WSM EQUILIBRIA IN AUTOCLAVED RUMEN INNOCULA.

	Cr-EDTA	Co-EDTA	Yb-EDTA	PEG
	% recovery in free liquid[2]			
Blank	103.0[b]	103.0[b]	102.0[a]	104.5[a]
Alfalfa	101.0[b]	99.0[b]	100.0[a]	102.0[a]
Prairie hay	102.0[b]	101.0[b]	101.0[a]	104.0[a]
Cottonseed hulls	83.0[c]	101.0[b]	96.0[a]	27.2[b]
Cellulose	100.0[b]	100.0[b]	102.5[a]	97.0[a]
Whole corn	118.0[d]	118.5[c]	119.0[b]	121.0[c]
Ground corn	107.0[b]	103.5[b]	108.0[a]	110.0[a]
Soybean meal	97.0[b]	98.5[b]	99.5[a]	104.5[a]
Pooled SE	10.5	6.0	7.0	12.3

[a]% recovery = ({weight liquid X marker ppm})/ug inital marker) X 100.

[b,c,d]Means in a column, within a marker heading, with unlike superscripts differ significantly ($P<.05$).

TABLE 6. FEED AND FERMENTATION EFFECTS ON WSM CONCENTRATION.

	Cr-EDTA	Co-EDTA	PEG
	% Recovery in free liquid[a]		
Blank	99.0^{bc}	99.7^{bc}	100.2^{a}
Glucose	1.7^{d}	86.7^{d}	98.3^{a}
Ground corn	94.7^{d}	88.7^{d}	99.2^{a}
Soybean meal	100.3^{b}	94.3^{cd}	97.8^{a}
Cellulose	102.3^{b}	99.7^{bc}	102.7^{a}
Alfalfa	100.3^{b}	100.0^{b}	104.6^{a}
Prairie hay	100.7^{b}	99.7^{bc}	103.6^{a}
Cottonseed hulls	95.0^{cd}	101.7^{b}	47.2^{b}
Pooled SEM	3.3	4.0	9.4

[a]% Recovery = ({weight liquid X marker ppm}/Mg initial marker) X 100

[b,c,d]Means in a column within a marker heading with unlike superscripts differ significantly ($P<.05$)

that either: 1) microorganisms metabolize the WSM destroying PEG and freeing metal from EDTA complexes which then binds to feed or is retained by the microbes, 2) that microbial fermentation creates feedstuff binding sites which will bind WSM, 3) that microorganisms absorb and concentrate but do not metabolize WSM, or 4) that a metabolite of fermentation forms an insoluble complex with WSM. The most feasible explanations are the latter two. Metabolism of WSM would be expected even in the absence of feedstuffs, but when feedstuffs were omitted no loss of WSM occurred. Feed digestion by microorganism could create new feed binding sites, but this would be unlikely for glucose. Glucose produced the greatest depression in WSM recovery. Increasing the digestible dry matter content of the incubation tubes would allow a greater microbial growth, and end-product accumulation of metabolites. These are both correlated with the decreased recovery of WSM. If the unrecovered WSM are still in the fluid phase, perhaps centrifugation at lower speeds to separate solids and liquids would increase recovery. If the unrecovered WSM is in the solid phase, *in vivo* marker disappearance from liquid phase would be misleading especially with high concentrate diets. Additional work is needed to understand behavior of WSM *in vivo*

WSM perturbation

Perturbation by addition of distilled H_2O to a steady state liquid-feed system instantly dilutes marker concentration in the free liquid phase (figure 1). If all fluid was in equilibrium with the added fluid, a new steady state concentration would be obtained rapidly. However, if some fluid is not in equilibrium with the pool to which water is added, time will be required to attain a new equilibrium. For the feed-liquid systems examined in this experiment, the second equilibria was established

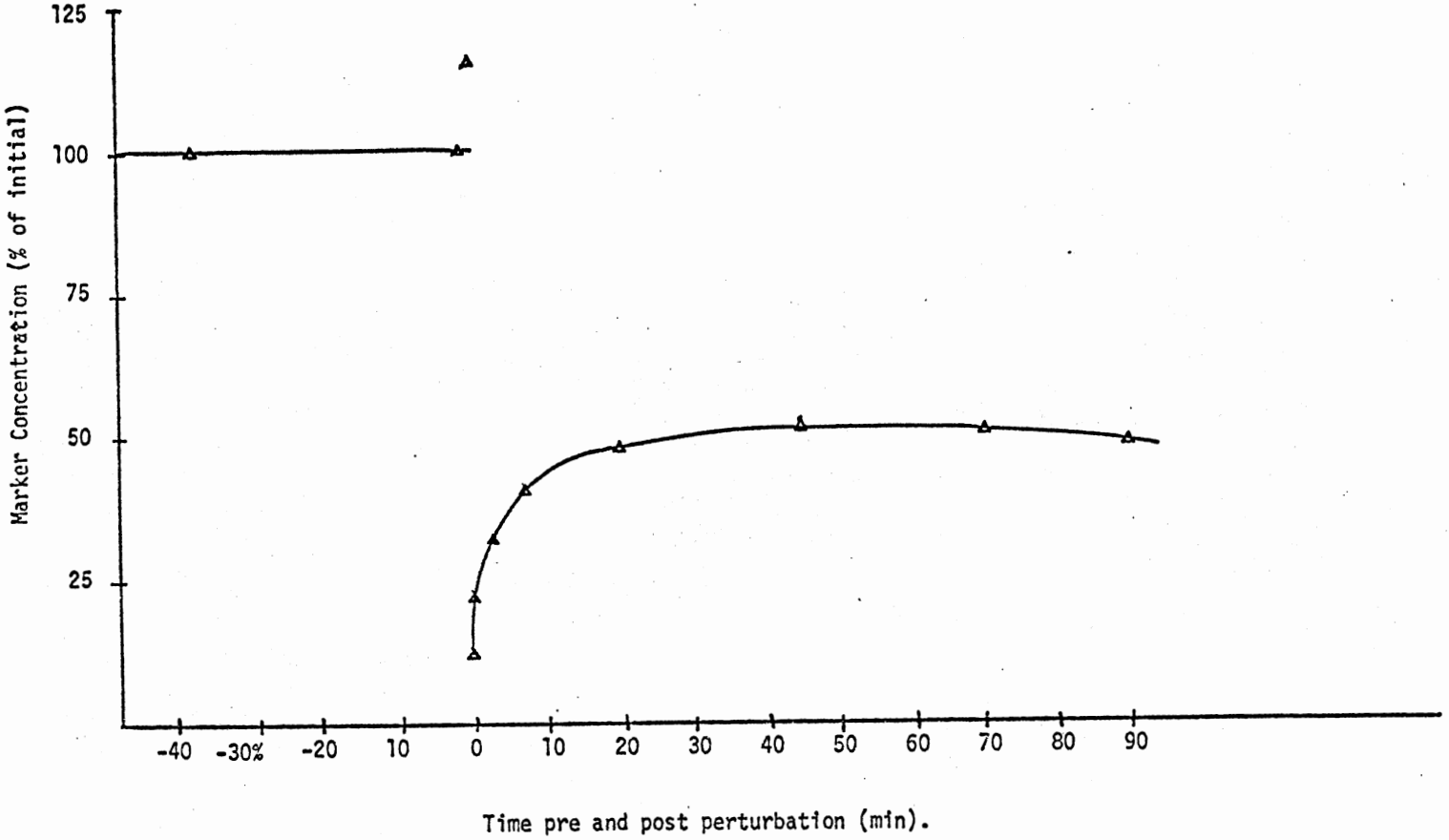

Figure 1. Typical markers perturbation kinetics.

within approximately 20 minutes after water addition. This contrasts with less than 30 seconds for distilled water not containing feed. Diffusion of WSM and presumably of liquid in and out of particulates delays equilibration. Concentrates, protein supplement and the roughages examined required similar times to restore equilibration. Results also indicate that rumen sampling immediately after water consumption by steers will cause a overestimation of fluid volume due to immediate dilution of the non-particulate fluid.

In vivo experiments

Experiment 1 and 2. Rumen volume as well as percent of free liquid in the rumen differed markedly between animals. But, estimates of rumen volume or dilution rate did not differ significantly ($P=.46$) between WSM (table 7). This suggests that all these markers tended to be biologically similar. Ruminal volume estimates measured by total evacuation were lower ($P<.05$) than volume estimates extrapolated from WSM concentrations. This discrepancy, which averaged 4.4%, could be the result of several factors. These include: 1) less than instaneous marker mixing, 2) binding of WSM by ruminal solids, 3) deleterious effects of marker on feed and water consumption or rate of passage, or 4) metabolism of WSM by rumen microorganisms. These factors will be considered individually. First, ruminally dosed WSM were assumed to be completely mixed only when their ruminal concentrations had reached a maximum. Only the decline after the peak was used for extrapolation to ruminal volume. The time required for marker concentration to peak ranged from .20 to 1.77 hours among steers and was correlated ($R=.67$) with the percentage of total ruminal liquid present with the ruminal solids. For each percentage more liquid associated with ruminal solids, mixing time increased by 1.6%.

TABLE 7. RUMINAL LIQUID VOLUME, FREE LIQUID, SOLIDS CONTAINED LIQUID, WSM ESTIMATED LIQUID VOLUME AND WSM DILUTION RATES FOR EXPERIMENTS 1 AND 2.

Experiment	Ration	Animal	Dry matter kg.	Liquid (l)	Calculated Volume at Time Zero					Dilution Rate (%/hr)					Mean Liquid outflow (l/hr)					Liquid phase (%)	
					Cr	Co	Fe	Yb	PEG	Cr	Co	Fe	Yb	PEG	Cr	Co	Fe	Yb	PEG	Free	Solids
1		1	6.0	71.0	69.5	68.8	88.1	70.2	70.1	4.3	4.8	5.3	5.1	4.9	3.1	3.4	3.8	3.6	3.5	73.0	27.0
	90%	2	8.1	48.6	51.2	56.3	55.6	52.7	53.4	4.6	4.8	5.3	5.2	5.3	2.2	2.3	2.6	2.5	2.6	26.0	74.0
	prairie	3	10.5	55.4	72.5	68.1	71.1	82.3	76.7	4.5	4.0	4.8	4.8	5.0	2.5	2.2	2.7	2.7	2.8	13.3	86.7
	hay, 10%	4	6.7	55.2	56.3	59.3	58.7	66.3	58.4	5.4	4.7	5.0	5.7	5.1	3.0	2.6	2.8	3.2	2.8	47.6	52.4
	supplement	5	9.2	83.0	72.6	71.1	78.6	79.4	79.6	6.6	7.0	7.8	6.7	6.8	5.5	5.8	6.5	5.6	5.6	44.2	55.8
		6	8.3	62.7	63.4	65.1	62.7	62.3	64.2	6.2	6.0	6.3	6.4	5.9	3.9	3.9	4.0	4.0	3.7	40.1	59.9
	Means		6.3	62.7	64.3	64.8	69.1	68.9	67.1	5.3	5.2	5.8	5.7	5.5	3.4	3.4	3.7	3.6	3.5	40.7	59.3
2-P1		1	6.9	52.4	53.4	51.7	52.5	53.1	52.6	3.1	2.9	3.4	3.3	3.0	1.6	1.5	1.8	1.7	1.6	50.3	49.7
P1	80% whole	2	5.3	40.4	39.2	40.1	40.7	38.2	39.6	2.7	3.2	3.1	3.6	3.3	1.1	1.3	1.3	1.5	1.3	46.4	53.6
P1	corn, 10%	3	6.4	41.9	41.3	42.7	44.6	42.4	43.2	2.6	2.9	2.7	3.0	3.1	1.1	1.2	1.1	1.3	1.3	42.3	57.7
P2	alfalfa	4	5.9	41.5	43.6	41.2	42.1	43.1	41.9	3.4	3.6	3.2	3.5	3.1	1.4	1.5	1.3	1.5	1.3	51.7	48.3
P2	hay, 10%	5	7.7	53.4	54.7	52.6	57.3	53.2	54.8	4.1	4.2	3.9	4.0	4.3	2.2	2.2	2.1	2.1	2.3	56.8	43.2
P2	supplement	6	7.0	50.0	50.2	53.4	51.3	54.6	51.7	4.5	4.3	4.6	4.2	4.7	2.3	2.2	2.3	2.1	2.4	57.4	42.6
	Means		4.2	46.6	47.1	47.0	48.1	47.4	47.3	3.4	3.5	3.5	3.6	3.6	1.6	1.7	1.7	1.7	1.7	50.8	49.2
2 P2		1	8.6	73.9	75.1	75.7	74.6	77.2	76.1	5.4	5.8	5.7	5.6	5.9	4.0	4.3	4.2	4.1	4.4	60.3	39.7
P2	90%	2	5.9	50.7	53.4	52.7	52.1	53.4	55.6	6.1	6.4	6.3	6.6	6.4	3.1	3.2	3.2	3.4	3.2	54.1	45.9
P2	alfalfa	3	8.5	61.2	65.6	63.2	64.1	64.0	64.9	6.0	6.2	6.0	6.5	6.3	3.7	3.8	3.7	4.0	3.9	47.6	52.4
P1	hay, 10%	4	6.4	50.8	54.2	55.1	53.6	51.9	52.4	5.8	5.7	6.0	5.9	6.0	3.0	2.9	3.1	3.0	3.1	50.1	49.9
P1	supplement	5	7.5	49.0	51.5	53.8	54.9	52.7	56.8	6.1	6.3	6.2	6.1	5.9	3.0	3.1	3.0	3.0	2.9	42.7	57.3
P1		6	8.6	59.6	62.7	66.3	60.1	62.3	63.2	7.3	7.7	7.8	7.6	7.6	4.4	4.6	4.7	4.5	4.5	47.6	52.4
	Means		5.4	57.5	60.4	61.1	59.9	60.3	61.5	6.1	6.4	6.3	6.4	6.4	3.5	3.7	3.7	3.7	3.7	50.4	49.6

Instaneous marker mixing is theoretically required for accurate volume estimation by regression. Any time delay in marker mixing and disuniform marker outflow can cause erroneous volume estimates. The magnitude of error is dependent upon the difference existing between the outflow rates of marker versus liquid during the equilibration time period. A slower rate of marker outflow relative to actual ruminal liquid dilution rate will result in overestimation of rumen volume while a faster rate of outflow would cause underestimation. Such errors may be influenced by rumen size, type and quantity of ruminal solids (ration intake) and dosing technique. Secondly, with the exception of whole shelled corn, feedstuffs used in these studies failed to alter recovery of WSM _in vitro_. Therefore, binding _in vivo_ would be surprising. Exclusion of WSM by whole shelled corn would cause underestimation of ruminal volume and would be dependent upon the quantity of whole corn in the rumen. Thirdly, no effect of WSM on feed or water intake was apparent. Fourthly, binding to fermentation metabolites or uptake by microorganisms is suspected _in vitro_ and may have occurred _in vivo_. Such an occurrence _in vivo_ would reduce WSM concentration at time zero if these fractions appear in the solid and not the free liquid phase. Underestimation of WSM at zero time would result in overestimation of volume.

Numerous conflicting reports concerning marker accuracy for predicting volume for steers fed high roughage rations are found in the literature. Czerkawski and Breckenbridge (1969) and Alexander _et al._ (1969) observed PEG to be excluded from feedstuffs _in vitro_ and _in vivo_. This resulted in underestimations of rumen volume. In contrast, Poppi (1980) observed PEG to overestimate rumen volume (15.8%) and presumed the effect to be due to feedstuff binding of PEG. Sutherland _et al._

(1962) noted binding of PEG by ruminal solids and Warner (1969) reported that Cr-EDTA is complexed by ruminal particulate matter. However, Bauman (1971) observed PEG to be an excellent marker for predicting rumen volume. Although, simultaneously dosing of WSM resulted in a slight overestimation of ruminal volume in our trials, the error was only 4.4%. With the high concentrate ration, the mean was only 1.7% above evacuated volume compared with 6.1% for roughages. Differences in the magnitude of error could be due to the differential absorption of H_2O and WSM rather than to greater marker accuracy under concentrate feeding conditions. Nevertheless, this magnitude of error would appear to be a small sacrifice for the labor saved in volume estimation with markers. Regressions of calculated volumes on measured volumes were high ($R>.89$) with all markers tested. Although the markers evaluated appear biologically similar, additional work is needed to determine sources of errors with markers dosed singly so that the reported discrepancies in the literature may be more fully understood.

Ruminal liquid volume, dilution rate and total dry matter in the rumen were 23%, 80%, 29% greater ($P<.05$) with the roughage diet than with the concentrate diet in trial 2 (table 8). This agrees with observations of other workers that adding roughage to a diet increases dilution rate of liquid. Total liquid outflow, the multiple of volume and dilution rate, was more than twice as great with the alfalfa than with the whole corn diet.

Experiment 3. Dilution rate of Co-EDTA was increased by 66% ($P<.05$) during the 4 hours designated as the feeding period (table 8). Animals had access to water ad libitum. Whether the increased dilution rate is due to increase in ruminal volume or an increase in liquid outflow rate

TABLE 8. CO-EDTA DILUTION RATE (%/HR) AS INFLUENCED BY FEEDING.

Time period	Animal 1	2	3	4	5	6	Mean[a]
10 hr before feeding	7.3	6.8	8.1	7.8	5.9	7.6	7.3[b]
Four hr feeding intial	12.7	11.3	14.6	13.4	9.6	10.7	12.1[c]
10 hr post feeding	7.7	6.3	8.3	8.1	5.6	7.6	7.3[b]
All samples	7.7	7.2	8.6	8.3	6.3	7.9	7.7[d]

[a]Means of the six animals for the specified time interval of the row.

[b,c,d] Means in a column with different superscripts significantly differ ($P<.05$).

is not known. Liquid outflow during the 10 hours prior to and after feeding was adjudged to be constant (R^2>.97) and slopes during those time periods were equal at 7.3% per hour. Warner and Stacy (1968) reported that liquid dilution rate was correlated with feeding. Increased liquid outflow also may enhance ruminal outflow of soluble or small particulate feed components. The effect of increased liquid dilution rate on particulate dilution rate has not been adequately investigated. This effect probably would be greatest with particulates that tend to have the greatest dilution rate. If passage rates of feedstuffs are soybean meal > whole corn = cottonseed hulls > prairie hay as indicated previously (Teeter, 1981), enhanced liquid outflow rates would influence soybean meal passage the most. Sequential feeding of ration constiuents might permit selective bypass of certain feed ingredients. This theory deserves further research attention.

Experiment 4. Adding WSM to the rations of steers fed high or low concentrate rations tended (P<.1) to decrease fecal dry matter (table 9). This may be an osmotic effect of the WSM. Very high concentrations of WSM could theoretically decrease fecal dry matter, cause diarrhea and alter dry matter digestibility. Recovery of dosed marker in feces ranged from 97 to 100% and did not differ significantly (P>.1) among markers. Detectable amounts of metals from all the EDTA complexes were found in urine but PEG was not detected in urine. Urinary loss of chromium and iron appeared greater than of cobalt and ytterbium. Total recovery in feces and urine ranged from 99.7 to 100.2% of dose.

In conclusion, the WSM examined all appeared well suited for experimental use. Although the markers overestimated volume, the errors were generally under 5%. Binding by particulate matter was minimal except for

TABLE 9. RATION DIGESTIBILITY, FECAL DRY MATTER, WSM INDIGESTIBILITY AND URINARY PASSAGE OF WSM'S IN EXPERIMENT 4.

Parameter	Ration					WSM					
	B	B + WSM	C	C + WSM	SEM	Cr	Co	Fe	Yb	PEG	SEM
Ration digestibility (%)	83.7[a]	85.2[a]	63.7[b]	62.2[b]	1.8	96.8[a]	98.9[a]	95.9[a]	99.1[a]	99.7[a]	2.0
Urinary marker excretion (%)	-	-	-	-		3.4[a]	0.8[b]	3.1[a]	1.2[b]	0.0[b]	0.1
Fecal dry matter (%)	29.3[a]	20.7[a]	32.4[a]	23.6[a]	1.4	-	-	-	-	-	

[a,b]Means in a row within ration or WSM having unlike superscripts differ significantly ($P<.05$).

PEG with some fraction of cottonseed hulls. All markers equilibrated and reequilibrated with liquid in both the free and solids fraction of all the feedstuffs tested except whole shelled corn. In light of the small errors associated with volume estimates, these finding provide indirect evidence that these WSM mimic flow of liquid in the digestive tract and have biologically similar flow properties. Errors associated with these estimates and those in the literature may be related to microbial digestion.

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CHAPTER III

YTTERBIUM CHLORIDE AS A PARTICULATE PHASE MARKER FOR RUMINANT ANIMALS

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Summary

Four *in vitro* and two *in vivo* experiments were performed to evaluate feedstuff binding strength and capacity of various feedstuffs for ytterbium, potential for Yb release from feed binding sites and subsequent migration to particulate and soluble fractions, and the effect of Yb labelling on *in vitro* and *in situ* digestion of feedstuffs. All feedstuffs examined exhibited a capacity to bind Yb, though the quantity binding to sites of similar binding strength varied considerably among feedstuffs. In general, feeds high in crude fiber or crude protein bound high quantities (76-205 μM Yb/g feed) of Yb at saturation while feeds high in starch content bound lower amounts (5-37 μM Yb/g feed). Each feedstuff examined had at least two types of binding sites for Yb that differed ($P<.05$) in binding affinity for the rare-earth. The weaker sites within a feedstuff had molar association constants ranging from 4.5 to 9.2 X 10^5 while stronger sites had association constants of 1.2 to 3.8 X 10^7. Dialysis of Yb tagged and washed feedstuffs against water (dilution rate 47%/hr) resulted in detachment of Yb from binding sites

at rates ranging from .09% to .57% per hour. To check such migration in the rumen, unlabelled prairie hay in nylon bags was suspended in the rumen of steers fed whole corn labelled with Yb. Yb migrated from the corn grain to the prairie hay resulting in Yb migration to a level of .18% of the corn. Data indicate that Yb bound to feed placed in an aqueous environment with disequilibria between bound and free Yb will release Yb from the feedstuff to approach a new equilibrium. Release of Yb from feeds was not well correlated with binding affinity demonstrating that binding strength across feedstuffs cannot be used to predict rate of Yb detachment. However, the molar association constant predicted the extent of detachment at equilibrium. Binding strengths of sites within a feedstuff may prove to be more highly correlated with Yb release rates. Soluble organics were observed to bind Yb. The ability of Yb to form water soluble complexes with organic solutes makes it imperative that marker release from labelled feeds be minimized. Relative solute affinity for Yb across five feedstuffs from greatest to least was lactate > acetate > lysine > glucose > glycine > sucrose.

Saturating feedstuffs with Yb reduced ($P<.05$) *in vitro* digestibility of dry matter (36%), neutral detergent fiber (22%) and acid detergent fiber (27%). Similarly, Yb labelling reduced ($P<.05$) *in situ* (nylon bag) disappearance of dry matter (36%) at 96 hours. Yb disappearance from nylon bags was correlated ($R>.99$) with dry matter disappearance and ranged from 9.3 to 25.3% at 96 hours of incubation. Reduced digestibility of Yb labelled feedstuffs indicates that Yb binding is stable to digestive processes. Altered feedstuff digestibility and the potential for Yb migration questions the validity of Yb data obtained after extensive time periods of digestion.

Introduction

Ideally, particulate markers mimic the flow of the particulate matter. Rare-earth elements have been used to label particulate matter because they possess adsorptive properties (Kyker, 1962). In contrast to nonabsorbed markers, adsorption of markers by particulate matter is desirable providing the marker does not influence digestion and passage. Radioactive cerium (Ellis and Huston, 1968; Huston and Ellis, 1968), dysprosium (Ellis, 1968) and ytterbium (Ellis *et al.*, 1979) are adsorbed by particulate matter *in vitro* and *in vivo* and have been employed as markers.

Although rare-earth elements are adsorbed by particulate matter, the potential for their detachment and movement to other particulates or soluble metabolites has received little quantitative attention. Detachment and subsequent migration to other feed particles has been observed *in vivo* and *in vitro* for three lighter rare-earths, namely samarium, lanthanum, and cerium (Hartnell and Satter, 1979b). Binding affinity of Yb for ethylenediaminetetraacetic acid (Spedding and Daane, 1961) and gluconic acid, glycolic acid, glyoxalic acid, ∝-Hydroxyisobutyric acid, lactic acid, malic acid and acetylacetonate (Sinha, 1966) is greater than the affinity of samarium, lanthanum and cerium, so differences in migration of rare earth elements might be expected. The following experiments were conducted 1) to estimate the binding affinity and binding capacity of several feedstuffs for ytterbium, 2) to examine the potential for marker migration to soluble or particulate matter, and 3) to evaluate the influence of marker adsorption on the rate and extent of particulate matter digestion *in vitro* and *in situ*.

Materials and Methods

Feedstuff binding strength and capacity for ytterbium

Cereal grains (3 g) and roughages (2 g) shown in table 1 were washed into dialysis bags (8,000 MW pore size[1]) with distilled H_2O. One bag of each feedstuff was placed in each of 13 glass beakers containing 750 ml of solutions of 13 different Yb concentrations (table 1) at pH 3.8. Samples were incubated at room temperature and the supernatant fluid sampled at 24, 48, and 72 hours. Following incubation, bags were dialyzed against water for 12 hours. Feed samples were removed from dialysis bags and analyzed for Yb by atomic absorption spectrophotometry with nitrous oxide combustion using the standard addition technique.

Detachment of bound Yb

Yb labelled, washed and dried samples of whole corn, ground corn, cottonseed hulls, cottonseed meal and prairie hay were dialyzed against deionized distilled water for 6, 12, 24, 36, 48, 60, and 72 hours. Residual samples were dried and analyzed for Yb.

Ruminal Yb migration

Nylon bags (105 μ pore size) containing 3 g prairie hay were placed in the rumen of eight ruminally cannulated steers fed a 91% whole shelled corn ration. Steers were fed 2,600 g of Yb labelled (2,526 ppm) whole shelled corn. Bags containing the prairie hay were removed at 3, 6, 12, 24, 48, or 96 hours after Yb administration. On removal from the rumen, bags were washed and dried. Residual hay was removed from bags and analyzed for Yb concentration.

[1]Scientific Products, 210 Great South West Parkway, Grand Prairie, TX 75050.

TABLE 1. EQUILIBRIUM MIXTURES: FREE AND FEED BOUND YB.

μ Mole equilibration media/ml				μ Mole Yb bound per gram of dialyzed feed							
Initial	24 hr.	48 hr.	72 hr.	WC[a]	GC[b]	WM[c]	GM[d]	CSM[e]	SBM[f]	CSH[g]	PH[h]
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.478	0.369	0.017	0.009	0.46	4.07	2.43	3.81	4.69	5.49	3.79	6.92
0.741	0.417	0.024	0.018	0.54	7.43	4.69	6.13	8.31	10.26	6.43	11.24
0.905	0.215	0.037	0.034	0.75	10.26	7.94	9.47	12.83	17.43	10.26	18.99
0.995	0.728	0.276	0.265	3.70	14.67	19.43	24.36	31.26	42.61	31.42	35.27
1.959	0.722	0.598	0.594	3.41	19.64	21.46	20.46	76.04	75.89	43.67	36.81
4.525	4.239	2.551	2.496	9.12	22.97	23.49	49.73	149.78	74.67	100.92	51.49
8.437	8.151	5.877	5.792	9.92	28.46	22.7	50.07	206.92	84.71	126.45	67.79
20.278	15.861	15.460	15.444	13.51	32.93	24.6	54.91	177.62	154.91	115.87	71.23
38.538	34.010	33.989	33.706	14.72	32.61	27.9	66.17	162.71	146.32	118.91	72.68
36.280	35.295	31.432	31.037	13.46	33.42	26.31	65.42	195.91	136.72	123.46	73.41
74.272	66.584	64.324	64.216	18.41	36.99	28.41	67.31	196.47	234.96	132.91	75.61
72.776	67.626	65.295	66.012	17.09	47.81	32.31	65.24	199.92	143.87	123.76	74.72

[a]Whole shelled corn grain
[b]Ground corn grain
[c]Whole milo
[d]Ground milo
[e]Cottonseed meal
[f]Soybean meal (44%)
[g]Cottonseed hulls
[h]Prairie hay

Affinity of solutes for Yb

Glucose, sucrose, L-lysine HCl, glycine, lactate and sodium acetate were dissolved in H_2O (20% W/V) and the pH adjusted to 6.0 by addition of HCl or NaOH. To these solutes were added an equal volume of distilled water (pH 6) containing 125 ppm Yb and 40 ppm Hg. Mercury was added to prevent fermentation. Following a 72 hr equilibration period, duplicate 10 ml volumes of each duplicate sample were added to test tubes containing no feed or 1 g dry matter from cottonseed hulls, corn starch, cellulose, cottonseed meal, or lignin[2]. The sample and feed were allowed to equilibrate for 72 hours. The supernatant fluid was then sampled and analyzed for Yb.

In vitro digestibility

Fifty gram samples of cottonseed hulls, cracked corn, chopped alfalfa hay, and chopped prairie hay were treated with either water, 28 mM hydrochloric acid, 28 mM $YbCl_3$ or 28 mM $DyCl_3$ for 24 hours. Feeds were then rinsed over a 2 mm seive with distilled water for three hours. Samples (2 g) of each feedstuff were placed in duplicate 50 ml centrifuge tubes containing 15 ml of rumen inoculum and 25 ml of McDougall's (1948) artificial saliva. Rumen fluid was a mixture of that obtained from a 517 kg fistulated steer fed 7 kg of an 80% concentrate diet twice daily and a 450 kg fistulated steer fed 4.5 kg alfalfa hay twice daily. Rumen fluid was strained through four layers of cheesecloth and equal volumes were mixed. Incubation was halted at 24, 48 and 96 hours by adding 1 ml of 5% H_gCl_3. Samples were stored at 10 C prior to filtration. Tube contents were filtered through number four Whatman paper,

[2]West Vaco, P. O. Box 70848, Charleston Heights, Charleston, South Carolina 29405.

dried first at 55 C for 48 hours and subsequently at 90 C for estimating dry matter disappearance. Feed samples, residual dry matter, and residual inocula from the 96 hour incubation were analyzed for neutral detergent fiber and acid detergent fiber (Goering and Van Soest, 1970).

In situ digestion

Duplicated samples (2 g) of the feeds utilized in the in vitro study above were treated with H_2O or 28 mM $YbCl_3$. Samples were washed, seived, and placed in nylon bags (105 μ) and incubated for 24, 48, and 96 hours in the rumens of three steers. Steers were fed 6 kg daily of an 80% concentrate diet. Bags were removed, washed, dried at 55C for 48 hours and subsequently at 100 C for 12 hours, and residual contents analyzed for Yb to estimate dry matter and Yb disappearance and rate of disappearance.

Results and Discussion

Binding strength and capacities

Feed adsorbed an increasing amount of ytterbium as Yb concentrate in the suspending fluid was increased, but uptake plateaued as binding sites were saturated (table 1). Ytterbium concentration changed little from 48 to 72 hr suggesting that equilibrium between feed binding sites and free Yb had been reached by about 48 hours. Rare-earth concentrations above the point of feed saturation increased the quantity of free Yb in solution but had little effect upon the quantity of metal bound to most feedstuffs. Following a 12 hr dialysis period against water to remove unlabelled and weakly bound metal from dialysis bag contents, ytterbium concentrations in feed and in the saturating solution were used to calculate the association constants of feed for ytterbium following the

Scatchard procedure (Ferdinand, 1976). This water dialysis period and analysis of bound ytterbium helps to avoid the rather substantial errors associated with calculating uptake as the difference between initial and final solute concentrations and also to eliminate weaker binding sites which would be of little concern.

Plotting the concentration (M metal/g feed) of metal bound to feed divided by the free rare-earth concentration versus the concentration of metal bound to feed produces Scatchard plots (figures 1-4) for feedstuffs. The slope represents the association constant of the rare-earth for feed and the abcissa intercept represents the point of saturation of feed with rare-earth. Lack of linearity in these Scatchard plots for the individual feeds indicate that binding sites for all feeds tested vary in strength and are not homogeneous. The Scatchard plots appear biphasic. This indicates the presence of at least two types of bonds which differ in binding affinity. Linear regression analysis of the linear portions of the Scatchard plots was used to estimate binding affinity and capacity for these sites (table 2). Diversity among feedstuffs indicates that specific functional groups, or the environment around the functional groups which bind rare-earths, varies within and among the plant products tested. Binding only to the higher affinity sites should minimize the opportunity for migration. Feeds exposed to low concentrations of rare-earth may have a large proportion of binding at the high affinity bonds. However, due to the low number of high affinity bonds and the high number of low affinity bonds, certainty of binding site at low levels of exposure is not assured. Saturation points of the low and high affinity binding sites for feeds tested are presented in table 2. If saturations are exceeded and the excess is not removed, the residual rare-earth in the feed is

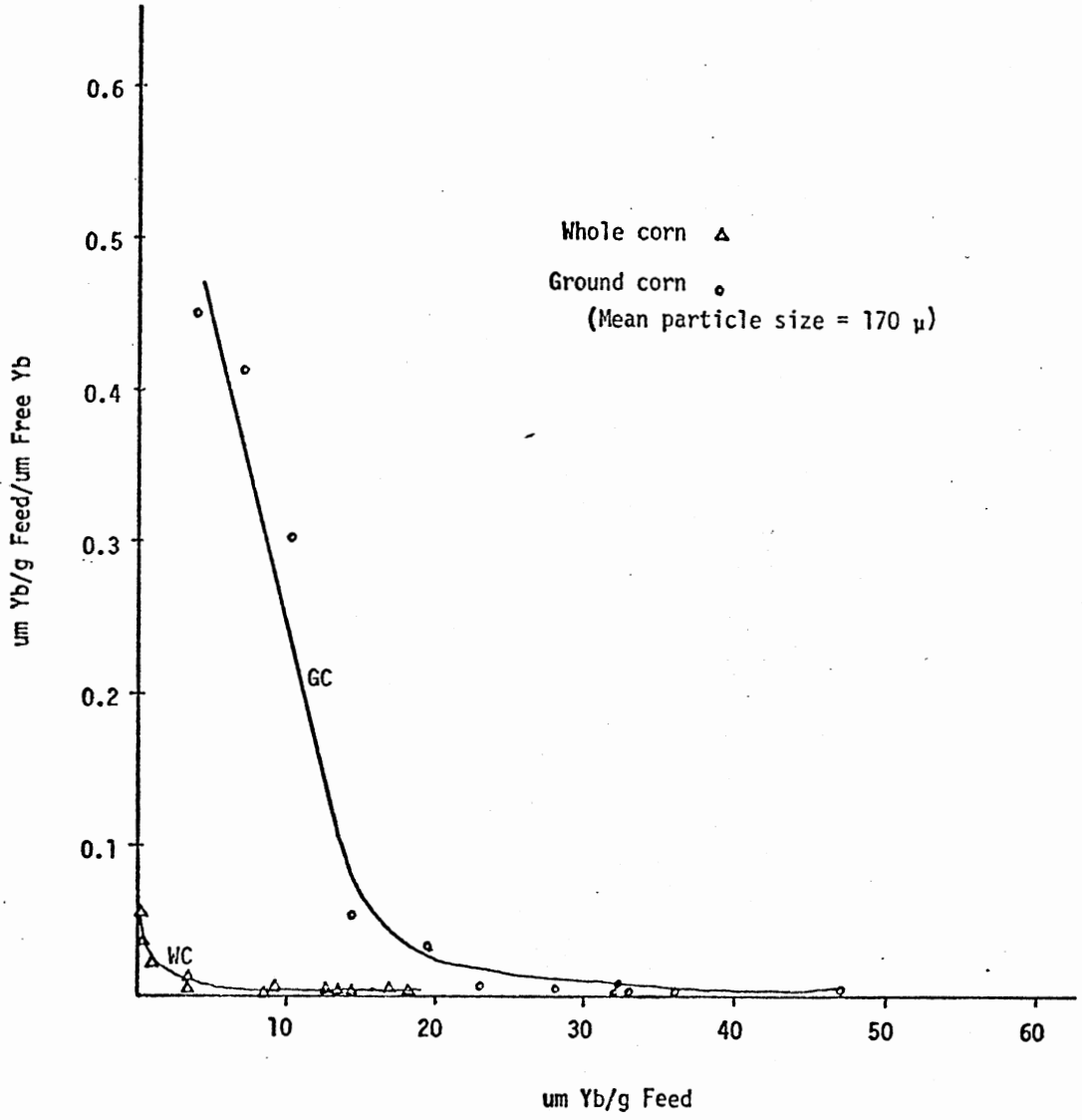

Figure 1. Scatchard plot for whole and ground corn grain.

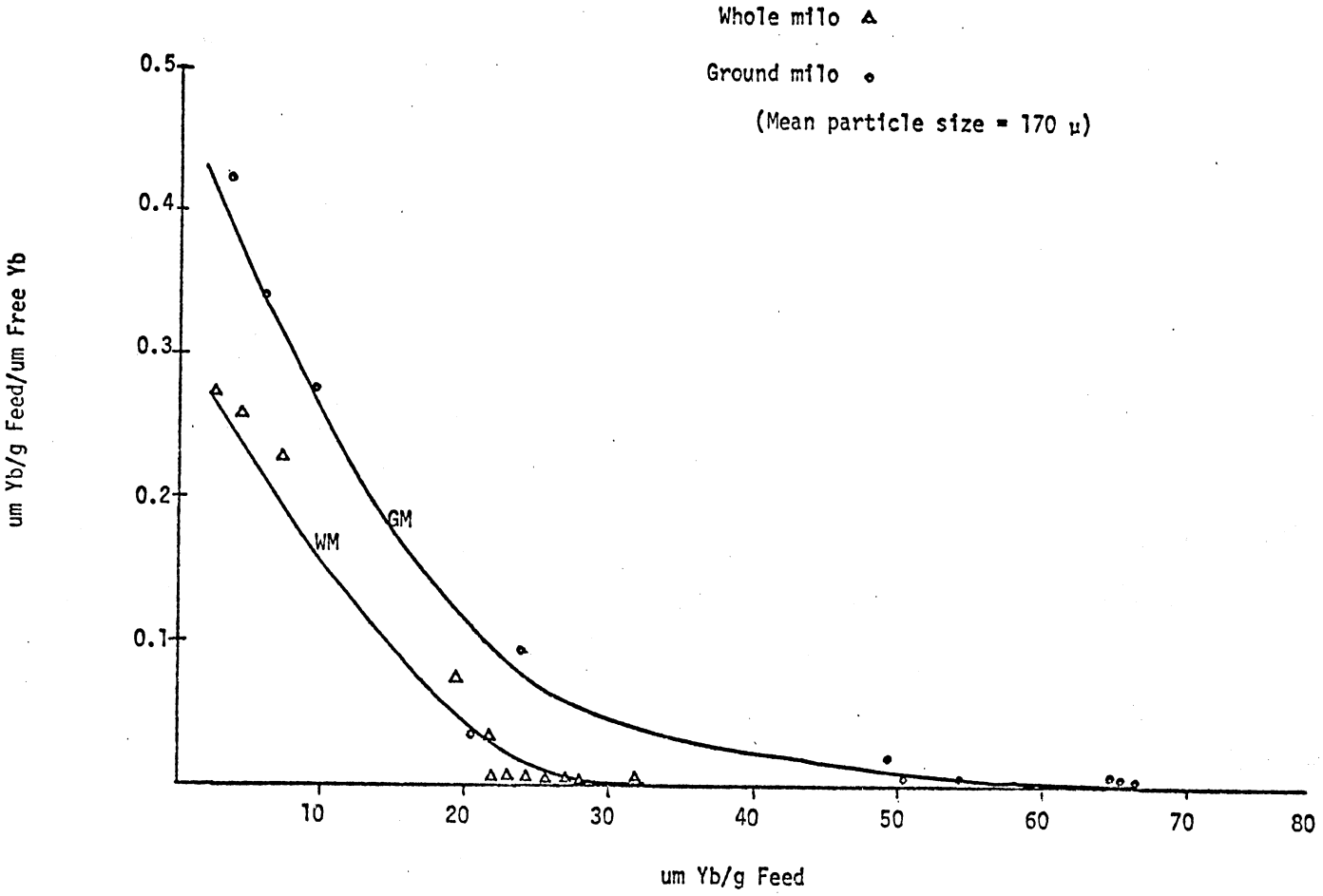

Figure 2. Scatchard plot for whole and ground milo.

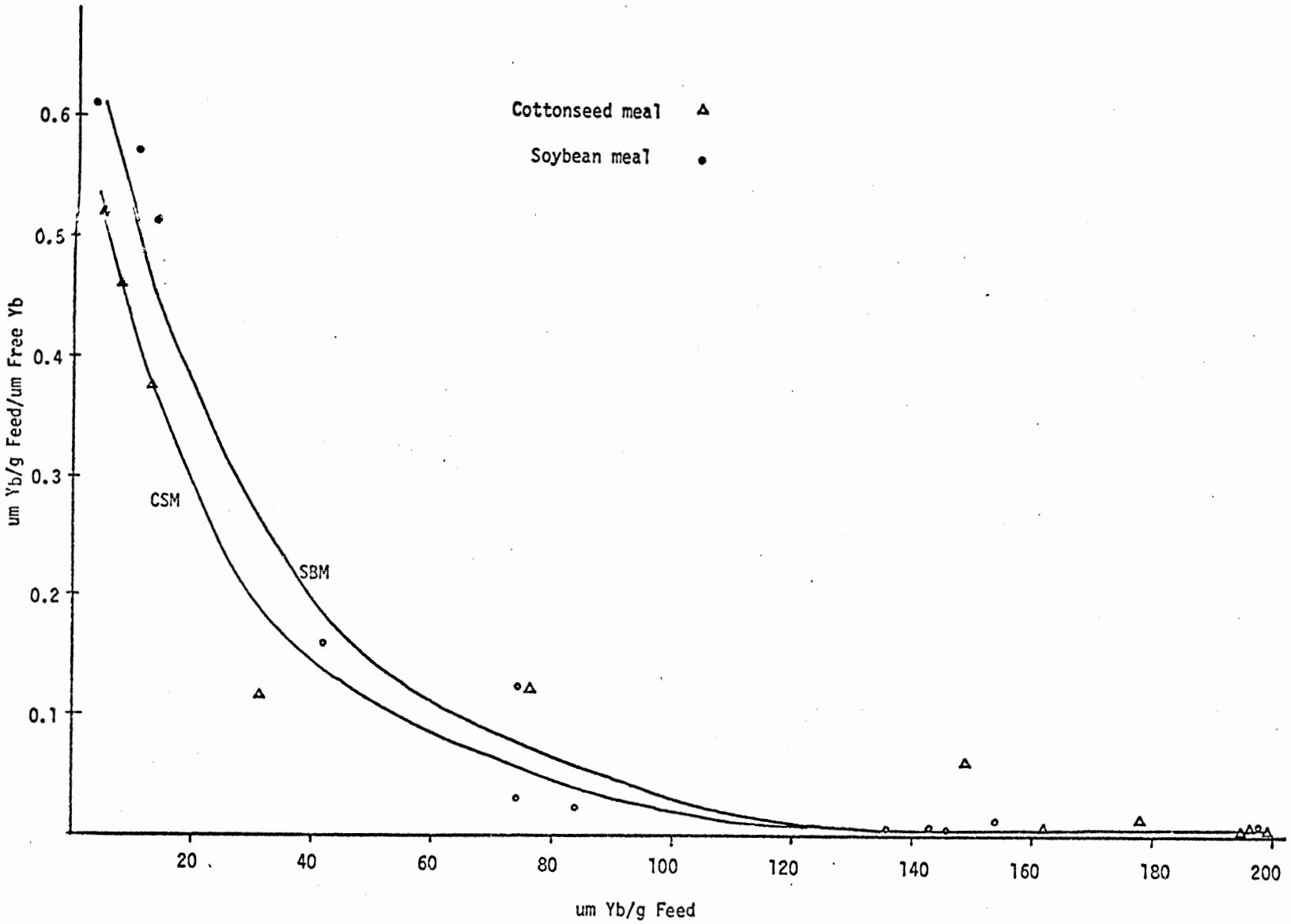

Figure 3. Scatchard plot for high protein feeds.

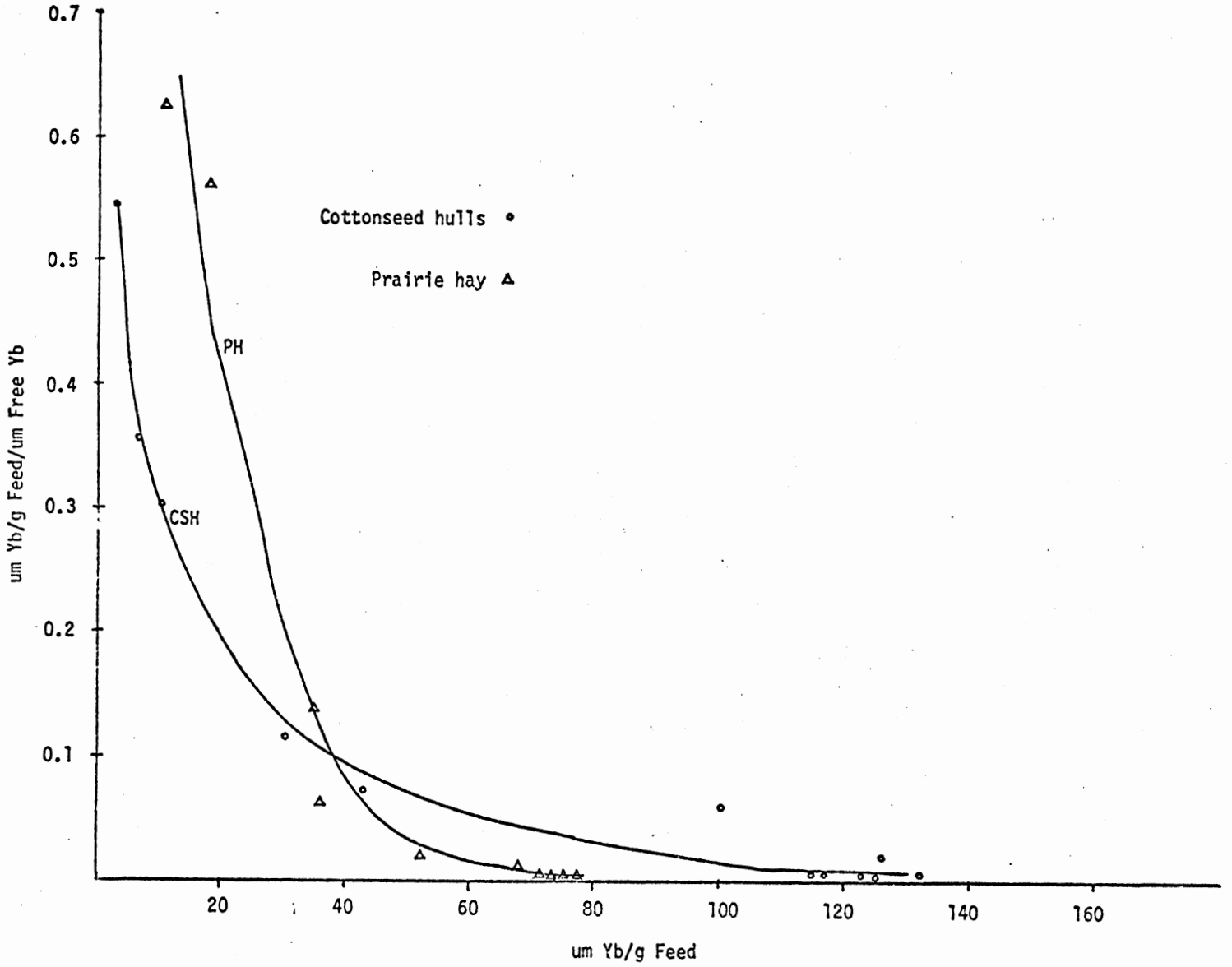

Figure 4. Scatchard plot for high roughage feeds.

TABLE 2. HIGH AND LOW MOLAR ASSOCIATION CONSTANTS AND THEIR BINDING CAPACITY.

Feedstuff	High affinity binding				Low affinity binding			
	Molar affinity K X 10^7	μ M/g capacity	N[a]	R[b]	Molar affinity K x 10^5	μ M/g capacity	N	R
Whole corn	3.8	0.98	3	-.88	6.85	14.93	9	-.82
Ground corn	3.83	17.09	4	-.96	9.20	21.9	8	-.72
Whole milo	1.26	24.96	5	-.99	6.33	5.53	7	-.68
Ground milo	1.50	29.89	3	-.98	7.29	37.50	9	-.95
Cottonseed meal	1.50	38.89	4	-.99	9.08	166.40	8	-.89
Soybean meal	1.19	57.26	4	-.99	4.48	131.14	8	-.69
Cottonseed hulls	1.77	34.22	3	-.99	7.44	176.18	9	-.91
Prairie hay	2.27	40.75	5	-.99	8.50	36.17	7	-.97

[a]Number of observation used in linear regression.
[b]Linear correlation coefficient.

certain to migrate to other feed or ruminal components following dosing

Techniques commonly employed to label particulate matter with rare-earths include oral administration of gelatin capsules containing water soluble rare-earth salts (Knapka et al., 1967; Miller and Byrne, 1970, and Miller et al., 1971), pouring rare-earth solutions on to the material of interest, followed by dosing or drying and dosing (Ellis and Houston, 1968; Olbrich et al., 1971; Harnell and Satter, 1979a) and immersion of feeds in rare-earth solutions followed by washing of feed residues to remove the unbound rare-earth (Teeter et al., 1979). Little is known regarding the influence of these different binding techniques on the amount of residual exchangeable Yb. Ellis and Houston (1968) studied fecal excretion of cerium that had been initially adsorbed onto alfalfa hay by drying a solution of cerium chloride onto the hay. Excretion curves for Ce and PEG were similar. This indicates that some exchange may have occurred. The optimal labelling technique may vary with the ration used. The immersion-washing procedure should minimize exchange. To adequately label a heterogenous diet, feed components should be individually labelled and then mixed together. Otherwise, labelling of ration ingredients will be disproportionate due to physical or affinity characteristics of ingredients. Development of techniques to label only the stronger feed binding sites should help reduce the potential for rare-earth detachment and migration to other feed particles or solute molecules.

Rate of ytterbium release was negatively correlated (R=.86) across feedstuffs with the number of weaker binding sites. Binding affinity, although a measure of the proportion of rare-earth bound at equilibrium, is not a measure of the rate at which feed-bound rare-earth is released from binding sites. Rate of Yb release differs with feedstuffs and is

independent of binding affinity. The quantity of Yb released at equilibrium, however, should be related to the binding affinity constant. The relationship between binding affinity and rate of Yb release under disequilibrium conditions needs to be evaluated within a feedstuff. If high affinity bonds within a feedstuff approach equilibrium at a slower rate than low affinity bonds, then preferential binding to high affinity bonds would be desirable because there would be less total release of marker during periods of in vivo incubation.

Detachment of bound Yb

Feed samples washed following exposure to ytterbium would be expected to contain little if any unbound rare-earth. Consequently, a decrease in the rare-earth concentration on dialysis can be attributed to release of the rare-earth from binding sites. Detachment of the rare-earth with water dialysis was measured (figure 5). Graphs indicate that the feed-Yb complex will dissociate in a low Yb environment towards an equilibrium between free and bound Yb, releasing from .1 to .6% of bound Yb each hour. A similar situation probably exists in the rumen. Therefore some marker migration would be expected. In this experiment, water was exchanged at a rate of 47% per hour. This is probably a more severe disequilibrium than exists ruminally and should overestimate potential marker migration. However, presence of feeds to bind released marker might speed migration. Dissociation is more drastic in the presence of .3 M EDTA, as this concentration of EDTA has been shown to quantitatively remove rare-earths from feedstuffs (Teeter, unpublished). This is not surprising since the EDTA molar association constant is approximately 10^{20} (Spedding and Daane, 1961) while feedstuffs have a maximum of about 10^{7}. EDTA more effectively lowers the free metal concentration than

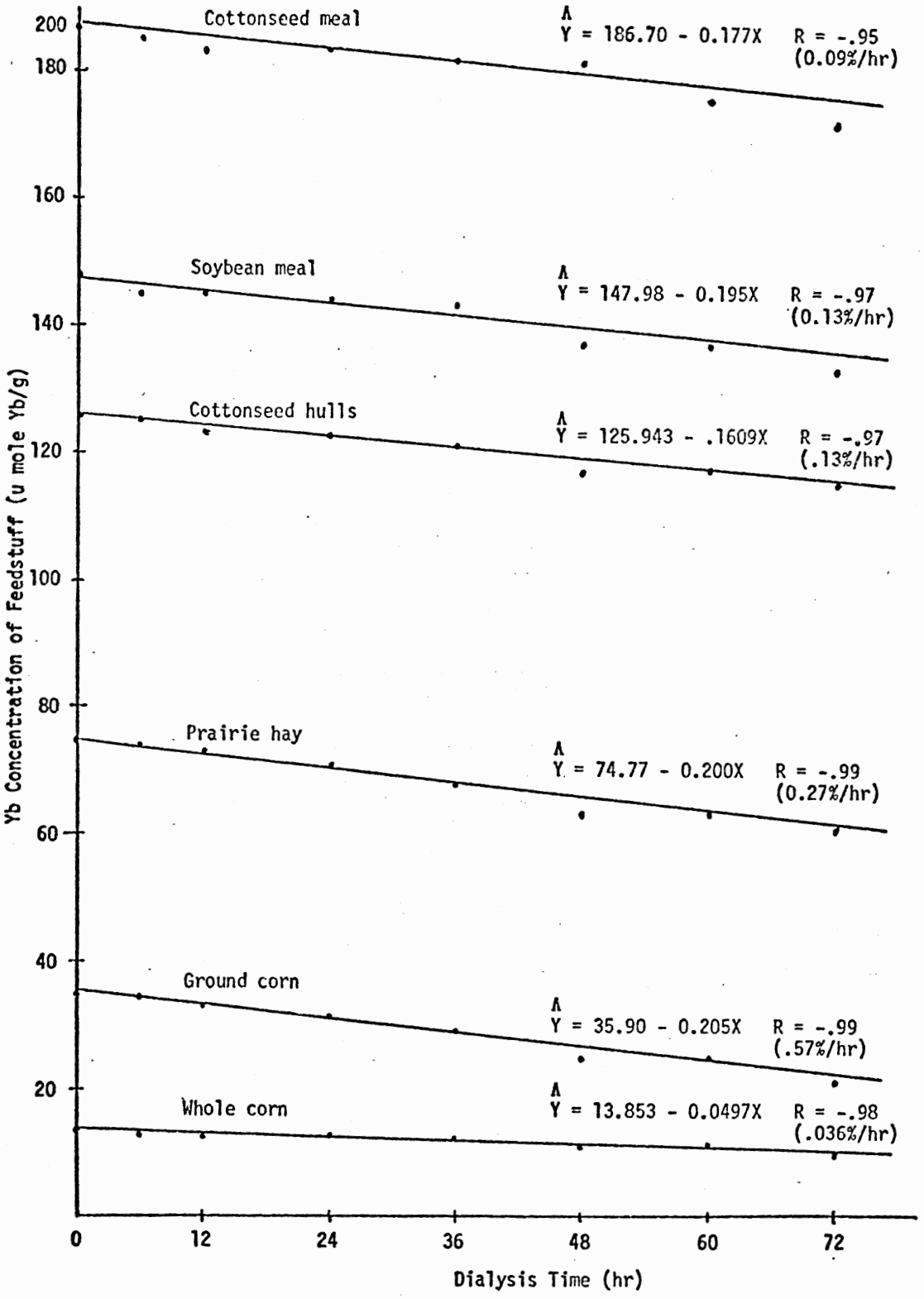

Figure 5. Release of Yb from labelled, washed and dried feedstuffs.

dialysis, thereby creating a more severe disequilibrium. If a metal-feed complex is stable in the presence of EDTA, this would indicate that binding of marker to feed component is very strong. Such stability has been reported with a Cr mordant-fiber (Uden et al., 1980). Whether such tight associations are required for useful digesta flow markers is unclear, though chelating agents (amino acids, lactate, sugars) normally found in the rumen would be expected to feed disequilibrium unless stability is high.

Ruminal Yb migration

Nylon bags containing unlabelled prairie hay, which is a feed with a high binding affinity and capacity, were suspended in the rumen of steers fed a dose of Yb labelled corn. Concentration of Yb in the hay increased over time of ruminal incubation (table 3). This indicates that ruminal Yb exchange occurs between these two types of particulate matter and that this rare-earth, after liberation, combines with other particles. In vivo marker exchange is difficult to quantitate. In this trial, only a fraction of the Yb migration was detected. The prairie hay attained labelling equal to .01% of the initial Yb dose after 96 hours or .18% of the projected concentration of Yb of corn dry matter assuming 3%/hr dilution of labelled corn. If marker exchange is a function of time, however, longer time periods increase the uncertainty of marker location. The magnitude of migration also can be influenced by the number and location of digesta pools. If digesta flow follows two-pool kinetics (Ellis et al. al., 1979) with the rumen constituting one pool and the rest of the gastrointestinal tract the second pool, the critical site for marker exchange is the rumen. If differential flow of digesta occurs post-ruminally, then marker exchange is critical throughout the digestive tract.

TABLE 3. RUMINAL YB MIGRATION TO PRAIRIE HAY.

	Incubation time (hr)					
	3	6	12	24	48	96
ppm Yb	.04[a]	.07[ab]	.08[b]	.13[c]	.14[c]	.27[d]

[abcd]Means in a row with unlike superscripts differ significantly ($P < .05$).

Affinity of solutes for Yb.

The results of adding 1 g feed samples to solutions containing 1 g solute and 126 ppm Yb are presented in table 4. If the solutes have little affinity for Yb, then Yb would remain free to bind with the added particulate matter. Increasing affinity of solute for Yb will decrease the quantity of Yb bound to particulates assuming that: (1) solute binding affinity is sufficiently strong, and (2) particulate matter does not bind with any solute-Yb complexes. Complete binding to the solute will increase the soluble non-bound Yb to a level of about 126 ppm, whereas with no binding, remaining soluble Yb should equal the value for water alone. High or low values for feeds indicate low and high Yb binding, respectively.

All the solutes examined formed water soluble complexes with Yb but binding differed markedly among solutes tested (table 4). Relative solutes affinity for Yb across feedstuffs from greatest to least was lactate > acetate > lysine > glucose > glycine > sucrose. Affinity or binding capacity particulate matter for Yb also varied, being from greatest to least, CSM > lignin > CSH > cellulose > starch. Interactions between solute and feed for Yb may be attributed to binding of soluble Yb complexes by the particulate matter. Formation of these water soluble Yb complexes makes it important to minimize the potential for rare-earth migration. Water soluble complexes would cause errors of much greater magnitude than migration from one feed particle to another. Lactate appears to have a stronger affinity for Yb than either starch or cellulose. Based on this study, functional groups involved in solute binding of Yb would include carboxyl and hydroxyl groups as well as α amino and epsilon nitrogen.

TABLE 4. SOLUTE YB BINDING.

Feed component	Free Yb (ppm)							
	Solute							
	H_2O	Glycine	Lysine	Glucose	Sucrose	Lactate	Acetate	$\Delta\bar{X}$
Blank	125.8^{a}	123.6^{a}	127.6^{a}	126.5^{a}	128.0^{a}	126.4^{a}	125.2^{a}	
CSH	3.7^{a}	35.6^{b}	4.7^{a}	18.0^{c}	18.9^{c}	46.5^{d}	3.8^{a}	18.7
CSM	1.8^{a}	4.5^{b}	5.0^{b}	12.3^{c}	6.5^{b}	16.7^{d}	1.2^{a}	6.8
Cellulose	10.6^{ab}	12.3^{b}	50.5^{c}	13.2^{b}	5.8^{a}	133.5^{d}	92.9^{e}	45.5
Starch	77.1^{a}	90.0^{b}	139.3^{c}	100.1^{b}	91.8^{b}	126.0^{d}	114.0^{d}	105.5
Lignin	6.7^{a}	5.6^{a}	6.0^{a}	5.3^{a}	5.8^{a}	70.2^{b}	5.7^{a}	15.0
$\Delta\bar{X}$	20.0	29.6	41.1	29.8	25.8	78.6	23.0	

^{abcde}Means in a row with different superscripts differ significantly ($P<.05$).

In vitro disappearance of Yb labelled feedstuffs

Addition of Yb or Dy to feed components decreased ($P<.05$) in vitro disappearance of dry matter, NDF and ADF when compared with controls extracted with either water or acid (table 5). This suggests that the metal complexes are reasonably stable to the digestive process since rapid release of bound metal or enzymatic displacement of rare-earth should allow digestion to proceed. Decreased digestibility of ADF (26.3%) and NDF (31.7%) compared to the H_2O control demonstrates that binding disrupts digestion of both fractions, suggesting that the metal attaches to sites in both fractions. Antibacterial activity of Yb might produce similar results, however.

In situ digestion

Dry matter digestion in situ (table 6) followed a pattern similar to the in vitro study. Yb addition reduced both the rate ($P<.05$) and extent ($P<.05$) of dry matter disappearance from nylon bags. Yb retention by nylon bag contents also was affected by feed source. Rate of Yb disappearance averaged 54% that of dry matter disappearance. This means that Yb concentration of labelled feed increased in the undigested residue which might be expected if Yb is bound to the fibrous fraction of a feedstuff. Relative release and exchange would be expected to depend both on feed being labelled and on extent of fiber digestion in the rumen. Whether this is due to Yb exchange or to digestion to small particles less than 105 μ containing Yb and exit of such particles from the bag is unknown.

In conclusion, rate of digestion and passage through the gastrointestinal tract are parameters which influence digestibility, ration intake and animal productivity. Yb readily complexes with particulate

TABLE 5. DIGESTIBILITY OF TREATED AND DIALYZED FEEDSTUFFS.

Feedstuff	Feed fraction											
	Digestibility, %											
	Dry matter				NDF				ADF			
	H_2O	Acid	Yb	Dy	H_2O	Acid	Yb	Dy	H_2O	Acid	Yb	Dy
Cracked corn	76.6^{a}	77.7^{a}	69.3^{b}	72.4^{ab}	71.4^{a}	58.6^{b}	45.1^{c}	43.3^{c}	86.6^{a}	72.8^{a}	74.8^{a}	61.1^{b}
Cottonseed hulls	19.7^{a}	27.1^{b}	1.5^{c}	6.3^{c}	59.1^{a}	59.6^{a}	35.0^{c}	43.0^{d}	65.4^{a}	64.7^{a}	42.0^{b}	50.3^{b}
Prairie hay	47.3^{a}	50.4^{ab}	24.0^{c}	25.9^{b}	66.7^{a}	69.4^{a}	54.1^{b}	51.1^{b}	64.3^{a}	61.4^{a}	56.4^{b}	41.4^{b}
Alfalfa hay	37.0^{a}	40.9^{ab}	20.1^{c}	28.9^{b}	64.0^{a}	65.7^{a}	44.3^{b}	38.6^{b}	63.3^{a}	53.8^{a}	32.8^{b}	28.4^{b}

^{a,b,c,d}Means in a feed fraction and feedstuff with different superscripts differ significantly ($P < .05$).

TABLE 6. IN SITU DRY MATTER AND YB DISAPPEARANCE.

		Incubation time							
		% dry matter disappearance				% Yb disappearance			
Feedstuff	Trt.	24	48	96	Rate	24	48	96	Rate/hr.
					%/hr				
Ground corn	H_2O	21.3^{b}	49.7^{c}	61.3^{d}	0.64	-	-	-	
	Yb	17.4^{b}	37.6^{c}	49.9^{d}	0.52	12.2^{a}	17.9^{c}	25.3^{d}	0.25
Chopped alfalfa	H_2O	17.9^{b}	36.7^{c}	43.8^{d}	0.45	-	-	-	
	Yb	11.1^{b}	29.8^{c}	31.7^{d}	0.34	6.3^{b}	11.6^{c}	15.7^{d}	0.16
Cottonseed hulls	H_2O	9.7^{b}	11.6^{c}	13.3^{d}	0.12	-	-	-	
	Yb	3.9^{b}	4.7^{c}	6.1^{d}	0.06	2.9^{b}	6.2^{c}	5.9^{c}	0.06
Prairie hay	H_2O	17.2^{b}	28.6^{c}	37.4^{d}	0.37	-	-	-	
	Yb	8.4^{b}	10.2^{c}	12.7^{d}	0.12	5.3^{b}	7.4^{c}	9.3^{c}	0.09

[a]Rate/hr calculated by regressing disappearance on Time, includes data point (0,0).
[bcd]Means within a row and heading followed by unlike superscripts differ ($P < .05$).

matter forming particles which should at least initially mimic digesta flow. However, due to the influence of Yb on particulate digestion and the migration of Yb to other feed particles and soluble components, values obtained after an extensive time period of incubation may not be fully representative of flow of unlabelled particles. Further information is needed regarding 1) methods of binding to decrease migration, and 2) quantitative significance of marker migration.

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CHAPTER IV

RUMINAL AND FECAL PASSAGE RATES OF PARTICULATE MATTER VARYING IN SIZE, DENSITY AND DIETARY CONCENTRATION

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Summary

Passage rates of liquids and rare-earth labelled feedstuffs were measured in four experiments. In trial 1, four steers were fed a 96% roughage diet at a level of 1.72% of body weight while in trial 2 steers received an 80% concentrate ration at a level of 1.66% of body weight. Yb labelled soybean meal (SBM), cottonseed hulls (CSH), whole shelled corn (WSC), and prairie hay (PH) were fed with one of the six daily meals. In addition, steers were ruminally pulse dosed with Co EDTA, a ruminal liquids marker. Rumen, abomasal and fecal samples were obtained over the subsequent 84 hours and dilution rates of Yb and Co, which may represent passage of feedstuffs and liquids, were calculated. Dilution rate of Co EDTA and Yb labelled feedstuffs was not affected ($P>.1$) by sampling sites. Liquids in both experiments had higher passage rates ($P<.05$) than the Yb labelled solids. With both the concentrate and roughage rations, flow rates of Yb labelled particulate matter, listed from greatest to least, was SBM > WSC = CSH > PH.

In trial 3 the influence of particle size of prairie hay on rate of passage was examined. Three duodenally cannulated steers, fed prairie hay were fed two particle sizes (17 cm and 1.9 mm mean particle length) of rare earth tagged hay. Duodenal and fecal samples were used to monitor marker passage. Passage rate of the short hay particles was 14% greater ($P<.25$) than for the long particles. Passage rates estimated using duodenal and fecal samples were similar ($P>.8$). Six ruminally cannulated steers, fed whole shelled corn were used in trial 4 to examine the influence of particle size on corn passage rate. Two rare-earths were used to label whole shelled corn and corn that had been ground through a 6 mm screen. Ground corn grain had a passage rate 6.1% higher ($P<.1$) than WSC. Passage rates calculated from ruminal and fecal samples following feeding the tagged corn was not influenced by sampling site though ruminal sampling tended to be more variable. Data from the four experiments indicate that particulate passage rate is influenced by both feedstuff type and particle size.

Introduction

Extent of digestion may be estimated by measuring feed consumed and feces excreted. Such calculations do not evaluate the dynamic state of digestion in the ruminant's multicompartmental digestive tract. The magnitude of digestion in any portion of the alimentary canal is the product of feed digestion rate and feed residence time. Residence time and digestion rate appear to be influenced by physical characteristics of feedstuffs which can be altered by processing (grinding, pelleting, and wafering; Evans et al., 1973). Increasing digesta flow without a concurrently increasing digestion rate will shift nutrients down

in the tract and can reduce the extent of digestion of certain feedstuffs. High levels of feed intake also can enhance feed passage rate and reduce digestibility (Weller et al., 1971; Grovum and Williams, 1977; Teeter, 1981). Roughage addition to a whole shelled corn diet also can alter the site (Cole et al., 1976) and extent (Teeter et al., 1980, 1981) of starch digestion. Knowledge of the factors influencing passage and digestion rate of feed particles is essential to maximize the utilization of feedstuffs by ruminant animals.

Rare-earth elements have been used to label feedstuffs and follow their passage rate with homogenous (Combe and Kay, 1965; Ellis and Huston, 1968; Miller et al., 1971) and heterogenous diets (Hartnell and Satter, 1979; Teeter et al., 1980). Although small amounts of rare-earth elements can migrate between feed particles (Hartnell and Satter, 1979; Teeter et al., 1979), passage of elements should represent passage rate of labelled constituents, if the marker does not alter intrinsic feedstuff characteristics, as 80 to 90% of the rare-earth remained bound to the original feed particle incubated in situ for 48 hours (Teeter, 1981).

The following experiments were conducted to monitor the rate of passage of ytterbium labelled feedstuffs differing in particle size, density, with different dietary fiber concentrations.

Materials and Methods

Ytterbium and dysprosium labelling of feedstuffs

Feedstuffs were individually labelled with ytterbium (Yb) or dysprosium (Dy) by immersing the feed in distilled water containing 22 mM Yb or 35 mM Dy. Mixtures were allowed to equilibrate 24 hours after which the supernatant was removed and the feeds washed for six hours to remove

~~and the feeds washed for six hours to remove~~ unbound and weakly associated metal.

Particulate passage with a high roughage diet

Four ruminally and abomasally cannulated growing steers (284 kg) were used in a 4 X 4 latin square experiment. Steers were fed feed at a level equivalent to 1.72% of body weight. The ration contained 90% prairie hay and 10% pelleted supplement (table 1). Steers were fed in six equal portions six times dally. Each 12 day experimental period consisted of 7 days for adaptation to ration and excretion of marker from the previous period. On day 8 of each period, steers received orally either Yb labelled cottonseed hulls, prairie hay, whole shelled corn or soybean meal and received ruminally a 500 ml dose of cobalt ethylene-diamine-tetra-acetic acid (Co-EDTA) prepared as specified by Dwyer et al. (1955). Ruminal, abomasal, and fecal samples were obtained just prior to marker administration and at 6, 12, 24, 36, 48, 60, 72, and 84 hours after dosing. Samples were analyzed for Yb and Co utilizing an atomic absorption spectrophotometer.

Particulate passage with a high concentrate diet

Four ruminally and abomasally cannulated steers (316 kg) were used in a 4 X 4 latin square experiment. Steers were fed at a level of 1.66% of body weight. The 84% concentrate ration (table 2) was fed in six equal portions six times daily. Experimental periods, labelled feedstuffs, and sampling procedures were as described above for the high roughage diet.

Passage rate of long and finely ground prairie hay

Three duodenally cannulated steers (207 kg) were used in a completely randomized experimental design. Steers were fed at a level of 2.8% of

TABLE 1. PELLETED SUPPLEMENT.

Ingredient	%
Ground corn	12.4
Soybean meal	55.0
Cottonseed meal	27.1
Potassium chloride	2.5
Salt	2.7
Vitamin A (10m/lb)	0.15
Trace mineral	0.15

TABLE 2. RATION COMPOSITION.

Ingredient	% of ration
Cracked corn	75.8
Cottonseed hulls	10.0
Alfalfa meal	4.0
Molasses, cane	4.0
Cottonseed meal	4.5
Limestone	0.75
Urea	0.5
Dicalcium phosphate	0.15
Salt	0.3
Vitamin A	0.015

body weight. The 96% unprocessed prairie hay, 4% supplement ration (table 1) was fed in four equal portions four times daily. The experiment lasted 14 days with days 1-11 for adaptation to ration and surroundings. On day 12, steers received either Yb or Dy labelled, unprocessed (17 cm mean particle length) prairie hay and Yb or Dy labelled finely ground (1.9 mm) prairie hay. Prairie hay particle sizes were randomly assigned to steers with the restriction that each steer received both particle sizes and one each labelled Yb and Dy. Duodenal and fecal samples were obtained at six hour intervals for 96 consecutive hours after dosing. Samples were dried at 55 C and stored in polyethylene bags prior to analysis for Yb and Dy determination.

Passage rate of whole and ground corn grain

Six ruminally cannulated steers (548 kg) were used in a completely randomized experimental design. Steers were fed at a level of 1.62% of body weight a 78% whole shelled corn ration (table 3) in two equal portions twice daily. The experiment lasted 21 days with days 1-14 being used for adjustment to diet and surroundings. On day 15, steers were fed labelled corn grain in the whole form or this same labelled corn ground through a 6 mm screen prior to feeding. Labelled corn treatments were randomly allotted to steers so that three steers received Yb labelled whole corn. Animals receiving Yb labelled whole corn received Dy labelled ground corn while animals receiving Dy labelled whole corn received Yb labelled ground corn. Rumen and fecal samples were obtained at 12 hour intervals for 84 consecutive hours after feeding the labelled meal. Samples were dried at 75 C and stored in polyethylene bags prior to analysis for Yb and Dy.

TABLE 3. RATION COMPOSITION.

	%[a]
Whole shelled corn	78.0
Cottonseed hulls	10.0
Pelleted supplement	
Ground corn	8.34
Urea	0.75
Dicalcium phosphate	2.10
KCl	0.75
Vitamin A	0.03
Vitamin D	0.03

[a]Dry matter base.

Results and Discussion

Particulate passage

Passage rate estimates for Yb which had been attached to various feedstuffs and fed to steers receiving either high roughage (Trial 1) or high concentrate (Trial 2) diets are presented in tables 4 and 5. In both studies, turnover rate of liquid was higher ($P<.05$) than turnover rate of feedstuffs. Neither estimate was influenced by sampling site ($P>.1$). Numerous workers have reported that liquid passage rate exceed that of the dry matter (Grovum and Williams, 1973; Miller et al., 1971; Ellis et al., 1979). Passage rates differed ($P<.05$) between feedstuffs. Relative rates of passage were similar for both studies: soybean meal > corn grain = cottonseed hulls > prairie hay. No obvious physical characteristics correlate with these passage rates, though wet density and wettability might be involved. As ruminal digestibility might follow this order, migration of the marker to solutes is of concern.

Differential passage rates for feedstuffs within a diet would make it necessary to couple rate of digestion estimates for a feedstuff of interest with the specific rate of passage estimates for that feedstuff, and not with overall solids flow rate. Identification of the physicochemical factors that enhance ruminal outflow may permit one to manipulate the turnover rate and thereby the time for and extent of feed digestion in the rumen. Increasing ruminal bypass may prove desirable for high quality protein supplements as well as intestinally digestible concentrate feedstuffs while longer retention of roughages for more extensive ruminal fermentation may be desirable if intake of digestible organic matter is maintained.

TABLE 4. RATES OF PASSAGE WITH A HIGH ROUGHAGE DIET.

Sampling site	Passage rate (%/hr)				
		Feedstuff			
	Liquids	Prairie hay	Cottonseed hulls	Soybean meal	Corn grains
Rumen	7.8 ± 0.7^{a}	3.0^{b}	3.9^{bc}	4.1^{c}	3.9^{bc}
Abomasum	8.1 ± 1.1^{a}	2.7^{b}	3.7^{c}	3.9^{c}	3.5^{c}
Feces	7.9 ± 0.6^{a}	2.9^{b}	3.9^{bc}	4.3^{c}	4.0^{c}

[a]Represents the mean of four observations per animal for the four animals ± SEM.

[bcd]Means in the same row which do not have a common letter in the superscript are different ($P < .05$).

TABLE 5. RATES OF PASSAGE WITH A HIGH CONCENTRATE DIET.

Sampling site	Passage rate (%/hr)				
		Feedstuff			
	Liquids	Prairie hay	Cottonseed hulls	Soybean meal	Corn grain
Rumen	5.1 ± 0.8^{a}	2.4^{b}	2.9^{c}	3.2^{d}	2.9^{c}
Abomasum	4.9 ± 1.4^{a}	2.6^{b}	2.7^{bc}	3.1^{d}	2.8^{c}
Feces	5.0 ± 0.7^{a}	2.6^{b}	2.9^{c}	3.0^{c}	2.9^{c}

[a]Represents the mean of four animals with four observations per animal ± SEM.

[bcd]Means in the same row which do not have a common letter in the superscript are different ($P < .05$)

Grinding effects on turnover rate

The passage rate of labelled long and finely ground prairie hay and whole and finely ground corn grain are presented in tables 6 and 7, respectively. No differences (P-.75) were detected between Yb and Dy labelled feedstuffs, indicating that these markers probably have similar validity. Grinding increased passage rate by 13% for roughages (P<.25) and 7% for corn grain (P<.10). Lack of differences significant at the 5% level of probability may be attributed to the low number of observations.

The time required for marker concentration in the duodenum or feces to peak (table 6) was influenced by sampling site and hay particle size. Peak concentrations at the duodenum for label on ground and long hay appeared at 14.0 and 18.3 hours after dosing, and fecal peaks were at 26.3 and 31.8 hours after dosing. Several authors (Blaxter et al., 1955; Grovum and Phillips, 1973; Grovum and Williams, 1973; Hartnell and Satter, 1979) have used the marker concentration during the early rapidly increasing portion of label excretion following a pulse dose of marker to mathematically describe a second marker pool which may be associated with the lower gut. Leverett, et al. (1977) have suggested that this gradual rise in marker concentration is an artifact of marker mixing in the reticulo-rumen or a second non-exiting coarse particle pool in the rumen rather than a second pool later in the digestive tract. The longer time required for large than for small particles to reach peak concentrations suggest that this time delay is related to ruminal particle size reduction. The similarity in estimates of turnover rate and time lag between particle sizes calculated from marker concentrations in digesta at along the digestive tract would question the existence of a post-

TABLE 6. PASSAGE CHARACTERISTICS OF INITIALLY LONG AND SHORT PARTICLES OF PRAIRIE HAY.

Animal	Marker	Initial particle size	Time (hr) to peak[a] marker concentration		Passage rate (%/hr)[b]			
			Duodenum	Feces	Duodenum	R^2	Feces	R^2
1	Yb	Short	11.2	27.3	3.9	>.99	3.9	>.99
	Dy	Long	16.4	32.7	3.0	>.99	3.1	>.99
2	Yb	Short	16.7	29.8	2.9	> .99	3.0	>.99
	Dy	Long	22.3	37.6	2.9	> .99	2.9	>.99
3	Yb	Short	14.1	21.9	3.4	> .99	3.5	>.99
	Dy	Long	16.2	25.2	3.1	> .99	3.0	>.99

[a]Values obtained by plotting ln makker concentration vs time and using best fit line to determine time required to achieve peak marker concentration.

[b]Values represent the slope of the ln marker concentration vs time (hr) decay curve after marker concentration had reached its peak.

TABLE 7. PASSAGE RATE OF CORN FED WHOLE OR GROUND THROUGH A 6 MM SCREEN.

		Ruminal passage rate (%/hr) for individual animals[a]											
Corn grain	Label	1		2		3		4		5		6	
			R		R		R		R		R		R
Whole	Yb	2.8	.94	3.7	.96	3.5	.93	-		-		-	
Ground	Dy	3.1	.97	4.0	.99	3.5	.98	-		-		-	
Whole	Dy	-		-		-		3.3	.92	4.1	.93	2.9	.96
Ground	Yb	-		-		-		3.8	.94	4.0	.97	3.4	.93

[a]Values represent the slope of the ln marker concentration vs Time (hr) decay curve after marker concentration had reached its peak.

ruminal mixing pool. Further, the time required for solutions of Co EDTA to peak in the rumen was longer when more solids were present in the rumen (R=.67; Teeter, 1981). With more solids present in the rumen, more time for mixing might be needed. Both particle size reduction and marker mixing therefore appear to be related to the time required for marker concentrations to peak following a pulse dose of marker.

Feed processing techniques that increase ruminal or total tract turnover rates can increase animal productivity only if rate of feed digestion is not proportionately decreased. Increased ruminal passage rates have been associated with increased feed intake of high forages rations (Baumgardt, 1970). But increased intake usually depresses digestibility (Blaxter, 1969). Some of the current feed processing methods (grinding, pelleting) increase animal productivity not by increasing digestibility, but by increasing the animal's ability to consume feed and extract only the most digestible feed fractions. Intake of digestible organic matter, not intake or digestibility alone, is the better predictor of animal productivity. Identification of physical factors controlling passage rate may permit one to develop processing techniques to increase both feed intake and feed digestibility.

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CHAPTER V

A DUAL MARKER--SINGLE SAMPLE TECHNIQUE FOR THE ESTIMATION OF RUMEN LIQUID VOLUME AND DILUTION RATE

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Summary

A dual marker-single sample technique to estimate volume and dilution rate (λ) of ruminal liquids was devised and tested in an in vivo experiment. The technique requires that two biologically similar digesta flow markers be pulse dosed in the rumen at intervals of 12 to 24 hours. Following marker equilibration, ruminal or fecal samples are obtained and the concentration of each marker relative to the dose is determined. Marker dosage and timing are crucial for accurate estimates and sample concentrations are expressed as a fraction of the original dose. Dilution rates and volumes are then calculated: Dilution Rate = (ln concentration$_2$/dose$_2$ - ln concentration$_1$/dose$_1$)/(Time$_2$ - Time 1); Volume = (Dose concentration)/(sample concentration/dose concentration e - $^{\lambda}$T).

In a trial with six steers (535 kg) ruminal liquid dilution rate and volume were estimated utilizing both conventional multiple sample methodology and the single sample technique. Results indicate that if the

two markers behave similarly, the single sampling technique has promise.

Introduction

Water soluble markers are frequently used to estimate volume and flow rate of ruminal liquids. Volume and dilution rate are important parameters since they influence the extent of ruminal fermentation, site of digestion, and efficiency of microbial growth (Bull et al., 1979). Historically, in vivo volume and dilution rate estimates have employed pulse dosing of a single marker followed by sampling of the labelled pool over a period of time. Marker concentration is analyzed and the natural log of this concentration is regressed against time. Hyden (1961) presented the mathematical derivation for these calculations. Dilution rate is estimated by the slope (percent/hour) and volume is calculated by relating the concentration at time zero to the original dose. Unfortunately, there are complications. These include: (1) labor and concentration differences at various sampling sites, (2) less than instantaneous marker mixing, (3) water and/or food consumption, and (4) alterations in dilution rate over time.

Precision in rumen liquid volume and dilution rate estimates is increased through obtaining repeated samples over a long period of time. It would be helpful, especially with non-fistulated animals, to sample ruminal contents only once to reduce animal stress. The objectives of this experiment were 1) to derive a dosing system to reduce the number of samples required to estimate rumen volume and dilution rate and 2) to test this system in vivo.

Materials and Methods

Multiple sampling technique

Mathematical considerations. The standard procedure for estimating pool volumes and turnover involves pulse dosing of a marker followed by multiple sampling of the pool. This can be called the multiple sampling procedure. Outflow of tracer (water soluble marker) and tracee (ruminal liquid) is considered to be a fractional loss (percent/hour). Consequently, during any given time, loss of tracer is the same as tracee. However, the pool size for tracer continually declines while tracee pool size remains constant. Therefore, marker concentration decreases exponentially over time: $q_t = q_0 e^{-\lambda T}$ where q_0 is quantity of tracer present at time zero with instantaneous mixing and q_t is the quantity present at time T. This mathematics is similar to that of radioisotope kinetics.

The slope (λ) of the tracer decay curve, which is used to estimate tracee dilution rate, becomes constant once the tracer is adequately mixed with the tracee. This slope is determined by measuring concentrations of tracer at two times, q_1 and q_2, at sampling times T_1 and T_2. Then:

$$\lambda = \frac{\ln q_1 - \ln q_2}{T_2 - T_1}$$

Regressions over longer times add to experimental but not necessarily to statistical confidence in the values.

Dual marker technique

A second more novel approach to the dilution problem is to dose the pool with a second marker at a specific time (T_2) after the first marker. This will be called the dual marker procedure. Tracer outflow

can be determined by difference in concentration of the two markers, a and b, expressed as a fraction of the dose of each marker, A and B. Then:

$$\lambda = \frac{\ln a/A - \ln b/B}{T_2 - T_1}$$

Knowledge of the size of the dose is crucial for both volume and dilution rate estimates with the double marker technique whereas dose size need not be measured to calculate dilution rate with the multiple sampling technique. Volume estimates are obtained utilizing the determined dilution rate (λ) and the exponential equation (Equation 1) to solve for marker concentration at zero time. Volume is calculated from initial marker concentration and marker dosage.

Experimental

Ethylene-diamine-tetra-acetic acid (EDTA) complexes of cobalt and chromium were prepared as specified by Dwyer et al. (1954) and Dowens and McDonald (1964), respectively. A ytterbium-EDTA complex was prepared by boiling .18 m ytterbium with .25 m EDTA for five hours. Any unbound ytterbium was removed by addition of 50 g cottonseed hulls per liter and allowing the mixture to equilibrate for a minimum of 24 hours before filtration and ruminal dosing with the filtrate. All solutions were diluted such that 500 ml would contain approximately 10 g of the respective metal.

Six mature ruminally cannulated steers (535 kg) were used in a completely randomized experimental design. Steers were fed at a level of 1.9% of body weight. The ration contained 45% roughage (table 1) and was fed in two equal portions twice daily for the first 10 days of the 15 day adaptation period and at four equally spaced times each day for

TABLE 1. COMPOSITION OF RATION USED IN EXPERIMENT 1.

Ingredient	%
Cracked corn	45.0
Chopped alfalfa hay	45.0
Supplement	10.0

the remaining five days of the adaptation and the two-day collection periods. On day 16, at a time equally spaced between feedings (time = 0 hr) all steers received intraruminally a 500 ml pulse dose of the ytterbium EDTA solution (20,191 ppm Yb); 6 hours later three steers (#1, 2, and 3) received 500 ml of the cobalt EDTA solution (20,097 ppm Co) while the other three steers (#4, 5, and 6) received a 500 ml dose of the chromium EDTA solution (20,018 ppm Cr). For the final marker administration, at hour 30 (day 17), the former three steers received 500 ml chromium EDTA while the latter three steers received 500 ml Co EDTA. Rumen samples were taken at 0, 6, 12, 18, 24, 30, 36, 42, 48, and 54 hours of the collection period. Sampling times were all 3 hours after a meal. All samples were analyzed by atomic absorption spectroscopy.

Results and Discussion

Estimates of ruminal liquid dilution rates are presented in table 2. Dilution rates for the water soluble markers, utilizing the single marker equations, were similar (P=.7) and dilution rate appeared to be constant throughout the dosing period. Flow rates estimated by the dual marker-single sample equations did not differ from estimates based on single markers using regressions across four to nine sampling times (P=.6).

EDTA complexes of cobalt and chromium gave similar rumen volume estimates (table 3) while volume estimates calculated from ytterbium EDTA were about 13.7% greater (P<.05). Volume estimates obtained previously with simultaneous dosing into the rumen of Yb, Cr, Co, and Fe EDTA complexes and PEG (MW 20,000) (Teeter, 1981) gave similar estimate volumes which closely approximated volume determined by total evacuation of rumen

TABLE 2. RUMINAL LIQUID DILUTION (%/HR) RATE FOR SINGLE AND DOUBLE MARKER TECHNIQUE.

Marker and Technique	Steer					
	1	2	3	4	5	6
Single marker						
Yb EDTA	8.6	7.5	8.4	9.6	7.6	6.5
Co EDTA	-	-	-	8.9	7.3	6.9
Cr EDTA	9.1	7.3	8.0	-	-	-
Dual marker						
Co EDTA/Cr EDTA						
@ 36 hr	9.2	7.0	7.7	8.9	7.2	6.5
@ 42 hr	9.9	6.7	8.6	9.3	7.1	6.4
@ 48 hr	9.9	7.5	7.5	8.0	6.7	6.2
@ 54 hr	9.4	7.0	7.9	8.4	7.2	6.3
Yb EDTA/Co EDTA						
@ 36 hr	9.2	7.0	8.3	9.6	7.0	6.7
@ 42 hr	8.9	7.0	8.4	9.8	7.2	6.2
@ 48 hr	9.1	6.3	8.9	9.3	7.0	6.1
@ 54 hr	8.0	6.1	8.1	9.4	6.6	5.7

TABLE 3. RUMEN LIQUID VOLUME ESTIMATES FOR SINGLE AND DOUBLE MARKER TECHNIQUES.

Technique Used for Volume Estimation	Steer 1			2			3			4			5			6		
	Yb	Cr	Co	Yb	Cr	Co	Yb	Cr	Co	Yb	Cr	Co	Yb	Cr	Co	Yb	Cr	Co
Single marker	63	60	56	55	48	53	67	65	65	53	45	47	52	51	54	64	59	56
Double marker Cr/Co																		
@ 24 hr	-	57	-	-	58	-	-	66	-	-	-	-	-	-	-	-	-	-
@ 36 hr	-	54	-	-	61	-	-	64	-	-	-	-	-	-	-	-	-	-
@ 42 hr	-	46	-	-	68	-	-	57	-	-	-	-	-	-	-	-	-	-
@ 48 hr	-	39	-	-	71	-	-	69	-	-	-	-	-	-	-	-	-	-
@ 54 hr	-	42	-	-	83	-	-	59	-	-	-	-	-	-	-	-	-	-
Double marker Co/Cr																		
@ 24 hr	-	-	-	-	-	-	-	-	-	-	-	46	-	-	52	-	-	64
@ 36 hr	-	-	-	-	-	-	-	-	-	-	-	44	-	-	51	-	-	65
@ 42 hr	-	-	-	-	-	-	-	-	-	-	-	41	-	-	49	-	-	72
@ 48 hr	-	-	-	-	-	-	-	-	-	-	-	56	-	-	59	-	-	82
@ 54 hr	-	-	-	-	-	-	-	-	-	-	-	49	-	-	48	-	-	90

contents. Ellis et al. (1981) has proposed that some feeds will bind water soluble markers and thereby cause erroneously high volume estimates. They have suggested that preliminary dosing with an innocuous marker can saturate these sites and solve this problem. Volume estimates obtained with the double marker technique were similar to single marker estimates, although precision for the estimates declined with increasing time after dosing, especially after 36 hours. This could be due to a differential affinity of solids for specific markers or to an increase in the relative sampling and analytical error as marker concentration declines. Markers exhibiting differential flow rates, due to binding, would be expected to have reduced precision as incubation time is increased and exhibit an increasing or decreasing estimates of ruminal volume over time. No trend of this type was apparent.

The double marker-single sample technique appears to be a viable alternative to the pulse-dose multiple sample procedure for the estimation of ruminal volume and dilution rate with samples taken up to 36 hours post-dosing. Costs in any marker trial include: (1) the marker, (2) marker analysis, (3) labor for dosing, and (4) labor for sampling. Use of the dual marker-single sampling procedure will increase costs for marker and analyses but could substantially reduce animal stress and human labor. The dual marker-single sampling procedure may prove useful for estimating ruminal turnover from fecal concentrations of markers with a large number of animals where animals are fed individually.

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CHAPTER VI

DIRECT RUMINAL ESCAPE

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Summary

A dual marker technique was employed to estimate direct ruminal escape of the liquid fraction of a ration fed to steers. The technique involved dosing of biologically similar digesta flow markers administered by two different methods. One marker was mixed with the meal and fed while the other marker was administered through a ruminal cannula. The amount of oral marker equilibrating with the ruminal marker was considered to be equal to the fraction mixing with ruminal contents. Bypass was calculated by difference. The following equations were employed: Marker bypass (%) = (Dose concentration, ruminal - dose concentration, oral)/ dose concentration, ruminal. Alternatively, if multiple samples are obtained and rumen volume is estimated, marker bypass = (Rumen volume from oral marker - Rumen volume from ruminal marker)/Rumen volume from oral marker. Bypass of Co EDTA, a water soluble marker, was estimated using six steers (504 kg) fed an 80% concentrate diet at a level of 1.5% of body weight daily. Markers were administered by alternating oral and ruminal doses within split periods of a replicated 3 X 3 latin square experiment. Bypass of Co EDTA was estimated to be 5.7%. Results indicate

that bypass of feed and liquids may be estimated with digesta flow markers.

Introduction

Optimal ruminant production efficiency is achieved if certain ration constituents are digested in the rumen while others are digested in the intestines. Hemicellulose and cellulose are used poorly past the rumen (1). But more readily digested carbohydrates yield 11 to 30% more energy (2) and well-balanced proteins have a higher biological value (3, 4) when digested post-ruminally. Physically bypassing the rumen for intestinal digestion of those nutrients which can be utilized post-ruminally should improve nutrient utilization.

Passage of some strained hay particles to the omasum has been observed to occur as soon as rations are consumed (5). Similarly, abomasal samples of forage-fed steers revealed protein supplement at the abomasum within minutes after the supplement was fed (6). This suggests that consumed feedstuffs may pass out of the rumen to the omasum without complete mixing with ruminal contents. Such physical bypass must not be confused with ruminal escape of digestion-resistant feed components. Such direct bypass needs to be quantitatively estimated and possibly employed to increase productivity of ruminant animals. The objectives of the following experiments were 1) to derive a technique for the estimation of direct ruminal bypass, and 2) to test this technique in vivo

Materials and Methods

Mathematical

Ruminal volume or pool size can be estimated from the quantity of

marker dosed and the ruminal concentration at zero time. Such calculations assume both instantaneous and quantitative mixing of the marker. However, site of marker administration might be expected to influence the quantity of marker equilibrating with the ruminal contents. Due to the anatomy of the rumen, an ingested marker has more potential for physical bypass than a ruminally administered marker. Physical bypass of the marker would cause overestimation of rumen volume. In contrast, delayed equilibration of a ruminally dosed sample would cause underestimation of ruminal volume. Physical bypass could lead to gross overestimates of volume when markers are fed rather than ruminally dosed. Alternatively, feeding one marker while ruminally dosing the other permits one estimate direct bypass of the fed marker since the ruminal pool size will be quantitatively overestimated.

Direct ruminal bypass was calculated as follows:

$$\% \text{ Marker Bypass} = \frac{\text{Dose Concentration}_{\text{Ruminal}} - \text{Dose Concentration}_{\text{Oral}}}{\text{Dose Concentration}_{\text{Ruminal}}}$$

or

$$\% \text{ Marker Bypass} = \frac{\text{Ruminal Volume}_{\text{Oral}} - \text{Ruminal Volume}_{\text{Ruminal}}}{\text{Ruminal Volume}_{\text{Oral}}}$$

Assumptions include: 1) that ruminally administered markers equilibrates completely with rumen digesta, and 2) equilibration of orally administered markers is solely dependent upon lack of rumen bypass.

Experimental

Six ruminally cannulated steers (604 kg) were fed twice daily at a daily rate of 1.5% of body weight. The 85% concentrate ration was fed

TABLE 1. RATION COMPOSITION.

Ingredient	% of Ration
Corn, rolled	75.8
Cottonseed hulls	10.0
Alfalfa meal	4.0
Molasses, cane	4.0
Cottonseed meal	4.5
Limestone	0.75
Urea	0.5
Dicalcium phosphate	0.15
Salt	0.3
Vitamin A	0.015

three weeks prior to initiation of this experiment. All feed was consumed within minutes after feeding. Animals were allotted to treatment utilizing a three period completely randomized design to permit multiple observations with each animal. In each 21 day period, the first 7 were for adjustment while days 8-14 and 15-21 were used for liquid marker administration and ruminal sampling. Co EDTA prepared by the method of Dwyer (7) was either incorporated into the feed or dosed into the rumen through the ruminal cannula. Site of marker administration (oral or ruminal) was randomized between the 8th and 15th day of each period. Rumen samples were obtained at 12, 24, 36, and 48 hours post-dose. Samples were analyzed for Co with an atomic absorption spectrophotometer and ruminal volume and marker bypass estimated.

Results and Discussion

Oral and ruminal volume estimates for steers dosed with Co EDTA are presented in table 2. Oral administration of marker tended ($P<.08$) to yield higher rumen volume estimates. This leads to an estimate of direct ruminal bypass of 5.7% for the Co EDTA. Alternative explanations for this observation include: 1) marker absorption in the mouth or esophagous, 2) failure of the dose to be quantitative, and 3) shorter equilibration time for oral dosing than ruminal dosing. These explanations are questioned by various other experiments. Only 3% (8) and .8% (9) of ruminal and orally administered Co EDTA doses respectively were recovered in the urine of cattle suggesting that absorption should be minimal. Finally, the dispersion rate of orally dosed liquid markers would be expected to be similar to those ruminally dosed since regressions of the logarithm of concentrations appeared linear over the 48 hour

TABLE 2. RUMEN VOLUME AND % MARKER BYPASS ESTIMATES[a] OBTAINED BY EITHER RUMINAL OR ORAL CO EDTA PULSE DOSING.

Site of Administration	Animal					
	1	2	3	4	5	6
Oral	71.9 ± 2.2	56.0 ± 3.0	117.6 ± 1.9	75.5 ± 3.3	67.9 ± 5.9	91.2 ± 3.1
Ruminal	64.0 ± .99	57.7 ± 6.5	101.6 ± 1.8	70.3 ± 6.5	70.2 ± 6.6	85.1 ± 4.2
% Bypass[b]	10.7 ± 3.9	-1.5 ± 9.0	13.4 ± 2.9	7.1 ± 5.8	-5.0 ± 4.7	9.4 ± 5.4

[a]Estimates represent the mean of three samples ± SEM.
[b]Mean bypass ± SED for each of the three periods.

sampling period.

If liquid markers bypass without equilibration with rumen contents, solids may also bypass. One might expect greater direct bypass with solids than liquids providing passage is not inhibited by particle size or density factors because dispersal by diffusion should be slower.

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CHAPTER VII

INFLUENCE OF ROUGHAGE SOURCE AND LEVEL ON DIGESTION OF STARCH FROM WHOLE SHELLED CORN

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Summary

The effect of roughage source on whole shelled corn (WSC) digestion was investigated in two experiments with steers. In the first trial steers received 7.3 kg of a basal diet (WSC plus supplement) or this basal diet with 4.3 kg of added cottonseed hulls or chopped alfalfa hay. Rate of ruminal disappearance of dry matter and starch from corn in nylon bags was greatest for steers receiving added alfalfa. Ruminal pH was increased by both roughages. Ruminal retention time of liquid and ytterbium labelled corn was reduced by adding either roughage. Fecal starch was decreased slightly with alfalfa added to the ration and decreased markedly by added hulls. Starch digestibility was significantly reduced by added alfalfa and increased by added hulls. Particles greater than 8 mm in size consisted essentially of whole corn and contained the majority of fecal starch. Coarse corn particles accounted for the effect of roughage source on starch digestion. In the second experiment, steers received the basal diet of the first experiment with chopped alfalfa or cottonseed hulls substituted for 10 or 37% of the ration. One group of

four steers was fed at a maintenance level of energy intake while eight steers were fed 1.8 times their maintenance energy requirement. Animals fed at maintenance rations of WSC, WSC + 10% Alf, WSC + 10% CSH, WSC + 37% Alf, WSC + 37% CSH produced feces containing starch percentages of 6.2, 7.6, 8.5, 7.0 and 3.7% of fecal dry matter for starch digestibilities of 99.2, 98.3, 98.1, 96.5 and 97.9%. Animals fed at 1.8 times maintenance produced feces containning 19.0, 10.3, 5.0, 11.4 and 4.9% starch for starch digestibilities of 96.3, 97.3, 98.9, 93.9 and 96.6. Results demonstrate that starch utilization from WSC rations differ with roughage source, roughage level, and feed intake level.

Introduction

Cereal grains

Cereal grains fed to both dairy and feedlot cattle are generally subjected to extensive grain processing. With increased costs of energy for processing, feeding of whole rather than processed grain may become economically advantageous if the reduction in starch digestion can be minimized. Though roughage levels have received research attention (Cole et al., 1976; Vance and Preston, 1971), roughage sources have received little research attention despite field reports of marked differences in the usefulness of various roughages with whole shelled corn diets.

Roughages are generally considered to be of limited energy value and often are more costly sources of net energy for gain than cereal grains. Roughages are included in corn-based rations to enhance palatability, and to supply bulk which aids in the prevention of displaced abomasums, acidosis, and liver abcesses. With high concentrate rations, roughage characteristics such as particle size, wettability, or density

generally receive less attention than when a higher level of roughage is fed. Yet, responses to added roughage have been variable. White et al. (1969) found that daily gain, ration intake, and rumen pH were considerably greater with 20% rice straw addition than with alfalfa hay, rice hulls, or oyster shells added to a 61% concentrate ration. Balch (1971) observed large differences between roughages in the time cattle spent eating and ruminating. This could alter the digestibility not only of roughages but also of other ration ingredients which benefit from particle size reduction prior to intestinal digestion. In situ studies with whole shelled corn (Srivastava and Mowat, 1978) indicate that intact corn grain is almost inert to ruminal digestion. Therefore, greater mastication to physically reduce the size of corn kernels could enhance utilization of nutrients from whole corn grain. The following experiments were conducted to evaluate the influence of two roughage sources which differ in physical characteristics on whole shelled corn digestion in the rumen and total digestive tract.

Materials and Methods

Experiment 1

Seven mature ruminally cannulated 600 kg steers were used in a 3 X 7 Youden square experimental design. The three treatments were: (1) basal ration (table 1) fed at 7.3 kg daily, (2) basal plus 4.3 kg cottonseed hulls, and (3) basal plus 4.3 kg chopped alfalfa hay. Steers were housed in metabolism crates and fed equal amounts of the basal ration twice daily. Each period of the Youden square consisted of a 28 day period with the first 14 days for adjustment to the new ration. Total fecal output was collected on days 15 through 20 of each period. Feces

TABLE 1. RATION COMPOSITION[ab]

	Basal ration, %
Corn grain[b]	89.21
Pelleted supplement	
Corn grain	7.39
Urea	0.74
Dicalcium phosphate	2.03
KCl	0.61
Vitamin A	+
Vitamin D	+

[a]Dry matter basis.

[b]Analyzed 9.8% crude protein and 67.1% starch on a dry matter basis.

were weighed daily and a 15% aliquot stored at -6 C in a polyethylene bag. The daily aliquots were composited. A subsample was washed consecutively through 8, 4, 2, and 1 mm screens and the residue caught on each screen was collected. Another subsample as well as the seived residues were dried at 55 C, ground, and analyzed for starch at total oligosaccarides (Macrae and Armstrong, 1969). Days 21 through 24 of each period were used for *in situ* incubation with six of the steers forming a replicated 3 X 3 latin square experimental design. Whole shelled corn (2.5 g), corn that had been scratched to remove a 25 X 5 mm section of the pericarp, and corn ground through a 6 mm or a 3 mm screen were placed in nylon bags with a pore size of 105 μ and incubated *in situ* for 1.5, 3, 6, 12, 24 and 48 hours. On removal from the rumen, bags were thoroughly washed under tap water and dried first at 55 C for 48 hours and subsequently at 95 C for six hours. Dried bags were weighed and contents were ground and analyzed for starch. On day 25 of each period, steers were fed whole shelled corn labelled with ytterbium (Teeter *et al.*, 1979) with their morning meal. In addition, each steer received 250 ml of Co EDTA (9 g Co) intraruminally. Co EDTA was prepared by the method of Dwyer *et al.* (1955). Rumen and fecal samples were obtained at 12 hour intervals for the next 72 hours. Samples were analyzed for Yb and Co using an atomic absorption spectrophotometer with nitrous oxide combustion.

Experiment 2

Twelve growing hereford steers with a mean initial weight of 277 kg were allotted to three groups of similar initial weight. Four steers were fed a maintenance level of intake, while the other eight steers were fed 1.8 times maintenance for the four experimental periods. Rations were divided into two equal portions and fed twice daily. Five rations were

fed to each group of four steers for four periods in a partially balanced incomplete latin square experimental design. The five treatments were: (1) whole shelled corn (WSC), (2) WSC + 10% cottonseed hulls, (3) WSC + 37% cottonseed hulls, (4) WSC + 10% chopped alfalfa hay, and (5) WSC + 37% chopped alfalfa hay. The pelleted ration supplement is shown in table 2. Each period consisted of 17 days with 12 days adjustment to the ration. Total fecal output was collected on days 13 through 17. Feces were weighed daily and aliquots of 100, 30 and 15% were obtained from the steers fed 0, 10 and 37% added roughage treatments, respectively. Aliquots were stored in polyethylene bags at -6 C, composited and a subsample washed through an 8 mm screen and the residue collected. A second subsample as well as the seived residues were dried at 55 C and analyzed for starch content.

Results and Discussion

Trial 1

Ration dry matter digestibility was reduced with roughage addition (table 3). This reflects lower digestibility of the roughage than of corn grain. Calculated by difference, the digestibility of the added alfalfa was 47% and of cottonseed hulls was 42%. The value is lower than the NRC value of 53% for TDN of midbloom alfalfa hay, but the cottonseed hull value is similar to the NRC value of 41%. These differences may reflect associative or level of intake effects on digestibility. Addition of either roughage reduced fecal starch percentage. This would be expected from dilution of fecal starch by additional fecal dry matter. Fecal starch is of greatest value for estimating digestibility when coupled with fecal output. Starch digestibility differed with roughage

TABLE 2. SUPPLEMENTED COMPOSITION (PELLETED).

Ingredient	%
Dehydrated alfalfa meal	1.04
Soybean meal	60.0
Cottonseed meal	22.7
Calcium carbonate	10.5
Potassium chloride	2.5
Salt	2.7
Rumensin (60 gram)	0.17
Tylan 40	0.09
Vitamin A 10m/lb	0.15
Trace mineral	0.15

TABLE 3. DRY MATTER AND STARCH DIGESTIBILITY.

	Roughage			
	Basal	+ 37% Alfalfa	+ 37% CSH	Pooled SE
Dry matter intake (kg)	7.3[a]	11.6[b]	11.6[b]	-
Fecal dry matter (%)	32.6[a]	25.5[a]	28.8[a]	1.54
Fecal dry matter (kg/day)	1.2[a]	3.5[b]	3.7[b]	0.24
Dry matter digestibility	82.3[a]	69.8[b]	68.1[b]	1.40
Fecal starch (%)	25.5[a]	20.4[a]	3.9[b]	1.80
Fecal starch (g/day)	362[a]	793[b]	174[c]	72.1
Starch digestibility (%)	91.7[a]	82.4[b]	96.2[c]	1.84
Ruminal pH	5.7[a]	6.5[b]	6.2[b]	0.12

[a,b,c]Values in a row with unlike superscripts differ significantly ($P<.05$).

source. Cottonseed hull addition enhanced ($P<.05$) while chopped alfalfa chopped alfalfa hay addition reduced ($P<.05$) starch digestibility. With alfalfa added to the ration, copious quantities of whole corn grain were caught by the 8 mm seive (table 4) during seiving of fecal matter while with cottonseed hulls in the ration, few corn kernels were detected. Starch distribution is illustrated graphically in figure 1. Starch analysis of samples retained by the 8 mm screen revealed 54.2% starch. The whole shelled corn fed in this trial analyzed 67.1% starch. These values support the suggestion of Srivastava and Mowat (1978) that only small quantities of starch can be digested from intact corn kernels.

Results of in situ incubation of corn samples of various particle sizes are presented in figures 2-5. The samples obtained at 1.5 hours of incubation were not influenced by diet ($P>.6$) and were assumed to have undergone no digestion. Dry matter or starch disappearance from the 1.5 hr samples is thought to be due to flushing of soluble components of corn grain or small particles of corn grain out of nylon bags either by rumen fluid or by tap water during washing. The 1½ hr disappearance of starch and dry matter therefore was used to adjust weight recoveries obtained at other incubation times so that digestion could be more closely estimated. Adjusted starch and dry matter (%) disappearance appeared linear with time for up to 24 hours. The 3, 6, 12 and 24 hr incubations were used to estimate rate of disappearance by regressing percent disappearance on time (table 5).

Dry matter and starch disappearance from nylon bags was altered by roughage content of the diet, grain particle size, and time. Alfalfa addition to the basal ration consistently enhanced the rate and extent of starch disappearance over cottonseed hulls and the basal diet. Starch

TABLE 4. SIEVED FECAL SAMPLES, TRIAL 1.

	Basal sieve size				Basal + 40% Alfalfa sieve size				Basal + 40% CSH sieve size			
	8	4	2	1	8	4	2	1	8	4	2	1
Trial 1												
Dry matter retained (%/day)	34.8± 5.9	22.8± 3.1	12.0±1.5	7.0± .8	26.5± 5.3	15.7± 1.2	19.4±2.9	11.7±1.1	4.4± 2.3	15.4± 3.9	26.0±3.3	22.4±4.0
Starch (%)	57.3± 2.1	9.6± 1.9	3.7±1.4	3.7±1.4	62.6± 1.8	33.7± 6.2	2.6±0.4	0.9± .3	42.7± 5.5	10.2± 2.0	2.9±0.43	2.0±0.3
Starch (g/day)	284. ±91.5	71.6±43.1	12.9±3.4	5.8±1.9	578.3±47.6	18.9±58.5	13.7±3.2	3.3±1.4	61.7±35.6	71.6±28.3	25.8±9.0	11.6±2.9

[a]Values expressed as means ± SEM.

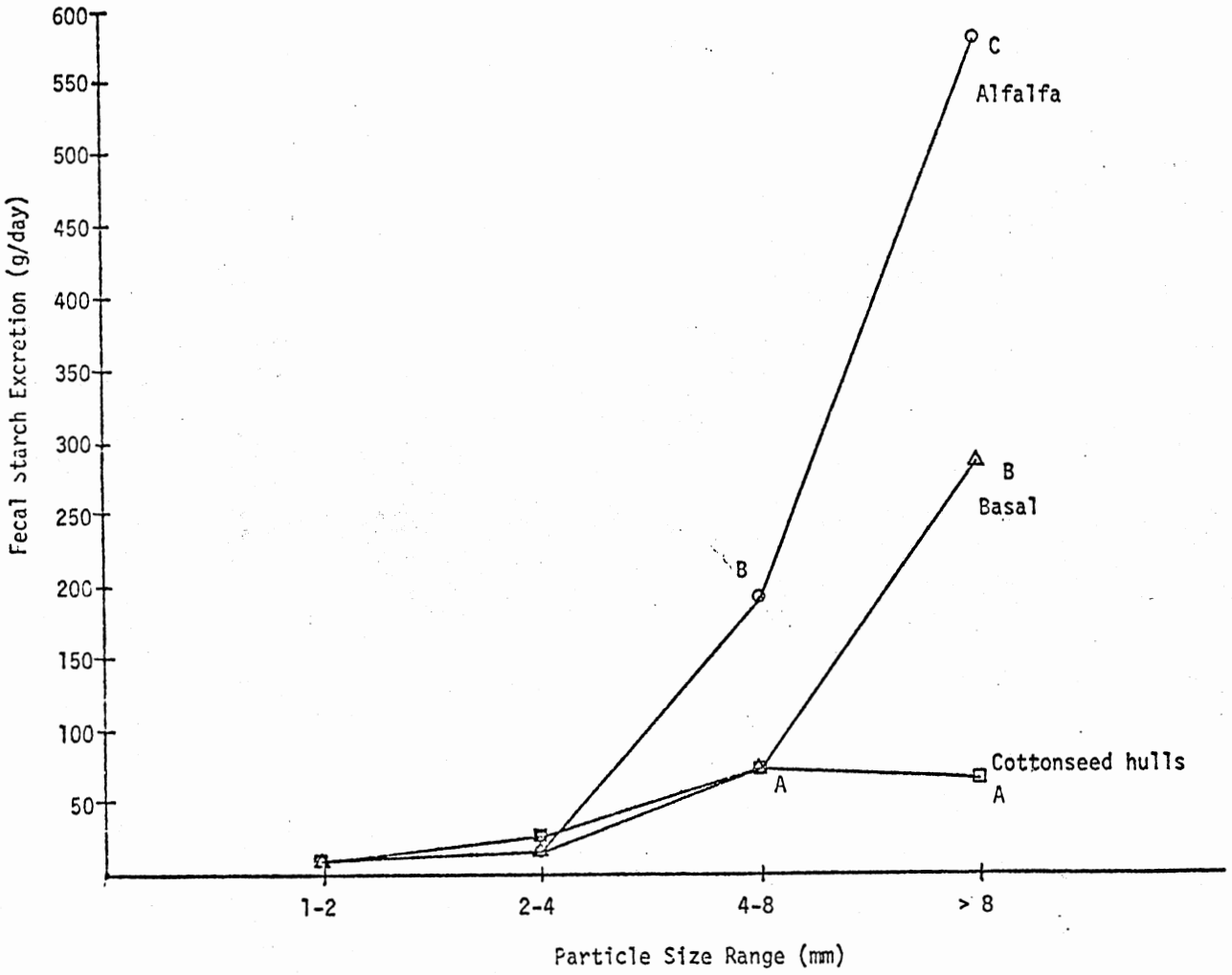

Figure 1. Starch distribution in feces.

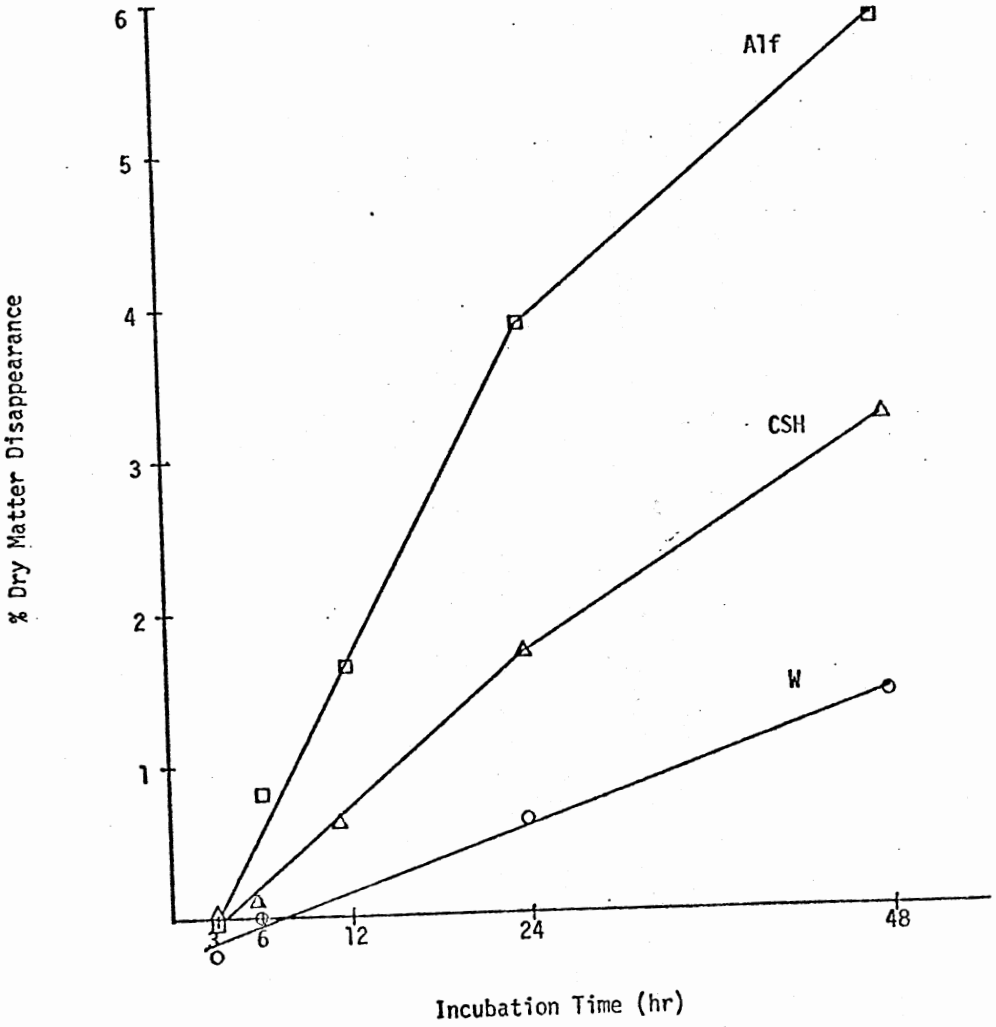

Figure 2. In situ dry matter disappearance of WSC.

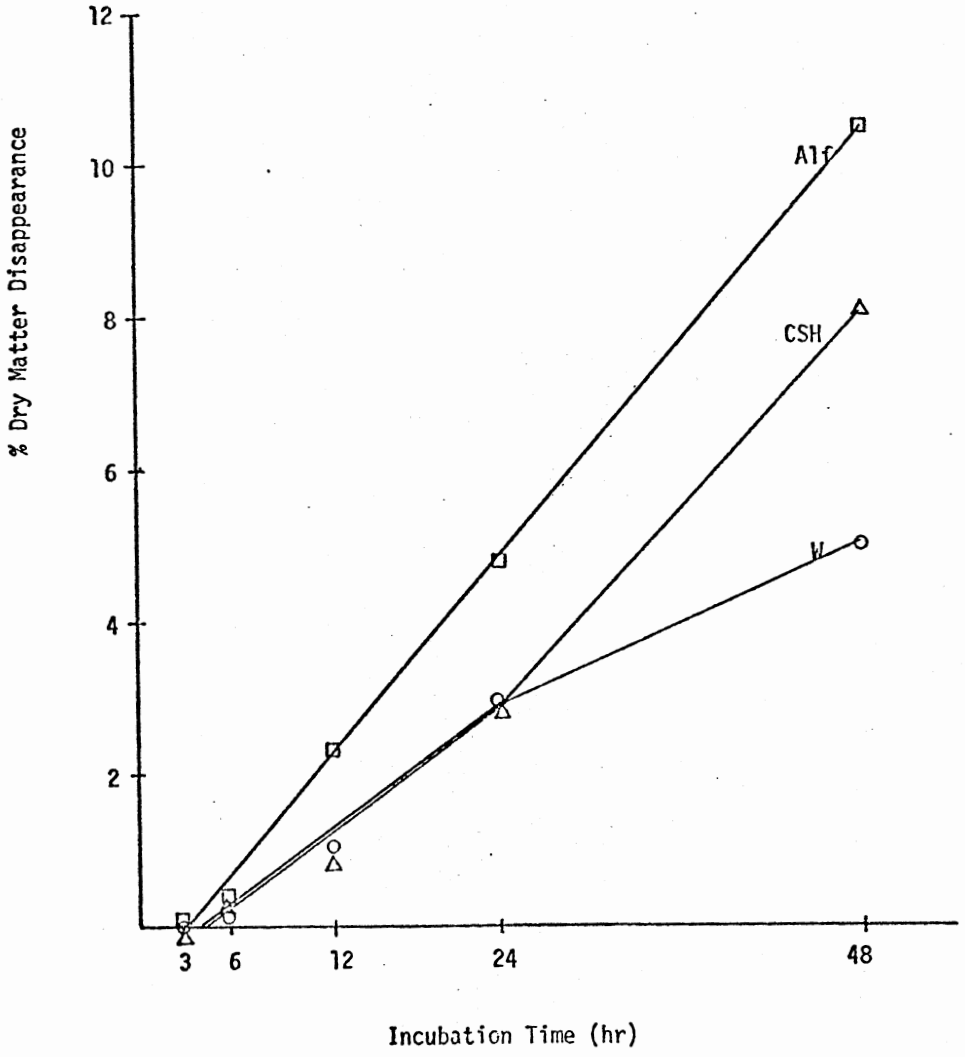

Figure 3. In situ dry matter disappearance of scratched WSC.

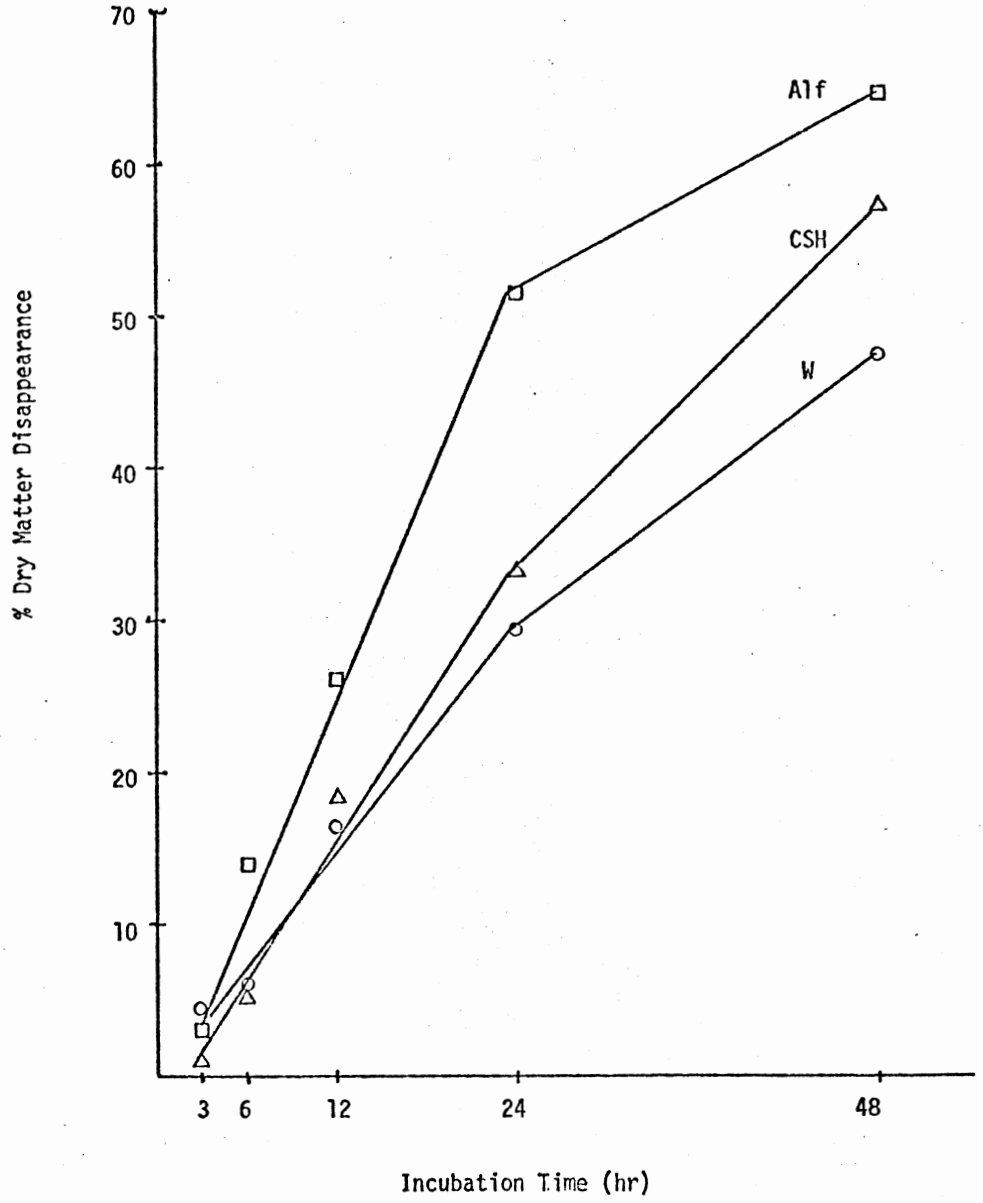

Figure 4. In situ dry matter disappearance of WSC ground through a 6 mm screen.

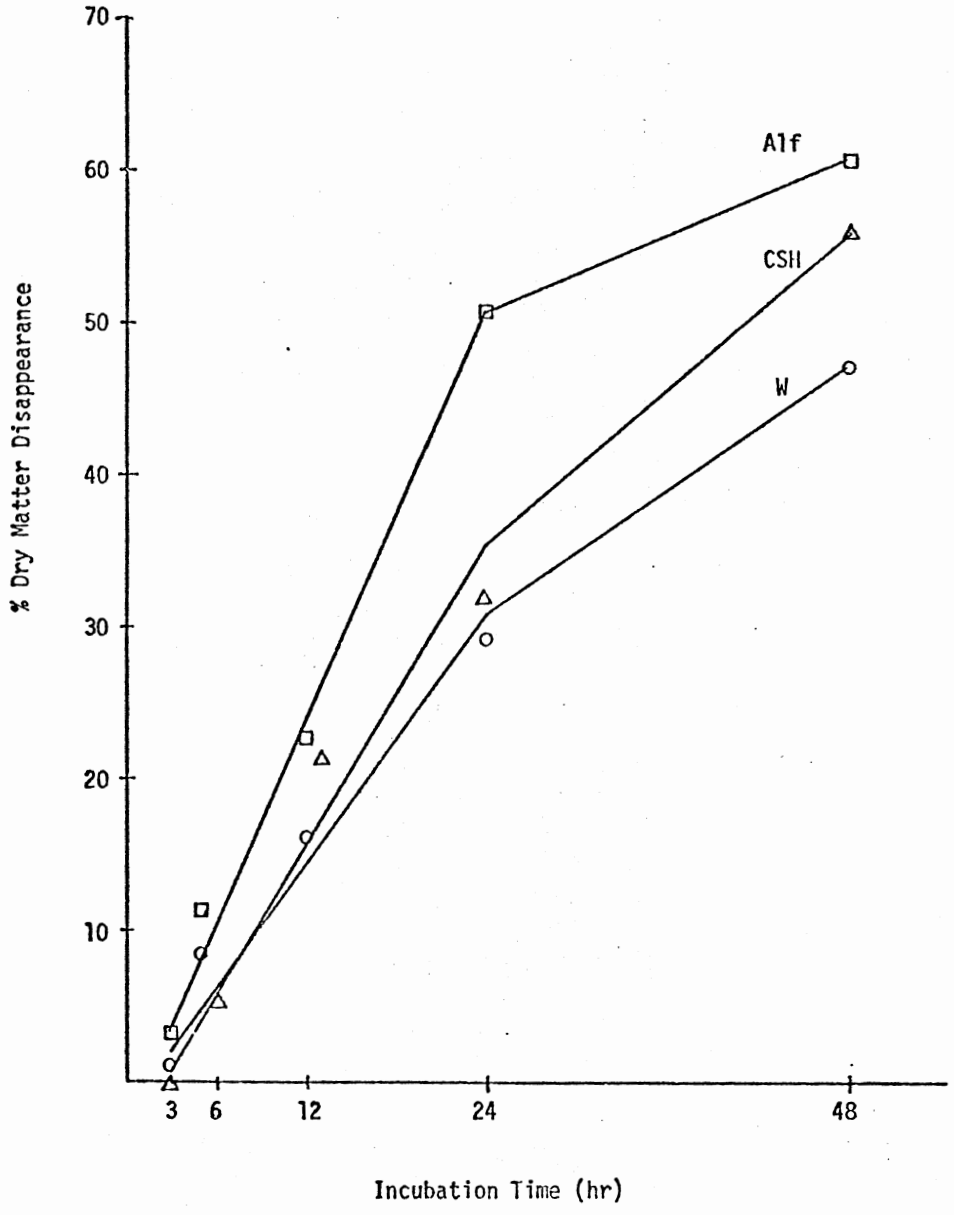

Figure 5. In situ dry matter disappearance of WSC ground through a 3 mm screen.

TABLE 5. RATE OF DRY MATTER AND STARCH DISAPPEARANCE FROM NYLON BAGS INCUBATED IN SITU.

	Diet of Steer			
	Basal	+ 37% Alfalfa	+ 37% CSH	SED
	%/hr			
Dry matter[a]				
WSC	0.08[b]	0.18[b]	0.08[b]	.01
Scratched WSC	0.14[b]	0.24[c]	0.15[c]	.03
Ground WSC (6 mm)	1.23[c]	2.23[c]	1.53[d]	.19
Ground WSC (3 mm)	1.28[c]	2.24[c]	1.52[d]	.19
Starch[a]				
WSC	0.04[b]	0.07[b]	0.06[b]	.02
Scratched WSC	0.08[b]	0.15[b]	0.10[b]	.01
Ground WSC (6 mm)	2.65[c]	3.26[c]	2.81[c]	.12
Ground WSC (3 mm)	2.90[d]	3.60[d]	3.40[d]	.14

[a] Values represent the mean slope of six regression of percent disappearance on time of incubation.

[b,c,d] Values in a row and classification with unlike superscripts differ significantly ($P<.05$).

content of whole kernels decreased by a mean of 1.8% while dry matter content declined by 3.8% at 48 hours. Disappearance of starch and dry matter for corn ground through 6 and 3 mm screens progressed at much higher rates than for whole shelled corn. Starch of whole corn kernels appear quite stable to ruminal digestion. Disappearance rate from nylon bags includes not only digestion but also filtration through the pores. Yet, comparisons within a particle size among steers fed different roughages should be valid. Ruminal digestion rate with steers fed the corn with 37% alfalfa added proceeded more rapidly than with steers fed no supplemental roughage. In the same comparison, CSH feeding increased starch disappearance from nylon bags by 50%. This difference may be attributed to the increase in ruminal pH (table 2), either due to reduced VFA production in rumen, to a greater salivary input of buffers, or to some factor intrinsic to the roughage.

Passage rates for the liquid marker and ytterbium which was attached to the fed corn grain are presented in table 6. Addition of either roughage increased ($P<.05$) dilution rate for both liquids and solids. An increased rate of passage of both liquid and solids might be expected as dry matter intake increases unless rumen fill changes. Dilution rates for liquid passage through the total tract averaged almost 10% greater than for the rumen alone and reflect greater variation in ruminal than fecal samples. Parallelism between dilution rates for the rumen and the total tract supports the concept that flow may be explained by a two pool model (Ellis et al., 1979). Cottonseed hull addition produced a greater ($P<.05$) total tract dilution rate of solids and liquids than alfalfa hay. This may be due to differences in physical or passage properties between the two roughages. The higher dilution rate for corn solids with cotton-

TABLE 6. WHOLE CORN AND LIQUID DILUTION RATES FOR TRIAL 1.

Fraction	Segment of digestive tract	Diet: Basal	Diet: Basal + 37% alfalfa	Diet: Basal + 37% CSH	SEM
		Dilution rate, % hr			
Liquid (Co EDTA)	Rumen	2.2[a]	6.4[b]	6.7[b]	0.18
Liquid (Co EDTA)	Total tract	2.4[a]	6.7[b]	7.7[c]	0.21
Yb labelled whole corn	Rumen	2.0[a]	2.2[a]	2.9[b]	0.18
Yb labelled whole corn	Total tract	1.9[a]	2.4[b]	2.9[c]	0.12

[a,b,c]Means in a row with different superscripts differ significantly ($P<.05$).

seed hull than alfalfa in the ration may be due to: (1) smaller mean particle size, (2) altered density, (3) changed ruminal mixing properties, or (4) greater influx of saliva due either to increased chewing or rumination time. Fecal starch distribution would support the first suggestion while the appearance of ruminal contents would support the third. When steers were fed alfalfa, a layered mat was noted in the rumen and whole corn was settled and separated from this mat. Increased mastication may have been responsible for greater starch digestion with cottonseed hull than alfalfa supplemented ration.

Increased rates of passage should reduce the time available for digestion and would be expected to reduce digestibility of whole shelled corn if digestion rate were constant. Considering first the enhanced digestion rate across particle sizes with the longer retention time with alfalfa than with cottonseed hull added to the diet, together with longer ruminal retention of corn with alfalfa than with cottonseed hulls, one might predict greater starch digestibility with alfalfa. This is contrary to the observed effect. This indicates that for such calculations to be meaningful, rate of digestion and dilution rate must be quantitated using particulates of the size being digested.

Trial 2

When feed intake was increased from 1 to 1.8 times maintenance, dry matter digestibility declined by a mean of 4.4 percent (table 7). This supports the concept that digestibility estimates determined at low levels of intake will overestimate digestibility at higher, more practical feed intake levels. The NRC (1978) projects a reduction in digestibility of 4% for each multiple of maintenance increase in intake based on data of Wagner and Loosli (1967). Fecal starch was lower when rations

TABLE 7. FECAL AND STARCH DIGESTIBILITY, TRIAL 2.

Measurement and diet	Energy intake level	
	Maintenance	1.8 Maintenance
Dry matter digestibility (%)		
Basal	93.1[a]	89.5[a]
10% Alfalfa	89.4[b] (56)[e]	86.3[ab] (58)
10% CSH	88.5[c] (47)	84.7[b] (41)
37% Alfalfa	86.3[d] (74)	83.3[c] (73)
37% CSH	78.4[d] (53)	72.8[c] (45)
Fecal starch (%)		
Basal	6.2[ab]	19.0[a]
10% Alfalfa	7.6[ab]	10.3[bc]
10% CSH	8.5[a]	5.0[bc]
37% Alfalfa	7.0[ab]	11.4[b]
37% CSH	3.7[b]	4.9[c]
Starch digestibility (%)		
Basal	99.2[a]	96.3[bc]
10% Alfalfa	98.3[ab]	97.3[ab]
10% CSH	98.1[ab]	98.9[a]
37% Alfalfa	96.5[b]	93.9[c]
37% CSH	97.9[ab]	96.6[ab]

[a,b,c,d] Means within a column and classification significantly differ ($P<.05$).

[e] Digestibility of added alfalfa or cottonseed hulls calculated by difference.

were fed at a maintenance level of intake but increased with feed intake. Percent fecal starch was negatively related with starch digestibility though the relationship was not ideal (R^2=.24). Low fecal starch values could reflect not only high starch digestion but also a low level of feed intake or a high intake of roughage.

Ration dry matter digestibility (figure 6) was generally reduced with addition of either roughage. This reflects the lower digestibility of the roughages than of the corn grain being replaced. Digestibility of roughage, calculated by difference, increased as more roughage was fed. Roughage effects, however, varied with level of intake, level of roughage, and source of roughage (figure 7). At maintenance intakes, addition of 10 percent roughage lowered starch digestibility while at 1.8 X maintenance intake, 10 percent roughage addition enhanced starch digestibility. Adding alfalfa hay above this level reduced starch digestibility at both levels of intake while cotttonseed hulls depressed digestibility only at the higher level of feed intake. Of the dry matter digestibility depression with increased feed intake, the portion attributable to reduced starch digestion was 20 to 50% with added alfalfa but less than 10% with cottonseed hulls. This suggests that reduced digestion of feed components other than starch is primarily responsible for reduced dry matter digestion with an increased level of feed intake. Added starch may depress fiber digestion due to reduced ruminal pH, ammonia, or other factors, though specific effects with various types of roughage have not been examined. One might conclude that with whole corn rations fed at a <u>low</u> level of feed intake, including even a low level of roughage, depresses starch utilization. In contrast, adding a small amount of roughage at a <u>higher</u> level of feed intake seems desirable, not only for health reasons

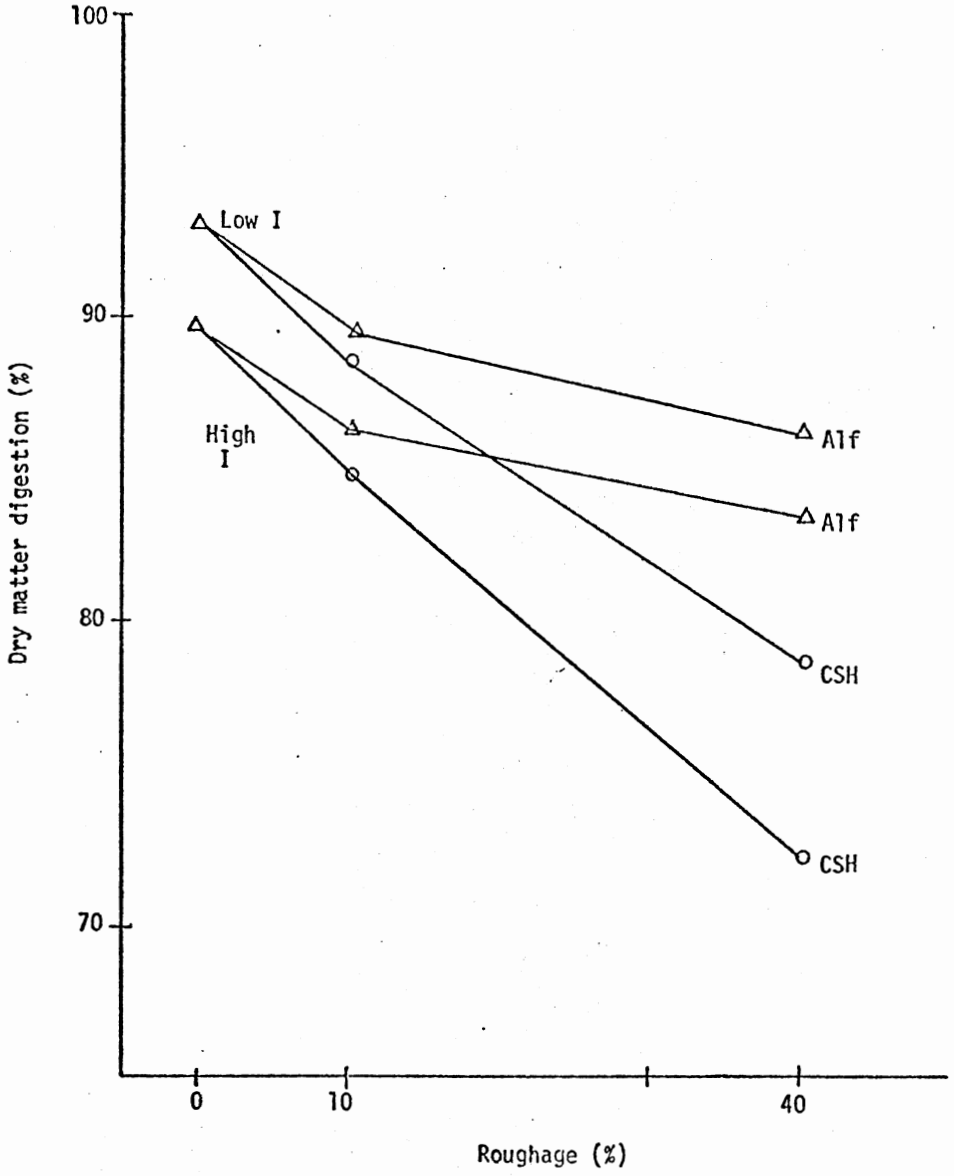

Figure 6. Dry matter digestion.

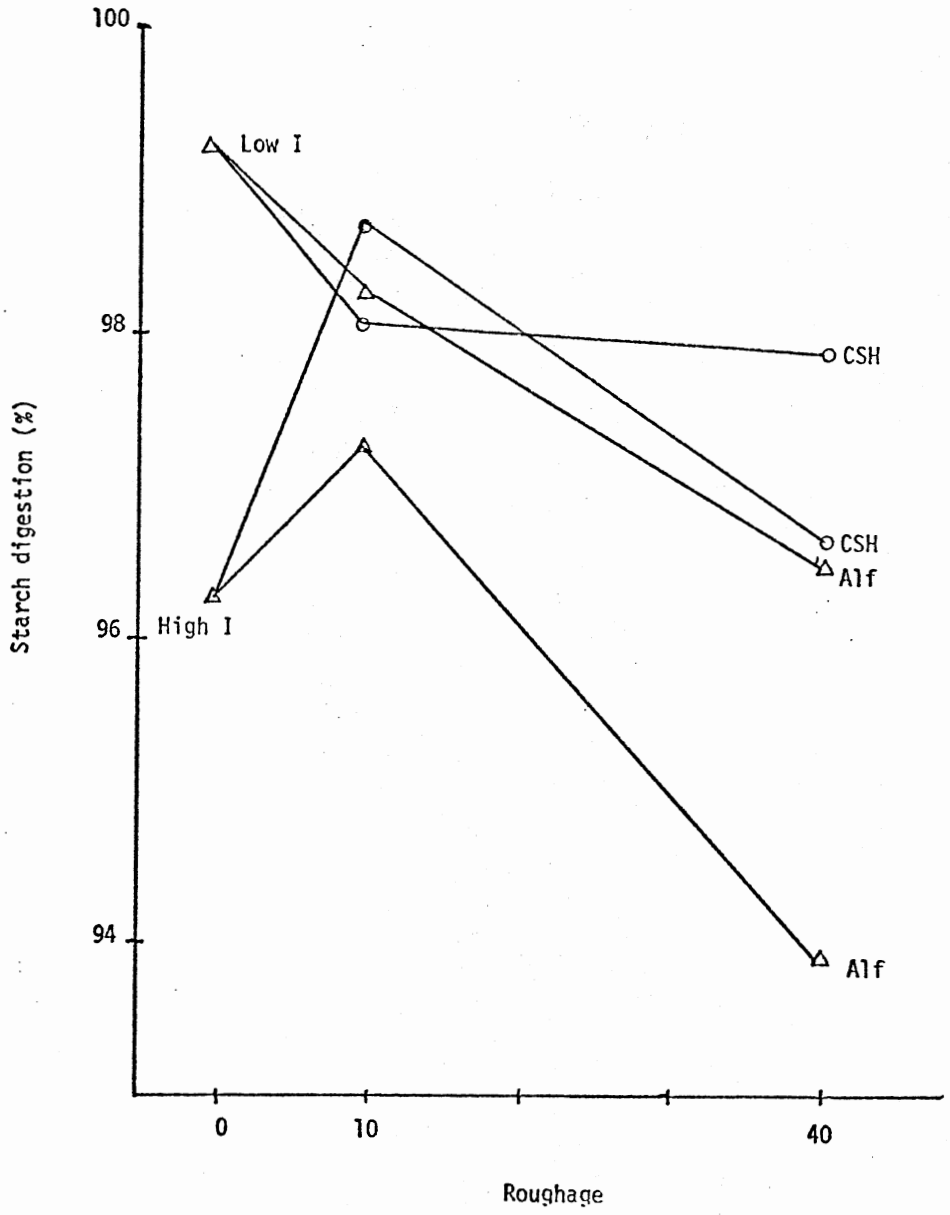

Figure 7. Starch digestion.

but also for starch digestion. When higher roughage levels are fed, source of roughage becomes more important. Cottonseed hulls caused less depression in starch digestion at both intake levels than did chopped alfalfa hay. In this trial, from 22 to 75% of the starch in feces was found in particles caught by an 8 mm screen. With 40% cottonseed hulls and the higher level of feed intake, the proportion of starch appearing as whole corn kernels in the feces of steers was much lower than with other rations fed. This may reflect increased chewing or rumination with cottonseed hulls in the ration, similar to trial 1. Further study of the effects of specific roughage sources and levels on site and extent of starch digestion is needed.

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CHAPTER VIII

SOLUTE AND PARTICULATE FLOW MARKERS

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Digestion in the ruminant's multicompartmental gastrointestinal tract is the net result of a number of dynamic processes. These must be individually measured to be adequately understood. Measurements of these dynamic processes requires the use of flow markers--substance which can be used to measure the flow (or turnover) of specific entities from a given meal. The requirements for a flow marker will vary depending upon the characteristics of the digestive process of experimental interest.

The extent of hydrolysis of potentially digestible entities (Waldo et al., 1972) in a feedstuff depends upon 1) rate of digestion, and 2) passage of the entity from the site of hydrolysis. Rates of hydrolysis vary for different entities within a feedstuff. However, passage rates of these entities tend to be similar since the undigested portion remains a component of the undigested feed residue. Undigested particulate residues comprise a part of the digesta but may have a flow rate different from the original feedstuff. Thus flow markers for undigested entities must remain associated with undigested particles derived from the specific meal to which the marker was originally associated. Association or attachment would ideally exist throughout the total gastrointestinal tract,

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though at a minimum, attachment must apply to the gastrointestinal segment of interest.

The specification of a test meal to which the marker was originally associated is important. Mastication during ingestion influences the particle size distribution entering the rumen and thereby the subsequent rate of passage. To consider the meal in toto, the marker should be uniformally distributed throughout the specific meal to achieve a single concentration of marker across all particle sizes.

Digestion in the ruminant is characterized by an 1) extensive feedstuff hydrolysis by microorganisms, and 2) postgastric enzymatic hydrolysis. Some microbes are closely adherent to the undigested particulate matter and have flow rates determined by the particulate matter to which they attach. Other microbes are free floating and have flow rates largely determined by the liquid phase. Still others are transiently adherent or free floating and have flow rates intermediate to that of particulate and liquid phases. Finally, some adhere to the ruminal wall for flow rates even slower than particulater matter. Postgastric digestion is characterized by hydrolytic digestion of metabolic products, microbial protoplasm and fermentation by-products, as well as soluble dietary entities escaping rumen metabolism and absorption.

Three different types of flow markers are needed to correspond to the three phases of interest in the rumen:

1. Particulate flow-- flow of undigested entities initially present in the particulate portions of the diet which remain insoluble until digested.
2. Solute flow-- flow of solutes derived from either the diet or

microbial metabolism.

3. Microbial flow-- flow of microbes irrespective of their phasic distribution or association.

This review will be limited to attributes which render certain substances appropriate flow markers for one of these phases in the gastrointestinal tract of ruminants. It will not consider techniques and methodologies for determining and expressing flow rate as these have been reviewed elsewhere (Faichney, 1975, 1980; Faichney and Griffiths 1978, 1980; Ellis et al., 1979). Unless otherwise specified, the term "flow rate" will denote the rate at which a particular substance is replaced due to passage from one site to a subsequent site in the gastrointestinal tract or to the feces. Thus flow rate is analogous to turnover rate. Flow rate calculations assume steady state conditions and represent the mean rate occurring over the period of measurements (Ellis et al., 1979).

PROPOSED FLOW MARKERS

Flow markers are classified on the basis of functional properties in table 1.

Water insoluble markers. Water insoluble minerals such as chromic oxide are used extensively as inert dilution markers. However, they are not suitable as specific flow markers due to their lack of association with either particulate or water soluble solute components of the digesta. Indeed, this lack of association can result in sedimentation in the rumen and sporadic transfer to the lower gastrointestinal tract and the feces. This problem is particularly evident with animals fed forage and limits the usefulness of chromic oxide as an indigestible marker.

TABLE 1. SOME PROPOSED SOLUTE AND PARTICULATE FLOW MARKERS.

H_2O insoluble

- Minerals
  - Cr_2O_3, Ti_2O_3, Si_2O_3
- Dyes
  - various
- Particles
  - plastic, rubber

H_2O soluble, non-absorable

- Polyethylene glycol (PEG)
- Chelates of trivalent Cr, Co, Fe
  - EDTA
  - DTPA

Metabolic isotopes

- ^{14}C, ^{15}N, ^{32}P, ^{35}S

Particulate bound markers

- Stains
- Polyethylene glycol
- Chelates
  - EDTA
  - tris-(1, 10-phenanthroline)-Ru
- Rare earths
- Mordant fiber
  - Cr (VI)
  - Ce(IV)

Synthetic polymers such as rubber and plastic have been used and have the advantage of controlled size, shape, and density. Variation in these attributes will influence their flow rate from the rumen. Again the lack of association with specific digesta components precludes their use if the experimental objective is to measure flow of specific dietary components.

Sudan III has been proposed as a particulate flow marker based upon its water insolubility and retention on feedstuff residues (Asplund and Harris, 1970). A number of other dyes have been employed to indicate feedstuff residue clearance time from the gastrointestinal tract and are completely recovered in the feces (Bradley et al., 1976). Such dyes have the advantage of ease of extraction with organic solvents for analytical determination and resolution by spectrophotometric methods. Some purification and concentration steps may be required to impart the specificity and sensitivity required for flow measurement by pulse dose procedures. Where other constraints limit use of other particulate flow markers, the dyes appear to offer considerable promise although experimental verification is limited.

Water soluble markers. Polyethylene glycol with a molecular weight mean of 4000 (PEG) was introduced by Hyden (1955). This material is extremely soluble in water and dietary doses are near completely recovered in the feces (95%+, Downes and McDonald, 1969). Analytical procedures for its determination are generally poor, but this disadvantage can be overcome through the use of ^{14}C labelled PEG. Some confusion exists regarding the utility of PEG for estimating rumen liquid volume and dilution rate. Czerkawski and Breckenbridge (1969) and Alexander (1969) have reported that PEG, presumably due to its large molecular weight, is excluded from

intratissue water of feedstuffs. In contrast, Sutherland et al. (1963) reported that PEG became attached to particulate matter. In each of these cases workers dealt with high fiber diets and determined PEG exclusion or association with solids by measuring the concentration of soluble PEG and total liquid volume. Teeter (1981) found no exclusion or association of PEG with feedstuffs high in crude fiber but PEG exclusion occurred to varying degrees with high concentrate feedstuffs. Whether these inconsistencies are due to methodology, characteristics of individual feedstuffs or analytical difficulties associated with PEG analysis is not yet known. Analytical problems have been noted with feeds high in tannins (Kay, 1969) which appear to precipitate PEG. A soluble component of cottonseed hulls (Teeter, 1981) also interferes with turbidometric analysis. Whether the soluble component in cottonseed hulls precipitates PEG or merely interferes with its detection is unknown.

The chromium chelate of ethylenedinitrilotetraacetic (Cr-EDTA) was proposed as a liquid flow marker by Downes and McDonald (1964). Recovery of a dietary dose in the feces was similar to PEG (90-98%). Since it has a smaller hydrated size then PEG, Cr-EDTA has the potential to distribute itself more extensively in intratissue water of feedstuffs (99%, Goodal and Kay, 1973). But molecular size has not been shown to inhibit entry of water soluble markers into feedstuffs.

Low concentrations of ^{51}Cr-EDTA appear to be bound to particulate matter in the rumen (Warner, 1969). Poppi (1980) observed rumen liquid volumes in cattle fed all forage diets were 15.8% higher when measured by dilution of Cr-EDTA as compared to manual emptying and measurement. Goodal and Kay (1973) reported rumen liquid volumes 15.2% higher in sheep when estimated from dilution of Cr-EDTA as compared to PEG. These

differences between observed and estimated volumes may suggest that Cr-EDTA binds to solids. Such binding would reduce the concentration of Cr-EDTA in the liquid phase. This may or may not cause dilution rate to be overestimated depending on whether animals are fed during the measurement period. Binding of Cr-EDTA must be rapid (less than 3 hr) or otherwise it would make the 3 to 24 hr regression coefficient curvilinear and the extrapolated initial concentration would not be affected by binding. Flow rate of Cr-EDTA may indeed represent its liquid flow rate when such flow rates are determined from post-dose changes beyond 3 hr if additional binding sites are not added by subsequent meals.

Though binding may indeed occur, it may not explain all the inaccurate volume measurement reported. Estimates of marker (EDTA complexes, PEG) binding have been found (Teeter, 1981) to be much less than 1 ppm across a series of roughage or concentrate feedstuffs. A typical ruminal dose might be 6 to 10 g of metal complexed to EDTA or 45 g PEG. Quantity of feed required to bind 15% of the marker dose, assuming 1 ppm marker bound by feed is 900 kg feed for 6 g marker, 1,500 kg for 10 g and 6,750 kg for a 45 g marker dose. Such amounts are physiologically unreasonable. Other factors that may be involved in marker inconsistency include 1) less than instantaneous marker equilibration with the rumen, 2) marker uptake by microorganisms, and 3) analytical anomalies. Ruminal mixing in 500 kg steers requires from 0.1 to 1.8 hr and is correlated with the quantity of solids contained in the rumen ($R = .67$, Teeter 1981). During the mixing period, disproportionate flow of marker and liquids would erroneously alter volume estimates. Volume errors associated with delayed mixing could range from 2 to 10%. Marker-EDTA uptake by rumen bacteria or complexing by fermentation products may be inferred from work by Teeter

(1981). Concentrations of Co-EDTA, Cr-EDTA and Yb-EDTA were reduced in McDougall's buffer containing glucose or feedstuffs and rumen innocula following a 96 hr incubation and centrifugation (20,000 g). Reduction in marker concentration was correlated ($R^2 > .98$) with *in vitro* dry matter digestibility. Microbial uptake or the formation of insoluble liquid marker-metabolite complexes *in vivo* could alter rumen volume and dilution rate estimates. If the liquid markers complexes still follow the liquid pool modification of analytical procedures may allow higher recovery and increased marker accuracy.

Based on stability constants for EDTA (Martnell and Smith, 1974) metals other than chromium (III) should have equal or superior utility. The stability constants of 23.4 for Cr (III) is exceeded by V (III 25.9), Co (III 40.7), Fe (III 25.1), Zr (IV 29.5) and is of the same order as Sc (III 23.1). Uden *et al.* (1980) has verified similar recoveries in the feces of several species for dietary Co-EDTA and Cr-EDTA.

The stability constants for the lanthanide series are quite high suggesting their potential for use in the EDTA form as solute markers. However, Miller and Byrne (1970) reported that daily doses of EDTA resulted in the appearance of ^{144}Ce in the urine of the calves receiving separate daily oral doses of ^{144}Ce in solution. In contrast, no urinary excretion was detected in the absence of EDTA dosing. It should be noted that extremely small amounts of Ce were involved since carrier free ^{144}Ce was dosed. This may be related to other observations (Lippke, 1979) that the amount of Cr-EDTA absorbed appears to decrease with increasing intake. This suggests that a small constant amount is absorbable while additional amounts are non-absorbable. Urinary excretion of EDTA complexes of Cr, Co, Fe, and Yb fed simultaneously ranged from

0.8 to 3.4% in a liquid marker balance trial envolving steers fed high roughage and high concentrate diets (Teeter, 1981). PEG was not detected in the urine. Fecal recoveries ranged from 95.9 to 99.7%.

Chelating agents other than EDTA might be predicted to have equal or superior utility to EDTA based on stability constants. As compared to EDTA, diethylenetrinitrilopentaacetic acid (DTPA) is capable of forming two additional chelation rings and provides two additional electron-pair donor atoms. This makes it capable of fully coordinating a metal having a coordination number of 8. The former property contributes increased stability for any metal. This property makes DPTA more appropriate as a chelating agent for metals such as the lanthanides which may have coordination numbers as high as 8 or 9. DTPA complexes of Cr, Co, and Ni have been routinely used at Texas A&M University over the past eight years. Similar recoveries and rates of passage in the liquid phase have been observed for Cr-EDTA and Ni-DTPA (unpublished observations). Rates of passage estimated from fitting Cr-DTPA concentration per unit fecal DM to a two compartment model (Ellis et al., 1979) yield equal rate constants for ruminal and lower gastrointestinal tract passage (Conner et al., 1977). This can be interpreted as evidence for a single flow compartment for this marker as compared with a differing rate constant when the same model was applied to Cr-EDTA excretion (Grovum and Williams, 1973). Grovum and Williams suggested the differing flow might be a consequence of imperfect mixing in the caecum and proximal colon. They also infer that bi-phasic distribution of Cr-EDTA between two phases having differing flow rates could be responsible. These considerations suggest that the comparatively more stable and higher coordinated complexes of DTPA may not bind to rumen contents to the extent which

complexes of EDTA may.

Disappearance of Cr from the gastrointestinal tract when dosed as Cr-EDTA is assumed to occur via absorption of the Cr-EDTA molecule (Downes and McDonald 1964, Dobson et al. 1976, and Uden et al. 1980). Less absorption would be expected of the larger DTPA chelating agent. The molecular size may be further increased by using even larger rare earths. Alternatively, if absorption of the metal occurs by displacement from the chelating agent, the more stable DTPA complexes (Martell and Smith, 1974) would be advantageous over EDTA complexes. Further, if displaced, absorption of a rare earth would be less than other metals. Displacement of metal from EDTA or DPTA by hydrogen ions can occur. This most likely would occur at the low pH of the abomasal or duodenal segment of the intestinal tract.

Regardless of the chelating agent used, care must be exercised to ensure that all the metal is fully coordinated since the non-coordinated metal ions may be absorbed or complexed with other metals. Three methods may be used to ensure full coordination. One is to use analytical grade metal salts and chelating agents and use a slight stoichiometric excess of the chelating agent. If excess chelating agent is a problem (Miller and Byrne, 1970a) the excess can be complexed with a metal of unrelated interest but lower stability constant than the chelated metal of primary interest. A second method, proposed by Downes and McDonald (1964), is precipitating the excess metal as the insoluble hydroxide. This can be accomplished by filtration after making the solution alkaline with ammonium hydroxide. A third, described by Uden et al. (1980) involves crystallization of the marker as the Li salt of Cr-EDTA and either the Li or Na salt of Co-EDTA. A fourth method is to add an excess

of a feedstuff to bind free metal and filter the solution prior to dosing (Teeter, 1981).

Metabolic isotope markers. Metabolic isotopes are incorporated into the molecular structure of the material of experimental interest. Since they are structural components of the material, they are indisputable flow markers. Their incorporation into the specific entity of interest only and their disappearance and reincorporation via metabolism must be quantitated and verified, however. Such markers can have further advantage of a uniform distribution of the isotope occurs throughout the entity of interest.

Smith et al. (1967) prepared uniformly ^{14}C labelled cell wall constituents (CWC) by extracting uniformally labelled ^{14}C plant tissue to remove the ^{14}C associated with non-cell wall material. The disappearance of CWC - ^{14}C from the rumen via digestion and passage could be resolved by a kinetic approach (Smith et al., 1979). Reincorporation could be precluded in this case by resolving rates of digestion and of passage of the conceptually indigestible cell walls (Waldo et al., 1972). This approach is only applicable where routes of disappearance can be confidently expressed qualitatively and quantitatively. Metabolic markers are most applicable if reincorporation can be precluded. Theoretically, reincorporation could be corrected for by a similar kinetic approach, but analytical requirements make such an approach either infeasible or extensively laborious.

The principal disadvantage of ^{14}C labelling is the cost and labor involved in uniform labelling or even non-uniform pulse labelling. Another possible but not unique disadvantage is that purifying the entity of interest may alter rates of digestion and passage.

Metabolic isotopes other than ^{14}C have been used primarily for entities which are highly digestible from feedstuffs and products of microbial digestion. The isotopes ^{14}C, ^{15}N, ^{32}P, and ^{35}S can be used to label microbial matter following injection in a molecular form uncommon to the feed or microbes but capable of metabolic incorporation into microbes. If subsequent degradation and reincorporation can be precluded or adequately described and measured, these isotopes can be used to measure rate of passage of the derived and labelled microbial entity. ^{35}S has been used extensively to measure flow of microbial matter (Walker and Nader, 1975).

Particulate bound markers. Stains were the first particulate bound markers used. The appearance of a stained residue at any gastrointestinal site quantitates transit time for the strained feedstuff between dosing site and recovery site of the specified residue. This transit time differs for different size residues (Ellis and Huston, 1967). All particulate residues must be counted to quantitate mean transit time and mean transit time differs from minimum transit time. Further bias is introduced if the concentration of strained residues is expressed as number of particles per unit weight of digesta dry matter. No satisfactory method has been proposed for quantitatively measuring recovery rate of the strain as has been proposed for water soluble dyes (Asplund and Harris, 1970, Bradley et al., 1976).

EDTA complexes and PEG, normally considered water soluble, may bind to particulates at low levels. The ruthenium (II) chelate of tris - (1, 10 phenanthroline) (Re-TP), in contrast, has a high binding capacity for particulate matter (Tan et al., 1971). The nature of the binding forces by Ru-TP might be due to hydrophobic groupings on the heteroaromatic

ring structure of this chelating agent. Tan et al. (1971) demonstrated that Ru-TP added as an aqueous solution sedimented with solids of rumen digesta. Subsequently, Faichney and Griffiths (1978) presented evidence for extensive migration of Ru-TP from larger to smaller feed particles and to microbes. They suggested that bacteria act as the vector for migration to smaller feed particles.

The rare earths have been proposed as markers which bind to particulate matter (Ellis and Huston, 1968). These were initially proposed based on observations that radioisotopes of these elements from atmospheric fallout were almost completely recovered on plant foliage (Morgan, 1959). More recent and specific criteria concerning binding of rare earths to particulate matter and their use as particulate flow markers will be discussed in subsequent sections.

Van Soest has proposed a procedure referred to as "mordanting" for binding certain elements to a number of entities including plant fiber and starch. Hexavalent chromium (dichromate) forms strong complexes with plant cell walls. The complex is stable in the presence of in vitro incubation with rumen fluid, 0.01 M HCl, or a pH 7 solution of 2.9% (W/W) of sodium lauryl sulfate (Uden et al., 1980). A minimum concentration of 12-14% (W/W) chromium was required to achieve this stability when exposed to extraction by a pH 7 solution of sodium lauryl sulphate containing 0.1 M EDTA. Concentrations of chromium exceeding 8% render the cell walls essentially indigestible.

Of the currently available particulate bound markers, the chromium mordant is unquestionably the most tenaciously bound and hence unquestionable flow marker. The low digestibility and high density of mordant fiber may adversely affect the flow rate, however. If digestion

facilitates particle size degradation and flow rate differs with particle size, then Cr-mordant residues would behave differently from the feedstuff being imitated. Another disadvantage of the Cr-mordant is the effect of the exhaustive chemical extraction of cell contents needed to remove cell components which may loosely bind chromium. Extraction may alter particle size as well as attack of digestive enzymes. Such extraction is not unique to Cr-mordants as it is practiced commonly for rare earths binding as well. However, the effect of the rare earth on digestibility appears to be much less than the effect of strong oxidizing agent, dichromate, and the amounts bound are also two orders of magnitude lower. For forages, extraction of unlabelled rare earth may be deferred to post-digestion samples of digesta of feces providing release and reattachment is not extensive. Another disadvantage of the mordanting process is the applicability of only one metal, Cr, as the mordant. This restricts use to a single marker. Where particulate digestion and flow are of interest multiple markers are needed. Since the rare earths and other metals have many advantages as particulate flow markers, the remainder of this review will be devoted largely to these metals.

BINDING STUDIES WITH METALS

Early studies with rare earths indicated 89-103% of ^{144}Ce associated with atmospheric fission product fallout was deposited on plant material (Morgan, 1959). Presumably, the rare eaths existed as oxides in the fallout. Subsequent studies indicated these rare earths were indigestible. Their tight association particulate matter continued during passage through the gastrointestinal tract of cattle (Garner et al., 1960). Further studies involving water soluble rare earth salts applied to forage showed complete recovery of the metal in feces (Ellis 1968, Bell

1969, Pfau and Abadir 1973) and suggested continued association with particulate matter in digesta (Ellis and Huston, 1968 and Pfau and Abadir, 1973) and feces (Huston and Ellis, 1968). Miller et al. (1966) observed negligible recovery of ^{144}Ce on a strong cation exchange resin when it was mixed with digesta of calves fed ^{144}Ce absorbed onto soybean meal. This suggests that the association may be due either to insolubility of the ^{144}Ce complex or to the strong chemical binding by ^{144}Ce to the feedstuff.

Rare earths exhibit colloidal behavior. At low concentrations, ($< 10^{-11}$ M) below the solubility of their hydroxides ($\sim 10^{-6}$ M) this behavior is referred to as radiocolloidal due to the low concentrations involved. Radiocolloidal behavior could possibly account for association at low concentrations of fission products with plant material (Morgan, 1959). A second type of colloidal behavior occurs at concentrations less than the molar solubility of the hydrolytic product. When a soluble lanthanum salt is administered intravenously to an animal, hydrolysis occurs promptly at physiological pH. However, the predicted insoluble hydroxides do not appear in plasma but rather the metal forms a stable colloid with some constituent of plasma (Kyker, 1961). These observations suggested that the soluble rare earth salts, upon entering the rumen, would hydrolyze to yield insoluble hydroxides which would be deposited, absorbed or adsorbed onto feed residues.

The specific nature of binding between rare earths and feed residues in ruminant digesta has not been investigated. Some observations suggest that binding is by coordinate covalent bonds in which the rare earth acts as an electron pair acceptor (or acid) and various ligands of feedstuff constituents act as the electron pair donor (or base). In this context,

rare earths act as hard acids and would form most stable coordination complexes with hard bases such as RNH_2, OH-, RO-, and ROH constituents of organic molecules (Ho, 1977). Most stable binding occurs via oxygen donors (p. 48, Bell 1977) leading to keto-enol chelates in which the "lanthanide shift reagents" (p. 102, Bell, 1977; p. 17, Ho, 1977) are examples. As a result, the variety of complexes which can be formed easily with rare earths is less than that for the d-transition elements (Thompson, 1979). Binding of rare earths has been studied with a number of specific proteins such as yeast and RNA in which up to 14 moles of rare earth bind at sequential sites having stability constants (log k) ranging from 8 down to about 5 (Kearns et al., 1978).

Martz et al. (1974) reported that forage cell wall constituents (CWC) will bind Au (II), Au (III), Ce (III), Ce (IV), and Cr (VI). Only complexes involving Cr (VI), (chromium mordants) quantitatively retained the metal on in vitro digestion and subsequent extraction with a neutral detergent solution containing 0.1 M EDTA. Undoubtedly the chelating agent, EDTA, was the primary if not the sole agent active in removal of the metal. Rare earth complexes have been demonstrated to be stable to in vitro incubation and neutral detergent extraction when EDTA was not present (Lascano et al., 1979). Refluxing for two hours with a 0.1 M, pH 7.0 solution of EDTA will remove from 50-80% of rare earths applied as trivalent metals to forage or digesta residues (Lascano et al., 1979).

Stability appears dependent on metal and coordination number since the complex formed by Cr (VI) is stable to EDTA extraction, the stability constant must be greater than 10^{23}. Hexavalent cerium, like hexavalent Cr, appears more tenaciously bound than the trivalent metal (Martz et al., 1974; Uden et al., 1980). The next most stable complexes would be those

involving tetravalent Ce and all the other rare earths since they have relatively similar chemical properties (Kyker, 1962), are all hard acids (Ho, 1977) and have similar large coordination numbers and chelation stabilities (Martnell and Smith, 1974). The next most stable complexes appear those formed by trivalent rare earths. Based on the similar recoveries reported by Martz et al. (1974) for Ce (III) and Au (II) and Au (III), these may be inferred to have similar stabilities.

Researchers at Oklahoma State University have studied more specifically the binding of rare earths to feed particles (Teeter et al., 1979; Teeter, 1981) at levels of binding below the saturation capacity of various feedstuffs, a small proportion (0-6%) of Yb (III) remained unbound or readily exchangeable and could be removed by repeated washing with distilled water over six hours. A similar amount of rare earth can be removed by refluxing marked feeds with phosphate buffer with or without 3% sodium lauryl sulphate (table 8).

Teeter et al. (1979) utilized the Scatchard procedure for determining association constants (ka) and binding capacities of Yb (III) with various feedstuffs. Based on the Scatchard plots, two or three types of binding appeared present for the feedstuffs examined. Averaged across feedstuffs tested, major proportion (71%) of the binding involved sites having molar association constants with Yb (III) in the range of 10^5 to 10^6 while a lesser number of bonds had binding constants of 10^6 to 10^7. With some feeds, a small number of sites formed complexes of even greater stability (10^{11} to 10^{16}). However, these sites were saturated by Yb (III) at levels of parts per billion and would be of little concern analytically.

Feedstuffs differed both in their association constant and binding capacity for Yb (III). In general, feeds higher in fiber tended to have

both higher association constants (higher stability constants, log ka) and binding capacities (table 2). Starch has a low binding affinity and capacity suggesting the importance of functional groups other than those presented by polyglucans. The strong binding affinity and capacity of purified cellulose (solka floc) indicated either that binding of Yb differs with configuration of the anomeric carbon of glucose and/or that adjacent polymeric chains of cellulose bind Yb. It should be remembered that feedstuffs are intact tissues and that surface components, such as epidermal tissues and fractured cell walls, are the primary cellular structures exposed to Yb. Hence, intracellular components of feedstuff such as protein and nucleic acids may have the capacity to physically bind Yb but the opportunity for exposure would be much lower than surface structures.

Marker migration. Based on the results in table 2, migration of rare earth from a labelled feed particle to a second kind of feed particle may be expected when or if:

1. The quantity of rare earth applied to the particle exceeds the particles' binding capacity.
2. The second kind of feed particle has an appreciably higher association constant for the rare earth.
3. Significant disequilibrium occurs between the particulate bound rare earth and the liquid phase.
4. The digestive process disrupts the molecular structure contributing to the binding force and/or capacity.
5. The easily exchangeable rare earth is not removed by prior washing and exchanges with and is bound to a second particle.

TABLE 2. HIGH AND LOW MOLAR ASSOCIATION CONSTANTS AND THEIR BINDING CAPACITY.

Feedstuff	High affinity binding				Low affinity binding			
	Molar affinity K x 10^7	μ M/g capacity	N[a]	R[b]	Molar affinity K x 10^5	μ M/g capacity	N	R
Whole corn	3.8	0.98	3	-.88	6.85	14.93	9	-.82
Ground corn	3.83	17.09	4	-.96	9.20	21.9	8	-.72
Whole milo	1.26	24.96	5	-.99	6.33	5.53	7	-.68
Ground milo	1.50	29.89	3	-.98	7.29	37.50	9	-.95
Cottonseed meal	1.50	38.89	4	-.99	9.08	166.40	8	-.89
Soybean meal	1.19	57.26	4	-.99	4.48	131.14	8	-.69
Cottonseed hulls	1.77	34.22	3	-.99	7.44	176.18	9	-.91
Prairie hay	2.27	40.75	5	-.99	8.50	36.17	7	-.97

[a]Number of observation used in linear regression.
[b]Linear correlation coefficient.

Hartnell and Satter (1979) observed negligable migration *in vitro* of rare earths from alfalfa hay to grain (0.8% over 24 hrs) but more migration from grain to hay (7.4% per 24 hrs). Since the rare earths (La, Sm, and Ce) were applied at concentrations of 1.4 g/kg feed, the binding capacities probably were not exceeded (table 2). However, the rare earth was applied to feedstuffs by spraying rare earth solutions onto the feed. Spraying, in contrast to immersion, would not be expected to expose all possible binding sites to rare earth metals for binding. The migration may have been due to 1) unbound rare earth, 2) higher binding affinity of hay, and/or 3) the easily exchangable rare earth which was not removed. Teeter *et al*. (1981) detected a small amount (0.27 ppm) of rare earth accumulation on unlabelled prairie hay incubated for 96 hours in a nylon bag in the rumen of a steer fed labelled, washed whole corn containing 573 ppm Yb. Migration may be accentuated by the higher association constant for Yb of hay than of corn. Alternatively or additionally, this migration may have been the consequence of rare earth binding to highly digestible entities of corn which on digestion, liberated the marker for migration. Microbes or small framents of a digestible entity marker complex would be highly mobile and readily penetrate pores of a nylon bag. This source of migration error can be reduced by extracting from the particle easily digestible entities before labelling or by extracting the digesta of such entities and microbes before marker analysis. Ellis *et al*. (1979) has proposed a buffered detergent solution for such extractions (see also table 8).

Migration of the marker due to disequilibrium from the solid to the liquid phase (distilled water; dilution rate 47%/hr) was observed to range from .09%/hr for cottonseed meal to 0.57%/hr for ground corn (Teeter,

1981). The data of Miller et al. (1966) precludes migration of ^{144}Ce from feed residues to the gastrointestinal epithelial tissue of calves fed natural diets.

EFFECTS OF METAL BINDING OR DIGESTIBILITY OF FEEDSTUFFS

Martz et al. (1974) and Uden et al. (1980) noted a depression in in vitro digestibility which increased with the level of Cr (VI) and other metals applied. The effect of ytterbium upon digestibility of several feeds has been evaluated (Mader, 1980). In vitro digestibility of wheat forage was lowered by 15, 21, and 34% for early, middle, and late maturity wheat forage by saturating binding sites with 69,000, 74,000, and 87,000 ppm ytterbium, respectively. As the wheat forage approached maturity, there was concommitant increase in crude fiber and ytterbium binding capacity. For prairie hay, in vitro dry matter digestibility decreased by 9, 14, 20, and 27% as the quantity of ytterbium label increased (10,000, 13,000, 19,000, and 32,000 ppm, respectively). Digestibility depression (4%) was much less with labelled and washed corn (497 ppm) than with wheat forage or prairie hay. This may have be due to either the low degree of starch binding or the low binding capacity of the corn. In situ (nylon bag, 105 μ pore size) dry matter disappearance of several feeds labelled with ytterbium revealed a slight depression (8 to 17%) in rate and extent of digestion following ytterbium labelling. This depression was less than the in vitro data would predict, probably due to the low precision generally obtained with nylon bags of pore size of 100 microns.

These results suggest that metal binding may render particles less digestible in the vicinity of the bound metal. If this is true, then marker migration due to digestion is reduced. Further, these results

would suggest that if normal digestibility is an important experimental objective, the minimum amount of marker feasible should be employed.

IN VIVO STUDIES ON RARE EARTHS AS PARTICULATE FLOW MARKERS

A number of studies have been conducted recently to test the degree to which rare earths remain associated with undigested fragments of particles throughout the gastrointestinal tract. These studies have been limited by the lack of an independent method for identifying residues from a specific feedstuff or meal.

Conner et al. (1977) used stained dietary particles as a means of identifying fecal residues. Two particles having different rates of degradation and flow were chosen. These were stained and different rare earths were applied. Particles were dosed via rumen cannulae without removal of easily exchangable rare earth. Fecal samples were collected serially, and dry seived as indicated in table 3. Stained particles were counted (no/g of specified size particles) and rare earths assayed for each specified particle size. The results are presented in table 3.

With the exception of one of 16 observations, the turnover rates of rare earth and the associated stained residues were within one standard error of the mean. Further, differences in turnover rate for stained residues from the two different dietary particles was reflected in turnover of the associated rare earths. Averaged across all observations, the turnover rate of stained residues was slightly (7 to 9%) lower than turnover of the rare earths as calculated by the regression coefficient or the mean ratio of fragment/RE. This difference could be due to errors in detecting and counting stained particles. Alternatively the faster turnover rate of rare earths could possibly be due to exchange of the rare earth since neither the dietary particles nor fecal samples were

TABLE 3. TURNOVER BY PASSAGE TO FECES OF STAINED FRAGMENTS AND RARE EARTH INITIALLY ASSOCIATED WITH THE STAINED DIETARY FRAGMENTS.

Animal	Fragment assayed[b]	Wood Chips ^{169}Yb	Wood Chips Blue	Forage NDF ^{141}Ce	Forage NDF Green
		k_2, hr^{-1}			
4	Feces	(.022)	NA	(.031)	NA
	1180/425	..013	.015	.036	.036
	425/250	.020	.025	.036	.037
	250/150	.018	.019	.030	.031
	<150	.022	.014	..030	.028
2	Feces	(.020)	NA	(.045)	NA
	1180/425	.026	.023	.034	.025
	425/250	.015	.014	.053	.045
	250/150	.018	.016	.032	.036
	<150	.016	.016	.031	.025
	$\bar{x}$	.021	.018	.035	.033
	r	.80	.93	.79	
	k_2Frag/k_2Rare earth	.86	.91	.94	

[a] From Conner et al. (1977).

[b] Feces = whole; Fragments indicated by range in seive size (screen opening, microns) used to obtain fragments.

washed or extracted.

Teeter (1981) measured turnover rate of four different feedstuffs labelled with Yb. Relative rates of passage across either high roughages or high concentrate diets were soybean meal > cottonseed hulls = whole shelled corn > prairie hay. Passage rate was also influenced by particle size with ground (2.5 mm) prairie hay flowing out of the rumen 14% faster than prairie hay fed in the long (17.5 cm) form. Similarly, ground corn had a ruminal passage rate 7% greater than whole shelled corn.

Lascano et al. (1979) seived masticated forage samples, applied rare earths and measured turnover rate. These particles were extracted with a neutral detergent solution without the EDTA chelating agent before binding the rare earth. The NDF of such dietary particles containing bound rare earths were not subsequently washed to remove easily exchangable rare earth. Subsequent to dosing, samples of rumen digesta and feces were collected serially and rare earths determined. If migration of the rare earth had occurred, one would expect at least a portion of the rare earths to be subsequently bound by non-NDF dry matter which might have a turnover rate (k_2) different from NDF. Results are summarized in table 4.

Lascano et al. (1979) also determined the proportion of rare earth in digesta and fecal DM which was recovered on NDF. These results are summarized in table 5. The recovery of essentially all the rare earth with the NDF of rumen digesta and feces precludes its migration to non-NDF components. This does not, however, preclude migration from NDF of the originally bound particle to NDF of another particle. If this occurred, the distribution of marker should be shifted to smaller size particles due to their greater binding surface area per unit weight.

TABLE 4. TURNOVER (K_2) OF RARE EARTHS DETERMINED IN WHOLE DIGESTA ON NDF.

Dietary NDF/ Rare Earth	site[b]	k_2 DM	k_2 NDF	r	$\frac{k_2 \text{ DM}}{k_2 \text{ NDF}}$
		-------hr^{-1}-------			fraction
c. leaf/^{169}Yb	R	.0351	.0352	.62	.997
	F	.0408	.0384	.64	1.063
stem/^{141}Ce	R	.0232	.0284	.40	.817
	F	.0279	.0291	.88	.959
fine/Yb	R	.0319	.0301	.63	1.060
	F	.0300	.0327	.71	.917

[a]NDF=residue remaining after 1 hr. reflux with ph 7 phosphate buffer containing 3% sodium lauryl sulphate.

[b]N=9/site; R=Rumen, F=Feces.

TABLE 5. *IN VIVO* RECOVERY OF RARE EARTHS (RE) ON *RUMINAL* AND FECAL NDF.

Dietary NDF/ Rare Earth	Rare Earth Recovery on	
	Rumen NDF	Fecal NDF
coarse leaf/^{169}Yb	1.13 ± .06	1.07 ± .09
stem/^{141}Ce	1.01 ± .08	1.09 ± .08
fine/Yb	1.01 ± .08	1.11 ± .13

[a] Recovery = $\frac{\text{RE on NDF/g DM}}{\text{RE/g DM}}$; N = 19 animals; x ≈ 10 samples/animal

[b] NDF=residue remaining after 1 hr. reflux with pH 7 phosphate buffer containing 3% sodium lauryl sulphate.

This was also investigated in the same experiments of Lascano (1979) and the results are summarized in table 6.

It should be emphasized in the results of Lascano (1979) that the rare earths were initially bound to the external surface of the CWC of the largest size particles derived from ingestive mastication of the long hay. Since these initially labelled particles were derived from seiving esophogeal samples, these particles are identical to the largest size particles entering the rumen from the long hay. They therefore have the potential for being further fragmented in an identical manner to other particles derived from the long hay. Thus, the distribution of rare earths on particles derived from these labelled fragment should be the same as the final particle size distribution of dry matter derived from the long hay if: a) migration to other unlabelled entities (CWC or non-CWC) was negligable, b) the largest size particle was subsequently fragmented in such a way that its initial surface (and hence bound rare earths) made a proportional contribution to all derived particles, and c) the extraction of non-CWC components and binding of rare earths did not affect its fragmentation. The concentrations were virtually identical across the particle size distribution summarized in table 6. This suggests that all these conditions were met.

It is difficult to conceive of a fragmentation mechanism whereby surface bound rare earths would proportionally contribute to all derived fractions. It is assumed the rare earths were largely bound to surface features since they were applied by sprinkling a solution at pH 4 onto the dried, extracted fragments. Soaking of particles in a neutral pH rare earth solution is now preferred compared to sprinkling. This yields a more uniform concentration of rare earth on the initially labelled and

TABLE 6. DISTRIBUTION OF DRY MATTER AND RECOVERED RARE EARTH BY PARTICLE SIZE IN THE FECES WHEN THE RARE EARTH WAS INITIALLY ASSOCIATED WITH MASTICATED PARTICLES > 1600 µm FROM A LONG HAY.

Fraction	Initial Particle	Fecal particle, µm					
		1600/1000	1000/800	800/500	500/300	300/160	<160
		----------%----------					
dry matter	long hay	8.9	8.7	18.9	20.6	22.2	20.6
^{141}Ce	stem	8.0	7.9	17.7	19.4	22.7	24.3
^{169}Yb	leaf	9.8	8.6	17.3	19.1	22.1	23.1

[a]Distribution of dry matter by particle size = $\bar{x}$ dry weight of specified particle size ÷ weight of fecal dry matter sieved; N = ∿ 144 samples per particle size.

[b]Distribution of recovered rare earth = output of rare earth by fecal particle size accumulated over six days ÷ output of rare earth in total fecal dry matter accumulated over six days ; N = 29 observations per value reported.

subsequently derived digesta particle. Such an application procedure also may allow binding to internal features of the CWC of plant tissues. Further, soaking as compared to sprinkling, would avoid localized concentrations of rare earth exceeding the feedstuffs' binding capacity.

Although the distribution of recovered rare earth was favorable when applied to masticated forage particles, caution should be observed to applying it to large dietary fragments. Conceivably, binding of rare earths to the external features of larger fragments could yield circumstances in which the externally bound rare earths would not proportionally contribute to all the derived particles.

TURNOVER OF RUMEN PHASES

Some recent measurements of turnover of particulate matter, microbes and liquid phase of rumen digesta are summarized in table 7 to further evaluate validity and potential application of solute, microbe and particulate markers. Turnover of the microbial phase was determined by a modification of the procedure of Walker and Nader (1975). A pulse dose of $Na_2\ ^{35}SO_4$ was given and turnover rate constants estimated as the first order rate constant from the specific activity of rumen dry matter between 12 and 90 hours post dose. Liquid turnover was similarly estimated from analysis of total Cr in rumen DM. All three markers were determined from the same sample following ashing and extracting with a mixture of 3 M each HNO_3 and HCl.

In the basal treatment, turnover of the microbial phase was slightly slower than of particulate matter ($^{35}S/Yb = 0.88$) and considerably slower than the liquid phase ($^{35}S/Cr = .421$). As compared to the basal, monensin feeding increased turnover of all phases but did so differentially. Turnover of microbe and water phases were increased relative to the

TABLE 7. FRACTIONAL TURNOVER OF FORAGE (Yb), MICROBIAL (^{35}S) AND LIQUID (Cr) PHASES IN RUMEN DIGESTION.

Treatment	Animal	Yb	^{35}S	Cr	^{35}S/Yb	Cr/Yb
Control	157	.047	.044	.088	.94	1.87
	158	.046	.037	.105	.80	2.28
	406	.045	.037	.143	.82	3.18
	117	.046	.044	.077	.96	1.67
	Mean	.046	.040	.103	.88	2.25
Monensin	156	.046	.059	.193	1.38	2.02
	161	.057	.057	.169	1.00	2.97
	425	.052	.059	.144	1.13	2.77
	1244	.054	.072	.198	1.33	3.67
	Mean	.052	.062	.176	1.18	2.86

[a] k_2.

[b] CrDTPA

[c] From Delaney (1980).

particulate phase. These results indicate interactions between these phases which necessitates their simultaneous determination for precise nutritional interpretation.

MARKING AND ANALYTICAL PROCEDURES

Procedures have been published recently on chromium mordants and water soluble chelates (Uden et al., 1980). The following discussion concerns the rare earths administered by pulse dose techniques. This technique is most appropriate for particulate flow measurement.

In addition to the usual need for specificity, accuracy and speed, analytical procedures for flow markers must also be sensitive. This requirement is more critical if flow is to be estimated by the pulse dose procedure (Ellis et al., 1979) than by continuous dosing (Faichney, 1975 and 1980). In the case of rare earths, the size of the initial dose is limited by binding capacity of the particular feedstuff (table 2). This determines the size of the meal to be marked. If multiple rare earths are to be used, the sum of all rare earth to be marked should not exceed the binding capacity of that feedstuff for a single rare earth.

The dose of marker required for a pulse dosing procedure can be estimated by assuming: a) rumen dry matter fill of 2% of body weight or undigested dry matter fill in the whole gastrointestinal tract (equivalent to fecal dry matter) of 1% of body weight, b) a daily turnover of $1/k_2$-day, and c) a serial diluting effect of 0.5. Then:

dose (µg/100 kg weight) = MAL X F/100 kg body weight X $1/k_2$-day X D X (1/.5)

where:

MAL = minimal analytical level for the marker, µgm/g DM

F = dry matter fill for segment of interest, g/100 kg body weight

k_2 = approximate turnover expected for the marker expressed in days

D = days post dose of last collection

The size of the meal to be marked can then be determined from the expected binding capacity of the feedstuff divided by the dose of marker required for detection on the last day of collection.

Until more is known concerning the specific binding sites, it seems advisable to confine the binding to specified, less digestible entities such as CWC. This requirement can be rather confidently met for less digestible (< 65% digestibility) forages (table 6) but may pose problems for feedstuffs high in starch.

The non-CWC of masticated or ground feedstuff can be obtained by extracting it with a pH 9 phosphate buffer containing 3% sodium lauryl sulphate. The neutral detergent solution proposed by Van Soest should not be used since it contains EDTA and this chelating agent is difficult to completely rinse away. Trace residues can bind rare earths applied subsequently. In the case of Coastal bermuda grass, the mastication, insalivation and water washing-seiving procedure will remove essentially all non-CWC for particles below 500µM and 60-70% of that of larger size particles.

The nitrates salts of rare earths are preferred due to their higher solubility (up to 700 mg Yb $(NO_3) \cdot 8H_2O$/ml distilled water). The pH may be adjusted to 4 with acetic acid if necessary to increase solubility but pH should not exceed 7. To avoid hydrolysis to and precipitation of the hydroxide the rare earth should be applied to the feedstuff as soon as possible after the solution is prepared. For storage of a rare earth solution, the pH should be adjusted to below 4 to avoid precipitation.

A disposable plastic drinking cup with sealing cap is a convenient container for marking a feedstuff. The rare earth is suspended for 12-24 hr in a sufficient volume of distilled water to completely immerse the feedstuff to be marked. Subsequently, the sample may be washed several times for 4 to 6 hr periods with a volume of distilled water at least twice that used to suspend the rare earth. A large Buchner funnel with coarse porosity filter paper is useful for washing. The sample may be dosed in the wet state or following drying.

Analytical procedures. The similarities in the chemistry of all lanthanides suggests that any lanthanide would be suitable as a particulate flow marker. Ytterbium and scandium occupy related positions in the periodic table and have similar chemical properties. An association with digesta similar to that observed for specific lanthanides has been observed for ytterbium (Marcus and Lengemann, 1962) and scandium (Miller and Byrne, 1970b). Scandium has been demonstrated indigestible by ruminants (Miller and Byrne, 1970b). The choice of a specific lanthanide, ytterbium or scandium will most often be determined by the most sensitive and practical analytical method available.

Radioisotopes offer high sensitivity for detection and speed of analysis. Gamma emitting isotopes permit automated multichannel counting. Most gamma emitting radioisotopes can be detected at levels of 10^{-6} to 10^{-7} μCi.

From a radiological safety standpoint, radioisotopes with low energy emissions and short half-lifes (< 30 days) may be preferred. Due to the time required for collecting samples, half-lifes greater than 4 to 6 days are desired. Where multiple isotopes are simultaneously administered, isotopes must be chosen which are resolvable by the detector to be

used. With sodium iodide crystal detectors and pulse height multichannel analyzers, ^{141}Ce, ^{169}Yb, and ^{147}Nd can be used simultaneously. ^{160}Yb is also useful but has a rather long half-life (73 days). ^{177}Lu is similarly useful if a 6.7 day half-life can be accomodated. ^{141}Ce, ^{169}Yb and ^{147}Nd are particularly useful since they have short biological half-lives and low and relatively simple gamma energy levels which can easily be resolved with multichannel analyzers.

If use of radioisotopes is not feasible, radioactivation analysis can be employed (Ellis, 1968; Gray and Vogt, 1974; Boynton, 1979). Radioactivation analysis requires more extensive facilities than radioisotopes. At Texas A&M University, the sample is subjected to two hours of irradiation at a thermal neutron flux of about 10^{13}. The solid sample (.5 to 2.0 g) is irradiated in a 2 to 8 gram vial, allowed to decay 8 days and counted for 5 minutes. The gamma radiation is detected with a 3 X 3 inch Ge-Li detector. The emission spectrum is stored and subsequently processed and analyzed with a computer based multichannel analyzer system which employs programs for curve smoothing, area computation, and mass calculation. Cost, including irradiation, is about $20 per sample but can include multiple isotopes per sample. Some suggested minimal analytical levels for use in this procedure are summarized in table 9.

Atomic absorption spectrophotometry by nitrous oxide flame provides marginally sufficient sensitivity for Yb and Er and Dy. Certain rare earths will interfere with detection of others so the "standard addition" approach in analysis is helpful. This also partially corrects for viscosity differences in samples being analyzed. Sensitivity can be enhanced by 10-100 times through the use of direct combustion rather than preashing samples. Plasma optical emission spectroscopy may yield slightly

TABLE 9. SOME SUGGESTED MINIMAL ANALYTICAL LEVELS

Element	Procedure		
	Radioactivation	Atomic absorption	
	µg/sample	µg/ml[a,c]	µg/g[d]
Co	.5	1	10
Cr	1.5	2	20
Dy	NA[b]	4	40
Er	NA	4	40
Eu	.02	4	40
La	.06	-[e]	-[e]
Lu	.03	-[e]	-[e]
Sc	.004	4	40
Sm	.004	-[e]	-[e]
Tb	.20	-[e]	-[e]
Yb	.07	2	20
Ho	1.5	8	80

[a]Minimal concentrations in acid extract of ruminant digesta yield an increase in O.D. of .05 units.

[b]Half-lifes too short for 8 day decay procedures.

[c]µg/ml aspirated or extracted.

[d]Assuming 2 g sample ashed and extracted by 20 ml and analyzed in nitrous oxide supported flame.

[e]Not generally of sufficient sensitivity.

greater sensitivity for some rare earths (Fassel and Kniseley, 1974 and Dekalb and Fassel, 1979). Another method of promise is X-ray excited optical luminescence (D'Silva and Fassel, 1979).

Specific extraction schemes to avoid dilution of rare earths offer operational opportunities for increasing sensitivity. The rare earth oxides are relatively soluble even in dilute acid and readily extracted by strong chelating agents such as EDTA or DTPA at neutral or slightly alkaline pH. These properties can be used to selectively and quantitatively extract the rare earths from organic samples or ash (table 8). The use of such "leaching" procedures has the advantage of reducing levels of undesirable salts in the extract to be analyzed. Salts, especially of rare earths, often alter absorption of rare earth elements. These same solvents can also be used to quantitatively remove Cr and $^{35}SO_4$ from ash. For liquid scintillation counting, an extraction mixture of 3 M each HNO_3 and HCl is preferred to DTPA due to the low solubility of the DPTA in polar scintillation solvents.

The following procedure is convenient for determing rare earths and other metals in feeds, digesta and feces. A sample (1-2g dry matter) is weighed into 50 or 100 ml beakers and ashed at 500 C overnight. If ^{35}S is to be determined on the same sample, .04 g $AgNO_3$ and .96 g Na_2CO_3 per g of sample DM is thoroughly mixed with the sample before ashing (Walker and Nader, 1975). Upon cooling, the ash is leached with slow oscillation for 12 hours with 20 ml of a mixture of 3 M each of HNO_3 and HCL (or 0.1 M DTPA with sufficient acid to yield a pH of 8 to 10 in the final extract). After allowing the acid insoluble ash to settle, an aliquot of the sediment free liquid is removed for analysis by atomic absorption or radioactivity assay. Standards are made up in a "blank" solution obtained

TABLE 8. SOLUBILIZATION OF ^{169}Yb FROM FECES.

	Solvent	Percent of Yb solubilized
1.	pH 7 phosphate buffer, 2 hr. reflux	3.6
2.	#1 + 3% sodium lauryl sulphate	6.7
3.	#2 + 0.1 M DTPA	30-90
4.	Ash + conc. HNO_3	98.0
5.	Ash + pH 4.0 HNO_3	89.6
6.	Ash + pH 6 HNO_3 + 0.1 M DTPA	74.6
7.	Ash + 0.1 M DTPA (pH 8-10)	98.2
8.	Ash + 3 M HNO_3 + 3 M HCl	98.8

by similarly treating a digesta or fecal sample obtained prior to dosing. This procedure is very useful for radioassay by automated gamma spectrometry since the liquid media gives a highly reproducible counting geometry. Some suggested minimal analytical levels by this extraction procedure followed by atomic absorption spectrophotometry are summarized in table 9.

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Footnotes

[a]Obtained from Research Chemicals, P. O. Box 14588, Phoenix, AR 85063

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Doctor of Philosophy

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