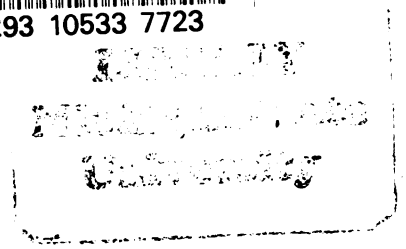

This is to certify that the

thesis entitled

Site and Extent of Digestion and Duodenal Digesta Flow Patterns in Steers Fed Alfalfa Haylage Diets

presented by

William Vernon Rumpler

has been accepted towards fulfillment
of the requirements for

Ph.D. degree in **Animal Science**

Werner G. Bergen

Major professor

Date 2/20/84

SITE AND EXTENT OF DIGESTION AND DUODENAL DIGESTA FLOW PATTERNS IN STEERS FED ALFALFA HAYLAGE DIETS

By

William Vernon Rumpler

A DISSERTATION

Submitted to
Michigan State University
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1984

ABSTRACT

SITE AND EXTENT OF DIGESTION AND DUODENAL DIGESTA FLOW IN STEERS FED ALFALFA HAYLAGE

BY

William Vernon Rumpler

Two experiments (exp.1 & 2) were conducted to examine the site (SOD) and extent of digestion in steers fed diets consisting of principally alfalfa haylage (AH). Four Holstein (both exp.) steers were fitted with intestinal cannulas (exp.1 - duod., exp.2 - duod. and ileal). The steers (both exp.) were fed at approx. 2% of body weight (DM basis). Samples (intest., fecal) were collected at 6 hr. intervals for 3 days resulting in one sample for every odd hr. in a day. Markers used were Yb and Cr.

In exp. 1 the diets consisted of two AH ensiled at two DM levels (30 and 60% DM). Total tract digestion (% of intake) (TTD) of dry matter (DM), nitrogen (N), acid detergent fiber (ADF) and neutral detergent fiber (NDF) was 62.1, 64.1, 66.9, 61.2, 62.7 respectively for the 30% DM AH and 38.7, 37.0, 45.2, 32.4 and 30.1 for the 60% DM AH. Ruminal digestion (% of TTD) (RD) of DM, OM, N, ADF and NDF was 70, 79, -1, 122 and 110 respectively for the 30% DM AH and 27, 43, -99, 157 and 143 respectively for the 60% DM AH.

In exp.2 the diets consisted of AH ensiled at either 30% DM or 45% DM. These AH were fed with or without supplemental high moisture corn (HMC). There were no significant differences between diets for any of the digestion parameters measured (either TTD or SOD) but there was a trend for the addition of corn to shift SOD. Average, across diets, TTD of DM, N, OM, ADF and acid detergent insoluble nitrogen (ADIN) was 63.8, 63.8, 69.3, 62.2 and -2.5. Average, across AH, RD (without HMC : + HMC) was 74% : 70% for DM, 96% : 85% for OM, 48% : 25% for N and 92% : 102% for ADF.

A third experiment was conducted to examine duodenal digesta flow patterns (DFP) of the steers in exp. 2 The animals were abomasally infused with PEG. The flow rate was calculated by dilution of PEG at the duodenum. Hourly flow represented between 2 - 6 % of the total daily flow. The effect of animal, diet and season on DFP and the effect of non-steady flow on estimates of SOD was discussed.

To my parents
Ronald and Beverly Rumpler

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INTRODUCTION

Providing animals with the proper balance and amount of nutrients has long been accepted as a means to improve production. To accomplish this the nutrient requirements of the animals must be known. In addition, the composition and nutrient availability of the feedstuffs in the diet must be known. Good approximations of the nutrient content and avalability of nutrients from feedstuffs can be achieved in monogastric animals by simple balance studies. The difference between intake and excretion can give reasonable estimates of nutritive value, since contributions from endogenous sources and alterations of composition, due to lower gut fermentation, tend to be small. Comparision of estimates of nutritive value and performance data can give reliable approximation of the requirements of the animals.

Due to the complex nature of the ruminant digestive system estimation of the nutrient content of feedstuffs for ruminants is complicated. Foregut fermentation alters the nutrient profile of the feedstuff before it passes to the small intestine. Hindgut fermentation alters the digesta passing out of the small intestine into the large intestine before it is excreted. In addition, a wide variety of factors

have been shown to affect the extent of digestion in the rumen (Bull et.al., 1979). A few of these factors are level of intake (Gaylean et.al., 1979, Blaxter,1961), diet particle size (Galyean et.al., 1979), forage to concentrate ratios (Potter et.al., 1971),NaOH treatment (Berger et.al, 1980) and mastication (Pearce and Moir, 1964).

Since foregut fermentation occurs and a wide variety of factors, in addition to diet composition, alter the extent of fermentation, the necessity of determining the site and extent of digestion is apparent. The determination of the site and extent of digestion is necessary if estimates of the nutrient availability of feedstuffs and the nutrient requirements of ruminant animals is desired.

LITERATURE REVIEW

SITE AND EXTENT OF DIGESTION STUDIES

IMPORTANCE

Digestion of a feedstuff makes the various components of the feed available to the animal for use in the maintainence and growth of body tissue and provides energy for metabolic processes and work. Total tract digestion estimates provide information as to the amount of nutrients which disappear, from the feedstuff, during passage through the total digestive tract. In monogastric animals, total tract digestion estimates represent the amount and composition of the nutrients absorbed by the animal, with minor corrections for endogenous components excreted in the feces. However, in the ruminant animal significant alteration of the nutrient profile of the feedstuff occurs in the foregut (rumen). This alteration in the nutrient profile of the feedstuff, in the rumen, affects quantification of several important aspects of digestion and efficency of utilization of nutrients. Some of the aspects of digestion and utilization of nutrients which are affected by the site of digestion include: Extent of fiber digestion; Efficency of carbohydrate utilization;

Efficency of nitrogen utilization; Estimation of the nutrient requirements of the animal. Each of these factors and how they are affected by site of digestion will be discussed below.

Fiber Digestion

Simple sugars are linked together to form complex carbohydrates used for storage and or stuctural support of plants. These plants are then consumed and digested by animals. Most animals synthesize enzymes capable of breaking down the principal storage form of carbohydrate (starch), which is a complex of alpha linked glucose units. The other and more prevalent form of carbohydrate is cellulose. Cellulose, a complex of beta linked glucose units, is synthesized by the plant as a structural component of its cell wall. This beta linked complex cannot effectively be broken down by enzymes produced by animals. However, bacteria do produce cellulases which can attack the beta linkages and breakdown the cellulose to its individual units (Hungate, 1966).

The diet of most domestic animals (cattle, sheep, horse, pig) consists primarily of plant material. Plant material contains a high proportion of its' carbohydrate as cellulose, thus, many types of animals have evolved digestive strategies which utilize bacteral digestion within their own digestive system. Hind gut fermentation can occur in a lower gut fermentation compartment (cecum) (horse, elephant) or in

the large intestine (pig). The ruminant (cattle, sheep, goats), utilizes a foregut fermentation compartment (rumen) and hind gut fermentation.

The foregut fermentation of cellulose is a much more effective strategy than hind gut fermentation. The useable products of fermentation are primarily volatile fatty acids and microbial cells. Microbial cells are very digestible (80%) (Bergen, 1978) and provide both lower gut digestible carbohydrates and protein. Hind gut and forgut fermentation produce the same products. However, when these products are produced anterior to the digestive and absorptive sites of the small intestine and stomach the animal only can utilize a small portion of the available nutrients.

In light of the above discussion, site of digestion of cellulose is important in the ruminant for two primary reasons. 1. Shifting cellulose digestion out of the rumen reduces the efficency of utilization of the cellulose which must be fermented to be utilized by the animal. This is due to lack of digestive and absorptive sites and digestive enzymes anterior to the small intestine, which can breakdown the microbial cells, produced by the fermentation process, into more readily absorbable components. 2. If cellulose digestion in the rumen is reduced significantly, it is unlikely, that fermentation in the lower gut will be able to compensate adequately. Thus, total tract digestion of cellulose will be reduced.

Efficency of Dietary Energy Utilization

Many of the feedstuffs in ruminant diets contain componenents which are capable of being enzymatically digested in the abomasum and small intestine (starch, protein, fat). Due to the nature of the ruminant digestive tract much of this fraction is fermented in the rumen. The relative proportion of the amount of the feed components which are fermented in the rumen versus digested in the abomasum small intestine can have a marked affect on the efficency of dietary energy utilization.

Black (1971) calculated the efficency of dietary energy use when the diet was either entirely fermented in the rumen or when digested entirely in the stomach - small intestine by the animals digestive enzymes (Table 1). The non-ruminant lamb realized nearly three times the productive energy from the same dietary energy as did the ruminant lamb. This difference resulted primarily from the losses due to methane production and heat of fermentation which the ruminant lamb incurred and the non-ruminant lamb did not. This indicates the relative inefficient use of diet components, which can be digested by the animals digestive enzymes, when fermented in the rumen. When ruminant animals are fed diets that contain components effectively digested in the animals abomasum - small intestine, shifts in the site of digestion could markedly affect the efficency of utilization of dietary energy.

TABLE 1. Theoretical utilization of dietary energy. Fermentation versus host enzyme digestion. (Black, J.L., 1971)

Energy partition (kcal/d)	Ruminant[a] Lamb	Non-ruminant[b] Lamb
Gross Energy	1780	1780
-fecal microbial residue	-18	--
-endog. fecal secretions	-27	-27
Digestible Energy	1735	1753
-methane	-174	---
-heat of fermentation	-104	---
-heat of digestion	---	-18
-energy in urine	-64	-41
Metabolizable Energy	1393	1694
-heat increment	-276	-229
-urea format. & excrt.	-29	-15
Net Energy	1088	1450
-maint. req.	890	890
Productive Energy	198	560

[a] assumes 100 % of diet fermented in rumen.

[b] assumes 100 % of diet digested in stomach - small intestine

Efficency of Dietary Nitrogen Utilization

A schematic of nitrogen utilization in the ruminant is presented in Figure 1. Dietary nitrogen can be classified as protein and nonprotein nitrogen (NPN). The microbes in the rumen can convert NPN into protein nitrogen during the fermentation process. Also, some of the dietary preformed protein is broken down and converted to amino acids and ammonia. Much of this amino acid and ammonia is used to synthesize protein. The protein which is produced by the microbes, from NPN and the breakdown of preformed protein, has a fairly high biological value (Bergen, 1978). Thus, fermentation of the dietary nitrogen, when high in NPN or low quality preformed protein, gives the ruminant animal a improved supply of protein for maintainence and growth of body tissue.

Even when the diet is high in good quality preformed protein, the breakdown and partial resynthesis of the protein occurs. Some of the ammonia excapes incorportation into microbial protein and is absorbed into the blood. This ammonia which gets into the blood is converted into urea in the liver and is either recycled into the rumen (via the saliva or across the rumen wall) or excreted via the urine. The breakdown and resynthesis of the high quality protein can have several negative features: 1. Urea can represent a significant loss of nitrogen to the animal. 2. The synthesis of the urea represents an energy cost to the animal. 3. The microbial protein may not be as digestible or

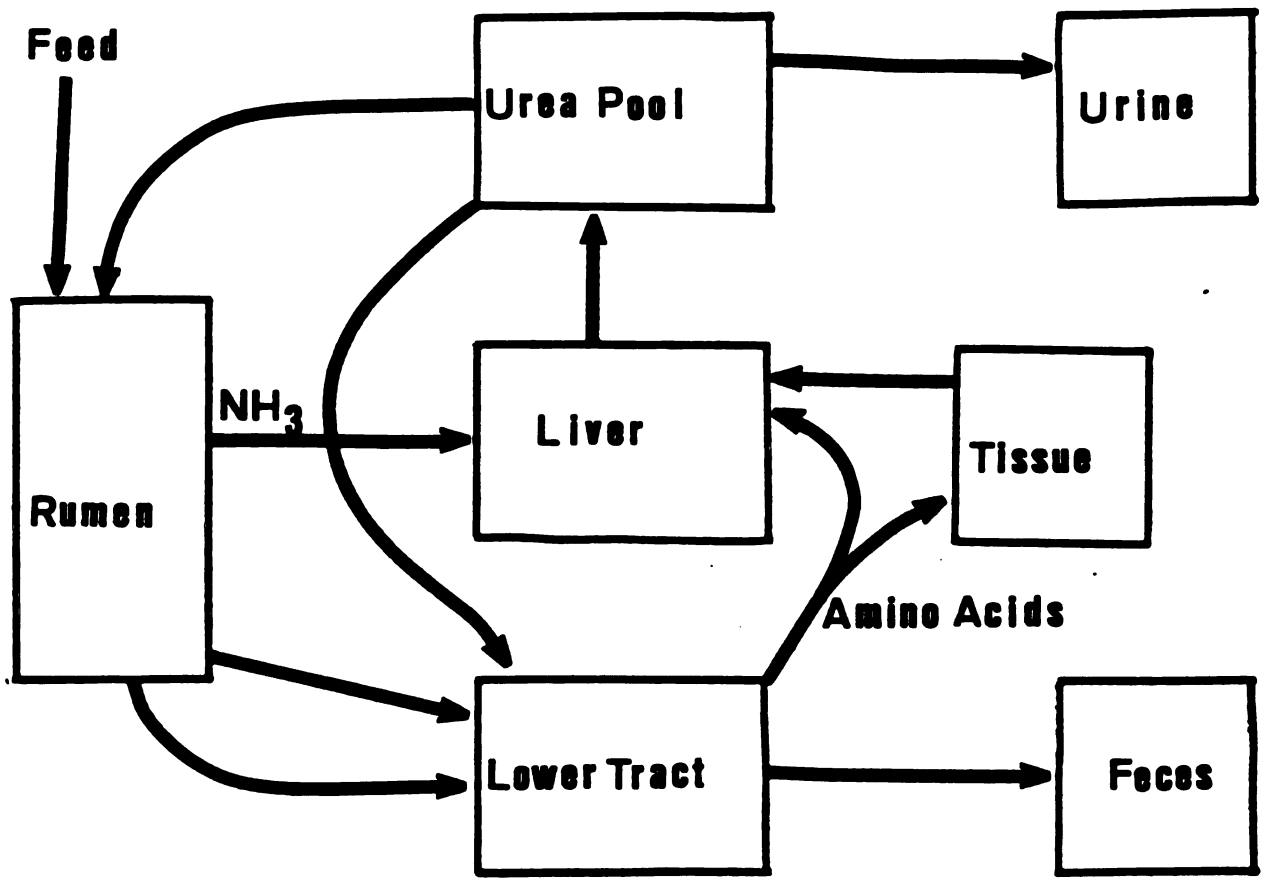


FIGURE 1. Nitrogen Metabolism in the Ruminant.
(Van Soest, 1982)

have as good as an amino acid profile as the diet protein. 4. During the synthesis of microbial cells a certain amount of the diet nitrogen is converted to nucleic acid and other nonprotein nitrogen compounds.

Estimating Requirements

The foregut fermentation which occurs in the ruminant poses a problem for the nutritionist attempting to estimate the nutrient requirements of the animal. The traditional method of estimating the requirements is to compare nutrient uptake with animal performance. From the previous discussion it is easy to understand how this is not straight forward in a ruminant animal. The fermentation of the dietary components can alter the composition of the nutrients in the diet. The result is that the composition of the digesta flowing out of the rumen will not be the same as the diet composition. Thus, nutrients absorbed by the animal cannot be calculated as the difference between the feed intake and fecal outflow. This requires some method of estimating the amount and composition of the flow out of the rumen.

TECHNIQUES

Three methods or approaches are principally used to determine site and extent of digestion: 1. Kinetic Technique; 2. Total Collection Technique; 3. Marker Ratio Technique. Each of these methods will be discussed below and the advantages and disadvantages of each technique will be outlined.

Kinetic Technique.

This technique involves the coupling of estimates of rate of digestion with estimates of residence time in the rumen to calculate the extent of ruminal digestion. The estimates of digestion are principally obtained by the in situ digestion technique. The residence time estimates are calculated from turnover rates of tracers placed in the rumen. The kinetics of these tracers (depending on the tracer), in the rumen, allow estimation of the turnover rate of the whole rumen or specific components in the rumen. Both of these techniques (in situ, turnover rate) will be discussed followed by an example of their use in estimating extent of ruminal digestion.

In Situ Technique

In situ digestion (i.e. nylon bag technique) has been used to estimate crude protein digestion (Kristensen et.al.,1982), fiber digestion (Van Hellen and Ellis, 1977) and effect of ruminal digestion on changes in amino acid profiles of feeds (Rumpler, 1979, Ganev et.al.1979). It involves the incubation in the rumen of feedstuffs, secured in porous polyester bags. Most workers using this technique have allowed free movement of the nylon bags within the rumen by simply anchoring the container to the rumen cannula and allowing sufficent nylon line to permit the container to move around in the rumen. These bags are removed, at discrete intervals, dried, weighed and analyzed for components of interest. Determination of the amount and composition of the residue, in the bag, after definite lengths of incubation, allows calculation of rates of digestion.

A number of factors affect the results obtained from this technique. These factors can be categorized as container, sample or rumen related. Container factors include porosity of the container material, sample size to container size ratios and position in the rumen. Van Hellen and Ellis (1977) found porosity of the material markedly affected digestion. They also reported a marked affect of the ratio of container size to sample size. Uden et.al. (1974) reported also reported an affect of container size and porosity on rate of digestion estimates.

Sample preparation has been shown to affect the estimates of rate of digestion obtained by this technique (Playne et.al., 1978). Fine grinding increases digestion in grains but had minimal affect on forages. Chewing of samples (obtained via esophogeal cannula) also increased digestion as did an acid pepsin predigestion. Drying of samples has been shown to markedly affect digestion (Orskov, 1982).

The advantage of this technique is that the degradation rate for a large number of samples can be determined with relatively little work. The major drawback is the uncertainty as to how well the degradation rate obtained estimates the real situation. An effort should be made to simulate the dietary conditions and intake levels under which the estimate will be used.

Rumen Turnover Rate Estimation

Bull et.al.(1979) defined rumen turnover as a measure of the time required for the outflow of enough of a component to equal that present in the rumen. Therefore, the amount leaving the pool per unit time is turnover rate. The amount of the total in the pool which leaves per unit time is refered to as the fractional turnover rate or k. The fractional turnover rate is generally the constant used to refer to turnover. To calculate the fractional turnover rate (k), generally, the rumen is considered to be a single pool

for the component with one input and one output (Figure 2).

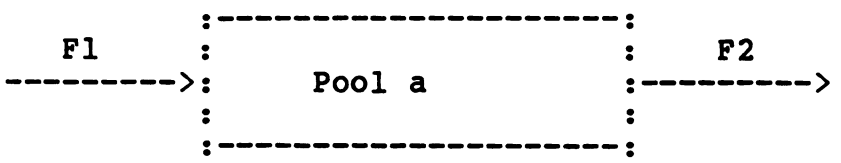


FIGURE 2. One Pool Model Representation.

Certainly there exists more than one entry and exit route in the rumen, depending on the component of interest. The use of this type of model is justified since the investigator is generally interested in a particular component which exists in the rumen and will have one dominant route of input and one or two dominant output routes. If more than one input or output routes does exist, the k value arrived at is a summation of the multiple routes and represents the overall phenomenon. If single pool kinetics are accepted, generally steady state conditions are considered to exist. Steady state refers to a condition in which pool size, inflow rate and outflow rate remain constant. Strictly speaking, steady state probably does not exist but if the rumen is viewed over a long period of time (i.e. 24 hours) it probably meets this criteria.

If the above conditions can be met reasonably well, there are two general methods for determining rumen turnover. Both methods involve a tracer (marker). The two methods

differ in the manner of introducing the tracer into the pool. The tracer is introduced, into the pool, either continuously or with a single dose.

A tracer is defined as a compound which can be differentiated from the component of interest but will behave chemically and physiologically like the component. Types of tracers and problems associated with them as they relate to digestion studies will be discussed in later sections.

Constant Infusion

This procedure involves the continuous infusion of a tracer into the rumen at a constant rate. Steady state conditions are assumed and no other source of tracer is present. Shipley and Clark (1972) detailed the model used and subsequent calculations and a general overview will be given here. An examination of the model (Figure 2) where component input rate (i.e. diet) F_1 is equal to outflow (turnover rate, F_2) with r being the rate at which the tracer is being introduced. From time (t_0), when infusion begins, specific activity (concentration of tracer in tracee) increases to a plateau value (SA_e) as represented in Figure 3.

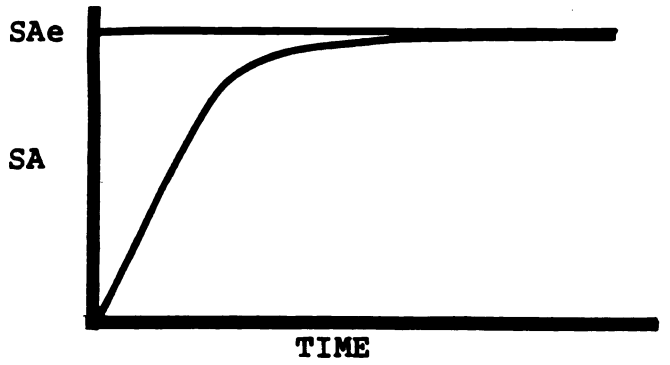


FIGURE 3. Specific Activity of Tracer in a Single Pool With Time After Start of Infusion

This is the specific activity at equilibrium. If the rate of infusion is known (r) and the specific activity at equilibrium is measured the inflow (F_1) rate can be calculated (equation 1).

$$1. \quad F_1 = r/SA_e$$

Since in steady state F_1 must equal F_2 the equation becomes

$$2. \quad F_2 = r/SA_e$$

This series of equations gives outflow rate if inflow is unknown (i.e. water intake) but does not allow calculation of pool size. If samples are obtained during the time when SA is increasing rate constants and pool size can be calculated. If k is outflow and r is rate of infusion the the amount (q)

of tracer in the pool is equation 3.

$$3. \quad q = (r/k)(1-e^{-kt})$$

Since k is fractional turnover rate and represented by

$$4. \quad k = F/Q$$
$$5. \quad SA = q/Q$$

equation 4, where Q is pool size, F is outflow or inflow and SA is the relationship (equation 5) between tracer and tracee. Division of both sides of equation 3 by Q yields equation 6.

$$6. \quad SA = (r/F)(1 - e^{-kt})$$

As a check at t = infinity (i.e. plateau) the exponential component in equation 6 drops out and gives equation 7, which is the same as equation 1.

$$7. \quad SA_e = r/F$$

To calculate k subtract SA_e from the values prior to the plateau and plot the natural log of $SA_e - SA_t$ versus time. The slope of this plot is k.

Several possible permutations of this exist. Priming doses can be used and calculations of multipool models can be

done. These are beyond the scope of this review however, and furthur treatment of this type of mathematics can be found in Shipley and Clark (1972).

Single Dose

This procedure involves the administration of a single dose of the tracer into the pool. Serial sampling following dosing will give a SA curve such as Figure 4.

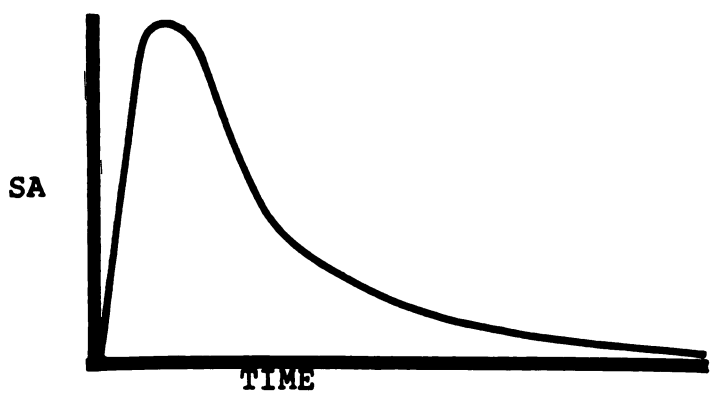


FIGURE 4. Specific Activity of a Tracer in a Single Pool System With a Single Dose.

Assuming a single pool model (Figure 2) fractional turnover rate and pool size can be easily calculated.

The initial rise in SA is due to non-instantaneous mixing. Since tracer as well as tracee are passing out at a constant rate and the tracer and tracee do not mix

instantaneously the peak concentration on the curve is some what less than dose divided by pool size. This necessitates the calculation of the fractional turnover rate first and then extrapolation back to zero time.

A log plot of the SA of the pool (Figure 4) results in a curve with the slope of the declining portion being the fractional turnover rate (k). Linear regression of the curve results in equation 1.

$$1. \quad SA_t = A + kt$$

SA_t is the specific activity at any time (t), k is the fractional turnover rate and a is the specific activity of the pool at time t_0 if instantaneous mixing occured. At time t_0 equation 1 becomes $SA_0 = A$. Pool size can be described by equation 2,

$$2. \quad Q = SA_0 / q$$

where q is the tracer dose, Q is the pool size and SA_0 is the specific activity at t_0. Since Q and K are now known the turnover rate (F) is simply the relationship shown in equation 3.

$$3. \quad F = kQ$$

Continuous Infusion or Single Dose ?

Each method of administering the tracer has its advantages and disadvantages. The continuous infusion has the advantage that after equilibrium is reached multiple samples can be taken which will give a more accurate evaluation of the plateau value of the tracer in the rumen. Nonsteady state is also not as great a problem with continuous infusion systems. Since the tracer is delivered continuously over a relatively long period of time small fluctuations in input or outflow can be dealt with as deviations from the mean plateau value. The principal disadvantage of continuous infusion is the need for an infusable tracer. Liquid phase tracers are readily obtained but solid phase tracers are more difficult. Solid phase tracers which have been used tend to bind in a non-specific manner to particulate matter and cannot be directed to any one component.

The single dose method is generally the method of choice. It requires no special infusion system and tends to be less stressfull to the animals. Tracer used in a single dose experiment can be solid or liquid and can be attached to a specific component of the pool or be nonspecific which ever is required for the experiment. The two primary disadvantages are the mixing problem and non steady state conditions. Since instantaneous mixing does not occur, the longer the tracer takes to become evenly distributed

throughout the pool, the greater the error in extrapolating zero time concentration. Non-steady state condition also presents a problem. With ever decreasing concentrations of tracer in the pool fluctuations in outflow and inflow rate will increase the error associated with the estimation of the decline. Deviations from linearity of the natural log concentration versus time plot increases error and decreases the confidence of the prediction of pool size and rate. One way to improve the estimate is to continue sampling for long periods of time after dosing. Since this is a natural log function as time increases the fluctuation in concentration are reduced in magnitude. Long sampling times however necessitate high concentrations early in the sampling period so that levels are still detectable at later times. These high levels could possibly affect the rumen and disturb outflow or inflow.

Both methods have three major disadvantages. They require cannulated animals, accurate sampling and a tracer which follows the component being studied. Effects of cannulation and tracer methodology will be discussed in later sections. Sampling accurately has always and will continue to be a problem with rumen studies. The heterogenous nature of the rumen and the diets fed make sampling difficult. Therefore, great care must be taken when sampling and interpretation of the results must be tempered with the sampling problem in mind.

Rumen Outflow Measurements

Both of the previous methods involve dosing and sampling in the rumen. Subsequent analyses of the tracee and the tracer permits calculation of decay curves and outflow rates. Rumen turnover rate (k) can also be calculated from tracer decay curves in samples obtained from abomasum/duodenum and or feces. The mathematics are very similar to previous discussions but methodology and implications are quite different.

Fecal Excretion Curves

Fecal excretion curves are generally based on a two pool model with a time delay (Figure 5).

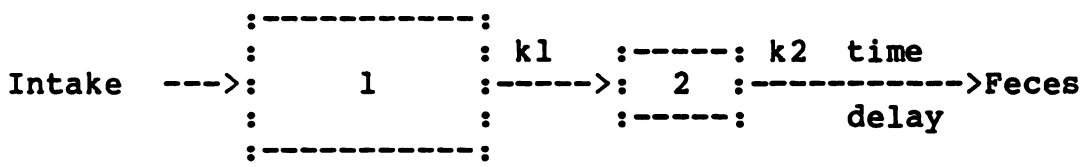


FIGURE 5. Two Pool Model Representation

The time delay is the length of time for digesta to pass from the proximal duodenum to the colon. There are differing opinions as to the nature of pools 1 and 2. Grovum and Williams (1973) postulated pools 1 and 2 represent rumen-

reticulum and cecum-colon respectively, with corresponding k values representing movement out of each pool. Hungate (1966) suggested pool 1 was the large particle pool and pool 2 was the small particle pool. Rate of particle size reduction would account for k1 and movement out of the rumen k2.

The mathematics of pool separation remain the same which ever model in accepted. A fecal excretion curve is obtained over a suitable length of time (Figure 6) when the rumen is dosed (single) with a tracer. The mathematics are discribed in detail by Shipley and Clark (1972). This review will present a general overview and adaptation with the reader directed towards a more general reference (as above) for more information.

The noninterchanging nature of this two pool system symplifies the mathematics. Grovum and Williams (1973) use a simple equation to describe the system.

$$1. \qquad y = Ae^{-k_1 (t-TT)} - Ae^{-k_2 (t - TT)}$$

Where TT is the calculated length of time to first appearance of tracer in the feces after a single dose. k1 and k2 are the rate constants associated with movement from their respective pools. A is the adjusted tracer concentration calculated as the intercept of the lines

derived from pools 1 and 2 (Figure 6).

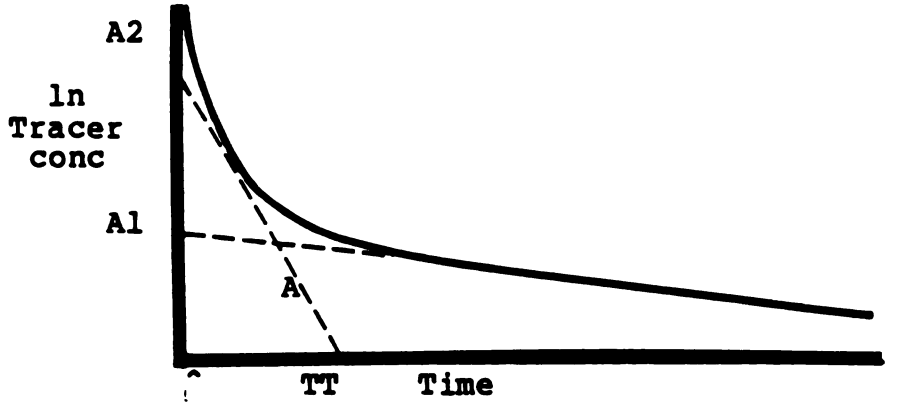


FIGURE 6. Decay Curve Based on a Two Pool Model

Curve peeling is used to generate the rate constants k1 and k2. Linear regression of time versus the natural log of the latter part of the concentration curve generates the line:

$$2. \quad y = A_1 + k_1 SA_1$$

This line is then used to calculate the expected specific activities for the rising part of the curve (pool 1). Subtracting the measured values from the predicted specific activities gives the predicted SA of the tracer in pool 1. Regression of the natural log of these values versus time gives the equation (equation 2).

$$2. \quad y = A_2 + k_2 SA_2$$

The intercept of these lines times gives the adjusted marker concentration A and the transit time (TT) . Pool sizes (Q) of 1 and 2 are represented by equation 4.

4. Q_1 = dose/inv ln A_1 and Q_2 = dose/inv ln A_2

and the sum of pool 1 and 2 is represented by equation 5.

5. $Q_{(1+2)}$ = dose/ inv ln A

Duodenal/Abomasal Excretion Curves

Two types of flow calculations are primarily made from duodenal flow studies, total digesta flow and marker decay rates. Total flow is an estimate of amount and composition of rumen outflow per day. Tracer decay rates are used to estimate rumen turnover.

To estimate rumen turnover, from doudenal-abomasal excretion curves, markers are added to the diet for several days until an equilibrium is reached. A sample (or samples) is obtained at the site of interest prior to withdrawl of the marker from the diet. After withdrawing the marker serial samples are obtained. Then the natural log of the concentration of the marker in the sample is regressed versus time. The slope of the line is the fractional turnover rate.

Rumen volume cannot be determined from these studies since samples are being collected anterior to the rumen and an unknown contribution of the abomasal secretions which adds to the volume as well as fluid absorption from the omasum which reduces the volume. The fractional turnover rate can serve as a relative indicator of the rate of movement of different components of the diet from the rumen.

Sample Calculations

The type of data obtained from this type of study is shown in Table 2. All subsequent calculations adapted from Orskov and MacDonald (1977).

TABLE 2. Protein Disappearance and Effective Protein Degradation of Soybean Meal (Orskov and McDonald, 1979)

Time After Feeding	Protein Disappear.(%)		Effective Degrad.(%)	
	Measured	Fitted	Restricted	Ad Lib
3	38	37	36	36
6	51	51	47	46
9	59	62	55	53
15	79	77	64	61
24	89	89	69	65
infinity		100	71	66

Effective degradation of the protein source (EDP) digested in the nylon bags is calculated from equation 1.

$$1.\quad EPD = \int_0^t e^{-kt}\, b\, e\, dt = a + \frac{b}{(1+k)}\,(1-e^{(1+k)t})$$

where p is calculated to give a and b (equation 2)

$$2.\quad p = a + b\,(1-e^{t})$$

where a and b are constants fitted by least squares linear regression of the measured extent of degradation (p) allowing predition of degradation (p). Thus extent of degradation up to any point in time becomes a function of rumen turnover rate (k) and degradation (b). When time (t) is taken to infinity the equation becomes

$$3.\quad D = a + \frac{b}{1+k}$$

and extent of degradation (D) is calculated from equation 3.

These equations can also be used to predict the amount of organic matter and nitrogen released into the rumen during any time interval. The difference between t_0 and t_1 (t_1 is any time after introduction of feed into rumen t_0) gives the amount released into the rumen. Coupling this with estimates of unit N incorporated per unit of organic matter fermented microbial protein production can be estimated. Estimated microbial protein can be used to estimate production of microbial cells. Very complex systems can be devised for

predicting rumen outflow and composition based on similar calculations.

The problems with these types of studies are obvious. Any error in estimation of rumen turnover rate or rate of degradability of the particular feedstuff will result in erroneous values. In addition, the differential digestion of fractions of the feedstuff, which have different compositions, complicate the estimation of the composition of the outflow.

Total Collection Technique

The total collection technique in digestion studies is based on a very simple concept. Digestion is calculated by determining the amount of a component which enters the pool and subtracting the amount which leaves and the difference is the amount digested. While the concept is very simple, the determination of the amount and composition of all of the outflow from a pool may not be simple.

If the whole digestive system is considered to be a single pool, digestion can be calculated by the difference between intake and fecal output of any particular component. Estimation of the amount and composition of fecal flow can be accomplished by collecting all the fecal output over a period of time and obtaining a subsample which will represent the total fecal collection. The analysis of the subsample will give a value, for the amount of a component of interest,

which can be used for the total collection. Thus, fecal output of a component is simply the composition of the subsample times the amount of fecal flow.

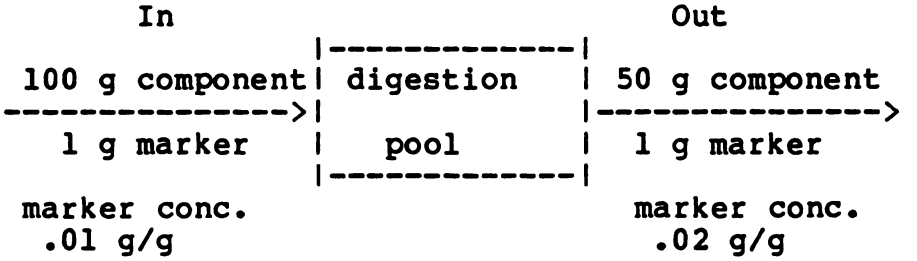
The same general principles apply for the calculation of digestibility within the rumen, by the total collection technique, as were applied for total tract digestibility. However, collection of total outflow from the rumen is not as simple as total fecal collection. The most common method used is to exteriorize the small intestine and insert a reentrant cannula. The use of this type of cannula allows total collection of the digesta flowing out of the abomasum and is assumed to represent, fairly well, the digesta flowing out of the rumen. The digesta collected is then subsampled and reintroduced into the small intestine. In a later section, the affect of cannulation by this method will be discussed but it is obvious that an animal altered in this manner may not exhibit normal digestive function.

The advantage of this technique is that, at least in theory, by collecting all of the feces and or digesta an accurate representation of the flow through the digestive system is achieved. However, it is difficult to collect all of the feces for a large animal without special facilities and these facilities usually require the restraint of the animals. Also, to collect all of the flow out of the rumen via a reentrant cannula requires the animal to be hooked up to an automatic sampling device which subsamples and

reintroduces the digesta into the small intestine. Attempting to relate data obtained under dramatically unnatural conditions to actual on farm situations is difficult. The requirement for restraint of the animal and radically altering the digestive system, with reentrant cannulas, are the major disadvantages to this type of study.

Marker Ratio Technique

This technique is based on a simple concept. Figure 7 is a diagramatic representation of the principle involved.



Digestibility = 1 - (marker conc. in/ marker conc. out)

FIGURE 7. Representation of the Marker Ratio Technique.

In this example there is 100 g of a component of interest entering a digestion pool. After digestion there is 50 g left. Thus, 50 percent of the component is digested away. If the amount flowing out (actually neither amount flowing in or out is needed) is unknown but the concentration of a

nonabsorbable, indigestible marker is known in the inflow and outflow, from a digestion pool, the digestibility can be calculated. The digestibility of the component is equal to one minus the concentration of the marker, in that component, going in to the digestion pool divided by the concentration of the marker comming out of the pool.

This technique is widely used in site of digestion studies. The use of this technique eliminates the need for total collection and allows the spot sampling of the outflow of a digestion pool. Since total collection is not necessary, T type cannulas can be used in place of reentrant cannulas. Much less radical alteration of the digestive system is required for T cannulation. Thus, the animal can be maintained in a more natural environment and under conditions likely to be seen in production situations.

There are certain assumptions which are made when utilizing the marker ratio technique and spot sampling. These are: 1. The markers are nonabsorbable and indigestible; 2. Samples obtained are representative of the digesta flow at the site of interest; 3. Steady state conditions exist.

The final section of this literature review will deal with markers which are available and the kind of information which can be derived from them. Sampling is a very difficult problem to address. It has been shown several times that spot sampling and the marker ratio technique is more than

adequate to estimate total tract digestion (Young et.al. 1976, Thonney et.al., 1979, Prigge et.al., 1981). The same consistancy cannot be demonstrated with duodenal sampling (Weber, 1983). Whether inconsistant results are due to poor sampling or other factors is unknown. Faichney et.al.(1980) presented a scheme for correcting digesta samples which may contain improper amounts of solids or liquids but did not determine if the problem was sampling or something else. The next section will deal with the steady state assumption and how it may account for inconsistant results.

Rumen Outflow - Steady State ? Non Steady State ?

One of the principal assumptions made in marker studies is that conditions have achieved steady state. As defined earlier, steady state is a condition in which pool size, rumen outflow and rumen inflow remain constant. In digestion studies both rate and composition of inflow and outflow need to remain constant. Much of the mathematics described previously require steady state conditions. In this section three questions will be addressed ; 1. Do steady state conditions exist for rumen outflow ? 2. What affect will nonsteady state outflow have on estimation of rumen outflow ? 3. With nonsteady state how can rumen outflow be estimated ?

Does Steady State Exist ?

The literature provides a very schetchy data base to evaluate this assumption. However, some data is available on continuous measurement of duodenal digesta flow which is an indication of rumen outflow. Poncet et.al. (1982) used an electromagnetic flowmeter to measure flow in both the ascending and the transverse duodenum in sheep. They attempted to evaluate the effect of different types of cannula on the flow of digesta through the duodenum. They reported no significant differences in the flow between types of cannula, except reentrant cannula gave lower flow rates. Flow in these sheep varied from 527 ml/hr with the Y type reentrant cannula and 802 ml/hr with the simple T type of cannula. In addition the standard deviation was between 10 and 20 percent within an animal.

Other workers have reported similar flow rates in sheep using continuous infusion techniques. James et.al. (1981) measured flow rates of 14.1 ml/min with a standard error of 0.31 by continuous infusion into the duodenum. The most extensive collection of observations on duodenal flow was reported by Corbett and Pickering (1983). These workers measured flow rates on 72 individual animal by continuous infusion into the rumen of Cr:EDTA. Hourly flow rate varied in excess of ±30 percent from the average flow over 24 hours. This variation in flow did not appear to be related to feeding behavior or diet. These studies indicate that flow rates vary significantly and that at least in these studies

flow was not constant.

Effect of Nonsteady State Flow On Rumen Outflow Estimates

To examine the effect of nonsteady rumen outflow on the estimates of daily digesta flow out of the rumen we can propose a hypothetical situation where flow and composition of the digesta vary (Table 3).

TABLE 3. Hypothetical Digesta Flow and Composition

Time	Digesta Flw.Rt.	Digesta DM Conc.
8am	1.0 l/hr	30.0 g DM/1000g digesta
2pm	0.7 l/hr	50.0 g DM/1000g digesta
8pm	0.5 l/hr	80.0 g DM/1000g digesta
2am	0.2 l/hr	40.0 g DM/1000g digesta

In the example above 4 samples were collected within a 18 hour period. If steady state flow exists, then each hourly sample represents an equal proportion of the daily composite and the composite should represent the average daily digesta, even if the composition of the digesta changes thoughout the day. In the example above, however, the flow and composition change. Intuitively, the amount of any individual hourly sample added to the composite should reflect the flow rate at that hourly sampling time. Thus, high flow periods would represent more of the composite than low flow periods. The results obtained with each method are simulated in Table 4.

TABLE 4. Effect of Compositing Method on the Dry Matter Content of a Daily Composite

Samp.Time	% DM	Weighting Factor *	% DM Weighted
8am	3.0	1.0/2.4 = .41	1.23
2pm	5.0	0.7/2.4 = .29	1.45
8pm	8.0	0.5/2.4 = .21	1.68
2am	4.0	0.2/2.4 = .09	0.36
average	5.0		4.72

The two methods result in somewhat different composition of the composite sample. This indicates that if nonsteady state exists, compositing by adding equal amounts of each individual sampling time to the composite, may result in a composite with a compostion different than that obtained for a weighted composite, in which the weighting factor is based on the hourly flow rate. The generally accepted method of compositing is by adding equal proportions of each sampling time to the composite. If flow and composition are not constant, compositing method may account for some of the inconsistant values obtain in this type of study.

MARKERS

Prior to this section much of the discussion has involved tracer kinetics. In digestion studies the class of tracers involved have generally been refered to as markers. These terms have often been used synominously in the literature but have much different implications. A tracer, as defined earlier must behave chemically and physiologically as the component it wishes to follow. A marker, however, generally has much different chemical and physical properties from the tracee and usually does not behave physiologically like the compound. A marker simply attempts to indicate the path and rates of the compounds' movement through that p_{ath}. Many authors have defined the charateristics of the "ideal" digesta marker. Faichney (1972) (Figure 8) outlined the charateristics of markers generally sought after in digestion studies.

FIGURE 8. Charateristics of an "Ideal" Marker

A Marker Must Be:
1. non absorbable
2. not metabolized
3. physically similar to or intimately associated with material to be marked
4. easily and accurately analyzable
5. not affect other analysis or total system

He also states " none of the available markers satisfy all these criteria. If this is not taken into account when selecting a marker for a specific purpose serious errors can

arise." This straight forward statement forces the researcher to ask a whole host of questions; What markers are available ? What information will a particular marker provide ? What are the limitations ? . In addition to choice of markers digestion studies require the researcher to address the problems of: How to incorporate and administer the marker; What type and amount of samples are needed; How to interpret the results. A thorough review of these question is well beyond the scope of this discussion. Several comprehensive reviews are available (Faichney, 1972, Ktob and Luckey,1977). Subsequent sections will to address some of these questions, as to how they relate to site and extent of digestion studies in the ruminant digestive tract.

MARKER CHARATERISTICS

Ruminant nutritionist generally classify markers as either liquid phase or solid phase.

LIQUID PHASE MARKERS

Liquid phase markers are considered to be water soluble and tend to follow the liquid small particle phase of digesta flow. In recent years the most frequently used markers in this category are: High molecular weight polyglycols (PEG) and ethelenediaminetetraacetic acid (EDTA) chelates of Cr, Co, or rare earths (Yb,Er,Ce,Sa,La).

High Molecular Weight Polyglycols

Polyethelene glycol (PEG) is the most used marker of this group. It is a heterogeneous compound or group of compounds ranging in molecular weight from 100 - 10,000 (Kotb and Luckey, 1972). Polymers greater than 1000 M.W. are considered to be nonabsorbable. Polymers with molecular weight less then or equal to 4000 tend to be water soluble (Clemens, 1980). To combine nonabsorbablility with water solubility the 4000 M.W. form of PEG has been used (Kotb and Luckey,1972).

Hyden (1955) outlined a turbidometric method for analizing PEG. He found the method fairly accurate at concentrations in excess of 300mg/100ml. Large ruminants (cattle) can have rumen outflow rates of 3- 5 l/hr (Weber, 1984). To maintain levels of 300 mg PEG/100 ml digesta in excess of 500 g PEG per day may be needed. This can represent, depending upon intake over 10% of total daily dry matter intake. However, high levels seem to have no apparent adverse effects (Sperber et.al., 1953).

Recovery of PEG is usually high and ranges from 93 - 100%. Smith (1958) reported 85-110% recovery from abomasal samples spiked with PEG. Other workers have reported even better recoveries; 94.8% (Nicholson and Sutton,1969); 89.9-97% (van Bruchem et al, 1981). However several factors have been shown to reduce PEG recovery. Repeated freezing and thawing markedly reduced recovery but long term freezing had

little effect (Bjornsson et.al.,1982). Soluble protein content of the sample is inversely related to recovery (i.e. higher protein :lower recovery) but precipitation of the protein fraction seems to alleviate the problem (Malawer et.al.,1967). There have been some reports of low recovery of PEG. Christie and Lassiter (1958) reported recoveries as low as 0.0% but averaging 71 -83%. However the levels used in the study were below those recomended by Hyden (1955).

The use of PEG as a liquid phase marker has been widely accepted. Numerous workers have used it (Neudoeffer et.al, 1982, Kay, 1969, Ulyatt, 1964). It has been shown to be practically nonabsorbable (0.5 - 4.2%)(Winne and Gorig, 1982) and as mentioned earlier it has good recovery rates. However, analysis methodology is tedious (Ktob and Luckey, 1972) and it has some sample handling concerns. It also due to its size, occupies only 90% of the water space (Ktob and Luckey, 1972). This can lead to underestimates of rumen volume when used in kinetics studies. Finally, the high levels needed to allow accurate detection may have some affect on the digestive physiology of the animal.

EDTA Chelates

Ethelenediaminetetraacetic acid (EDTA) is a powerfull chelating agent. Cations are bound tightly with EDTA and form soluble (Cr:EDTA, Co:EDTA) or insoluble (Ca:EDTA) complexes which are virtuallu nonabsorbable (Broad, 1974).

The principal complex used in marker studies has been Cr:EDTA (Ktob and Luckey, 1972). However other complexes have been used by several workers. Ellis (1968) reported the use of rare earth (Yb,Er) complexes. Co has also been complexed to EDTA and used in nutrition studies (Uden et.al., 1980).

The principal advantage to the use of Cr:EDTA is the ease of analysis. Samples are acid digested then analized for Cr by atomic absorption. Alternative methods are also available. Kennelly et.al. (1980) used neutron activation analysis. Downs and McDonald (1964) were amoung the first workers to use Cr:EDTA and used a radioactive isotope (Cr 51). All reported methods are very sensitive and can detect accurately levels below 1 ug/g. This allows the worker to use very low levels ,relative to PEG, in the diet.

The principal limitation to Cr:EDTA as a marker is the variability and extent to which it is absorbed in the intestinal tract. Downes and McDonald (1961) reported of between 2.5 and 5.0% of the Cr(51) appeared in the urine when the rumen was dosed. Binnerts et.al.(1968) reported similar results (3-5% absorption with the stable chromimum complex. Absorption of the marker will result in lower recoveries. Low recoveries result in overestimation of outflow and rumen volume.

Cr:EDTA has also been reported to bind to the solid phase in the rumen (Warner and Stacy, 1968). Hogan (1964) showed no such binding. It may be that feeds have low levels

of binding sites for Cr:EDTA and that saturation of these sites and maintaining adequate levels of Cr:EDTA in the diet would alleviate this problem. Binding to feed particles would lower estimates of turnover rate but would have no affect on total flow or rumen volume estimates.

The use of Cr:EDTA as a liquid phase marker is certainly acceptable. It occupies 95-98% of the water space (Warner and Stacy,1968), is eaisily analized and generally has high recoveries (95-97%) with slight absorption. In addition no toxic effects have been reported in the literature. However, digestive disturbances have been observed when Cr:EDTA is fed or infused (1-2g Cr/day) in cattle. The animals seem to adapt in a three week period (Weber, 1984).

SOLID PHASE MARKERS

Solid phase markers associated with the dry matter particulate phase of the digesta. Markers in this category can be divided into two categories, external and internal (Kotb and Luckey,1972).

Internal Markers

Internal markers occur naturally in the diet. They must be non digestible and nonabsorbable. Since, internal markers are an indigestible fraction of the diet, when used in rate studies they would be expected to represent the

slowest moving portion of the diet (i.e. low k values). They tend to follow the fibrous portion of the diet and could be used to mark crude fiber or acid detergent fiber fractions. The two most commonly used internal markers are lignin and acid insoluble ash.

Lignin

The two principal methods for determining lignin are permanganate (VanSoest and Wine,1968) and acid detergent lignin (Georing and VanSoest,1970). A variety of analytical difficulties have been noted. Reviews on the analytical procedures are available (Muelier,1956).

The composition of the fraction isolated by the two methods mentioned above, as lignin, has a variable and uncertain composition. VanSoest (1982) presents an extensive discussion on lignin composition and digestibility. He points out that the usefullness of the lignin fraction as a digestion marker is related to the type and age of the plant source. Diets composed of young forages or grains which are low in lignin tend to be unsuitable as experimental diets in which lignin is the marker. As a general rule if lignin is to be used as a marker it should be in the diet at levels in excess of 6% (VanSoest, 1982).

Diet - lignin digestiblity interaction can be supported from available data. Galyean et.al.(1979) feeding 72% concentrate diet found lignin digestiblily ranging from 27.9-

53.3. Fahey et. al. (1979) reported lignin digestibility in diets containing strictly alfalfa or ryegrass of 5.8±5.2 and -45.7±4.5 respectively. Interestingly the alfalfa diets contained 6.06% lignin and ryegrass only 2.36% lignin. All other diets (mixed diets), in Faheys' study, had lignin values of less than 3% and lignin digestibilities ranging from -79% to +28%. Kennedy (1982) fed alfalfa hay or pasture hay diets containing 7.66% and 9.7% lignin respectively. He was able to recover virtually 100% of the diet lignin at both the abomasum and feces. From these studies it is evident that diet composition must be considered before using lignin as a marker.

Acid Insoluble Ash

Acid insoluble ash (AIA) is a fraction derived from an acid digest of the sample and subsequent ashing. The residue is basically silica. There are several slightly different methods for determining AIA. The methods differ in the concentration of the acid used to digest the sample. VanKeulen and Young (1977) compared a method proposed by them (2N HCL) with the use of concentrated HCL (Shrivastava and Talapatra, 1962) and 4N HCL (Vogtman et.al.,1975). There were no significant differences between the three methods in determining the amount of AIA. Fecal recoveries, of AIA, in Vankeulen and Youngs' study ranged from 84.0 - 106.1% depending on the diet. These results agree with those of

Thonney et.al.(1979) who reported fecal recoveries of AIA (2N method) of 90.12 to 105.97%. Other workers have reported similar results in rabbits (Furuichi and Takahashi,1981). Penning and Johnson (1983) reported a slight variation in the method of AIA determination. Indigestible ADF was determined by incubating ADF residue in a cellulase solution for 4-5 days and recovering the residue. This method also resulted in high total tract recoveries of AIA.

Problems with AIA procedures could result from contamination of either feed or feces. Since the analytical procedure primarily isolates silica contamination from sand and dirt will alter the results. However this procedure certainly does seem to be more consistant than lignin. Thonney et.al. (1979) compaired AIA with permanganate lignin by total fecal collection AIA accurately predicted total tract digestion. Permanganate lignin greately overestimated digestion, which is an indication of low recovery.

External Markers

External markers are compounds which are added to the diet but are not normally found in the feeds. These markers are intended to follow the excretion path of the solid phase. The principle markers in this category are chromium sequoxide (Cr2O3), chromimum mordented fiber and rare earth metals. These markers have much different physical charateristics and may follow different types of

feed particles through the rumen. The implications of the results obtained by using these markers can be much different. Therefore, choice of marker will be predicated on the types of data the researcher requires.

Rare Earths

Rare earth is a general term applied to the lanthanide series of elements. The principle elements in the class used in nutrition studies are Lanthanum (La), Cerium (Ce), Dysprosium (Dy), Yttrium (Y), Ytterbium (Yb), Erbium (Er) and Prasodinium (Pr). Kyker (1961) presented ar extensive review on the chemical and physical properties of rare earths.

Ellis and Huston (1967) observed that rare earths had several attractive features which make them ideally suited for use as digestion markers. They are nonabsorbable, bind to particulate matter and are easily applied to the diet. They worked primarily with Ce and Pr but subsequent work has shown other rare earths to have similar properties: Dy (Ellis,1968), Y (Sklan et.al.,1975), Yb (Prigge et.al.,1981).

The property of rare earths to bind to particulate matter is usefull. It allows the tracing of individual dietary components through the digestive tract. For example Yb could be applied to a particular component of the diet and Ce to another. Examination of the turnover rates of Yb and Ce would give residence time of its respective dietary component. Coupling this (as discussed in previous sections) with digestion rates could give estimates of extent of

digestion in the rumen.

Movement of rare earths from one component to another has been observed. Moverment would result in erroneous estimation of the turnover rates of a particular particle. The extent of movement would influence the accuracy of the residence time estimate. Several workers have discussed this problem of marker movement (Hartnell and Satter,1979, Teeter et.al., 1979, 1981) and presented data on marker diet interactions.

As a total flow marker rare earths are ideally suited. A number of workers have shown them to be nonabsorbable (Sklan et.al.,1975, Henrickson and Stacy, 1971) which is critical for a digestion marker. In addition analysis is both sensitive and accurate. Early workers used radioactive rare earths (Ellis, 1968, Ellis and Huston, 1967). Neutron activation analysis has also been used to determine Dy (Young et.al., 1975), Ce, Sa and La (Hartnell and Satter, 1979). Neutron activation analysis is very sensitive as long as conditions are well controled. It allows detection as low as 0.001 ug/g for several commonly used rare earths (La, Sm, Yb) (Michigan State University Neutron Activation Publication 1980). The major difficulty is its lack of availability and cost since it involves the use of a neuclear reactor. More recently atomic absorption spectrophotometry has been used (Wray, 1981). Detection levels are limited for some elements (La = 500 ug/g) but are excellent for others (Yb = 1 ug/g). All the major analysis schemes are effective and allow the

researcher to select a system which will work in his situation.

Effect of Cannulation

Many of the types of studies which attempt to determine the site and extent of digestion of feedstuffs in ruminants require the use of surgically modified animals. Several different sites in the digestive tract are routinely cannulated. Samples collected at these sites, in the digestive tract, are used to estimate the flow and/or composition of digesta moving through the digestive tract. These values are then related to normal animals under production conditions and it is assumed the animal and conditions of the experiment mimic the normal situation.

Three segments (sites) of the digestive system in the ruminant have been cannulated with the greatest frequency ; rumen, abomasum and duodenum. The primary types of cannulas used in these site are fistulas and T or reentrant type cannulas. Fistulas are large, cylindrical cannulas which are mainly used to gain access to the rumen. T and reentrant cannulas are smaller and are usually placed in the abomasum or small intestine. T cannulas are inserted in to the gut by splitting the wall of the gut and sliding the cannula through the hole. Since, as the name indicates, T cannulas have two lips (the arms of the T) the lips hold the cannula in place.

Reentrant cannulas require the exteriorization of the gut. After a small portion of the gut is exteriorized, the gut is severed and a plastic tube is used to connect the two free ends. This plastic tube is refered to as the reentrant cannula.

Hayes et.al.(1964) demonstrated with four sets of twin steers, that total tract digestion was not altered by rumen fistulation. These workers also investigated the affect of abomasal and intestinal cannulation alone or in combination with rumen fistulation. They were unable to detect any difference between multicannulated, singley cannulated and uncannulated animals in respect to total tract digestion. Other workers have shown similar results (Reid et al. 1961, MacRea, 1974). These studies indicate that total tract digestion is not affected by cannulation but does not give any information on the affect on site of digestion.

The affect cannulation on the flow of digesta through the small intestine has been investigated by a number of workers. Wenham and Wyburn (1980) reported disturbances in digesta flow through the small intestine when a reentrant cannula was used. They also reported much less disturbance in the flow with the use of a T - type cannula. Poncet et.al. (1982) using electromagnetic flow meters implanted into the small intestine of sheep demonstrated similar results. Cannulation altered flow patterns in the sheep but the affect of T cannulation was much less than that of

reentrant cannulation. However, Sissons and Smith (1982) reported no differences in abomasal empting and secretion between animals fitted with abomasal or abomasal and duodenal reentrant cannula in the preruminant calf.

Generally with the use of reentrant cannula researchers have reported low intakes (Zinn, et.al. 1979). With T cannulation more normal intakes have been reported and workers have even been able to maintain milk production (Merchen and Satter, 1983). This would suggest that a more nearly normal animal can be achieved with a T type cannula than a reentrant.

HAYLAGE STUDIES

EXPERIMENT 1.

This study was conducted to evaluate the effect of moisture content, at the time of ensiling, on the site and extent of digestion of alfalfa haylage. The study was also intended to identify the procedures and problems involved in a marker ratio type of site and extent of digestion study, in the metabolism room facility at Michigan State University.

MATERIALS AND METHODS

Animals and Facilities.

Four Holstein steers were used in this study. The steers weighed approximately 300 kg at the start of the experiment. By the end of the study they weighed approximately 350 kg. Each steer was fitted with a intestinal T type cannula in the proximal duodenum. The animals were individually housed in an indoor facility.

The indoor facility used in this study was the metabolism room at the Beef Cattle Research Center. The Beef Cattle research Center is located at the Michigan State University campus in East Lansing, Michigan. The metabolism room consisted of 24 pens, completely enclosed with concrete

slatted floors, over a manure pit. These pens were washed daily and the manure pit was pumped out as needed. Each pen contained a feeder separate from the other pens. Water was provided by automatic watering cups positioned between two pens.

Fresh feed was furnished twice daily (8:00am, 8:00pm). Animals were provided feed, in excess of ad libitum intake, for a period, at least 16 days, prior to the start of the collection period. For five days prior to and during the collection period, the animals were restricted to approximately 90 percent of intake. This was an attempt to insure that the animals would consume all the feed offered to them.

Haylages

The following information on the preparation of the haylages was obtained from Nahara (1981). The haylages used in this study were primarily alfalfa (80 %) with some orchard grass (20 %). They were harvested June 13, 1980 through June 18, 1980. The alfalfa was in the early bloom stage of maturity. A New Holland mower - conditioner (model 49) was used to mow, crimp and windrow the forage in one operation. The windrowed forage was allowed to wilt, until it reached approximately 30 % dry matter. Alternating rows were then harvested. Harvesting was accomplished using a New Holland (model 392) forage harvester which chopped the forage into

approximately 0.65 to 0.97 cm pieces. The high dry matter forage was allowed to wilt for a longer period and then was harvested in the same manner. Both forages were ensiled in separate concrete stave silos (15.24 m X 3.66 m).

Diets

The diets consisted primarily of the haylage described above, supplemented with salt at 0.25 % of the diet dry matter. Table 1.1 presents the average analysis, of both experimental periods, of the haylages after ensiling.

TABLE 1.1. Composition of Haylages (Experiment 1)

Component	[-----Haylage----] 30 % DM	60 % DM
Dry Matter(%)	29.15	60.70
Organic matter (% of DM)	87.31	91.28
Nitrogen (% of DM)	12.89	14.74
Acid Detergent Fiber (% of DM)	45.86	45.56
Neutral Detergent Fiber (% of DM)	56.71	54.56

The 60 % DM haylage appeared dark brown in color and had a caramell like smell. This indicated excessive heating during the ensiling process (Thomas, et.al.1982).

Sample Collection and Handling

After the 21 day adaption period, a four day collection period was used. Samples were collected every eight hours during the four days, with one six hour interval per day

(Figure 1.1). This provided one sample for every odd hour in a reconstructed theoretical day. At each sampling time a fecal grab sample (500 g) and a duodenal digesta sample (350 ml) was obtained and frozen until composited.

Day 1	Day 2	Day 3	Day 4
7:00 am	5:00 am	3:00 am	1:00 am
3:00 pm	1:00 pm	11:00 am	9:00 am
11:00 pm	9:00 pm	7:00 pm	5:00 pm

FIGURE 1.1. Sampling Scheme

Compositing of duodenal samples involved homogenization of the whole digesta sample in a large blender after thawing. Equal amounts (100 g), of the wet homogenate, were then added to a composite sample. Fecal samples were also composited in this manner, but, without prior homogenization. Composited samples and feed samples, obtained during the collection period, were then freeze dried and analyzed for dry matter, ash, nitrogen, acid detergent fiber, neutral detergent fiber, ytterbium and chromium. In addition, a portion, of the wet homogenate, was oven dried (digesta dry matter determination) and another centrifuged. The centrifuged sample (supernatant) was used for ammonia determination.

At the end of the four day collection period, markers were withdrawn from the feed. Duodenal samples were then collected at 12, 24, 36 and 72 hours, after withdrawl of the

markers. These samples were handled as described above.

Markers

Two markers were used in this study. Ytterbium (Yb) was used, as a solid phase marker, for digestibility determination (as described previously) and for solid phase turnover. The chromium complex of ethylenediaminetetra-acetic acid (Cr:EDTA) was used to determine the turnover rate of the liquid phase. The markers were included in the diet, at each feeding, for 10 days prior to the collection period.

Duodenal samples collected after the withdrawl of the markers, were analyzed for both Cr and Yb. This allowed the calculation of the turnover rate of both the solid and liquid phase of the rumen digesta (as describe previously).

Rumen and total tract digestibility were calculated by the marker ratio technique, described in previous sections. This technique was used, instead of the total collection method, for total tract digestion, due to the difficulty involved in the quantitative collection of feces in a slatted floor facility. Ruminal digestions were calculated by the marker ratio technique, since, as described in previous sections, total collection is not possible with T type cannulas.

Experimental Design and Statistical Analysis

This experiment was designed to evaluate the effect of

moisture content at the time of ensiling on the digestibility and factors effecting the digestibility of alfalfa haylage. The design used was a replicated 2 X 2 latin square, in which four animal recieved two diets in two periods (Figure 1.2).

Period 1		Squares	Period 2	
An 1 Diet 1	An 3 Diet 2	<-- 1 --->	An 1 Diet 2	An 3 Diet 1
An 2 Diet 1	An 4 Diet 2	<-- 2 --->	An 2 Diet 2	An 4 Diet 1

FIGURE 1.2. Design Model (Experiment 1)

This design permits the separation of period, square, animal and diet effects in the analysis of varience (Figure 1.3) for dry matter, organic matter, nitrogen, acid detergent fiber and neutral detergent fiber. This analysis of varience was calculated for total tract digestion as a percent of intake, ruminal digestion as a percent of intake, lower tract digestion as a percent of intake, ruminal digestion as a percent of total tract digestion, lower tract as a percent of total tract digestion and lower tract as a percent of that component which reached the lower tract. The model was set up and analyzed using the Genstat program available on the Michigan State University Cyber 750 computer.

ANOVA

Source of Variation	Df	SS	MS	f Ratio
Period	1			
Squares	1			
Animals	3			
Treatment	1			
Residual	1 (1)			
Total	2			
Grand Total	8			

FIGURE 1.3. Sample Analysis of Varience Table (Experiment 1)

The loss of one degree of freedom is due to a missing cell. In the first period, animal 1 escaped from his pen and consumed feed other than the experimental diet. This occured during the collection period. Due to cost, feed and time restraints, it was not possible to restart the study which resulted in the loss of one cell of the experiment.

Analytical Procedures

All analyses was performed on the freeze dried samples, prepared as described in the sample handling section. Dry matter determinations were made on the freeze dried composite samples and all subsequent analyses were corrected for dry matter. Nitrogen was determined by Kjeldahl digestion (AOAC, 1970) followed by ammmonia nitrogen determination via a colorimetric method (Technicon Auto Analysis). Acid and

neutral detergent fiber analysis was performed as described by Georing and Van Soest, 1970. Sample were ashed at 600 C and organic matter was calculated by 100 % minus the percent ash.

The markers Cr and Yb were analyzed by neutron activation analysis. This procedure involved exposure of the sample to a neutron flux generated by the Michigan State University nuclear reactor. After activation for a specific length of time the emission of gamma radiation was measured. Each element emits at a specific wavelength which is a known physical constant and the intensity of the emission is directly proportional to the amount of the element present and the length and intensity of the elements exposure to the neutron flux. Including a group of samples, of known concentration, in with the unknown samples at the time of activation allows a standard curve to be calculated. This curve is used to determine the amount of a specific element in the sample. Most elements emit at several different wavelengths and the choice of emission wavelength to measure is based on the intensity of the emission, the half life of the isotope and consideration of possible interferences which may occur. In this study Cr was measured at 319.8 kev and Yb was detected at 198.1 kev.

RESULTS

Tables 1.2 - 1.6 contain the average daily intakes, the duodenal and fecal flow and the site and extent of digestion, of the various components in the two diets (30% DM haylage ,60% DM haylage). These components include dry matter (DM), organic matter (OM), nitrogen (N), acid detergent fiber (ADF) and neutral detergent fiber (NDF). The digestion coefficients are expressed three ways, each providing some insight into the nature of the digestion of the various components of the diet. 1. Expression of digestibility as a percent of the intake of a particular component gives an estimate of the apparent digestibility of that component. Digestibility as a percent of intake also indicates how much of the dietary component is digested, at each site, in the gastrointestinal tract. 2. Expressing the amount digested in the rumen and lower tract as a percent of the total tract digestion indicates the proportion of the available component which is digested in the rumen and lower tract. 3. Calculation of the digestibility of the components which reach the lower tract results in an estimate of how digestible the material flowing out of the rumen is in the lower tract. In addition, each table contains the standard error of the diet means and the significance level for the differences, in the digestion coefficients, between diets for each of the various site of

digestion estimates. References to specific values for comparison between diets will present the value for the low DM haylage first and then the value for the high DM haylage diet (i.e. 30% DM, 60% DM).

Dry matter intake, flow and site of digestion is presented in Table 1.2. Intake was generally in excess of two percent of body weight (intake/body wt). Intake (9750, 9635 g/day) and duodenal DM flow (5323, 5257 g/day) were very similar for both diets. However, fecal DM flow (3485, 6367 g/day) was only half for the low DM haylage diet compared to the high DM haylage diet.

TABLE 1.2. Intake,Flow and Site and Extent of Digestion of Dry Matter (Experiment 1).

Item	[--- Haylages ---] 30% DM	60% DM	SEM	Sig.
Intake (g/day)	9750	9635		
Duodenal Flow (g/day)	5323	5257		
Fecal Flow (g/day)	3485	6367		
Digestibility (% of Intake)				
Total Tract	62.13	38.70	0.26	P<.05
Ruminal	44.53	10.24	0.32	P<.05
Lower Tract	18.60	28.46	0.07	P<.05
Digestibility (% of Total Tract Digestion)				
Ruminal	69.84	26.55	0.30	P<.05
Lower Tract	30.14	73.44	0.30	P<.05
Digestibility of Component Reaching Lower Tract	31.49	31.15	0.06	NS

Large differences in the digestion coefficients occured between diets. Total tract digestibility was significantly ($P<.05$) higher for the haylage ensiled at 30% DM. This is most likely due to the significantly ($P<.05$) higher ruminal digestion (44.53, 10.24 as a % of intake). This was offset, somewhat, by higher lower tract digestion of the high DM haylage diet (18.60, 28.46 as a percent of intake). These two factors combined to give a large difference in site of digestion. Ruminal digestion accounted for 69 percent, of the total tract digestion, in the low DM haylage diet but accounted for only 26 percent in the high DM haylage diet. The interesting feature of this, is that as a portion of the total tract DM digestion, 31 percent occured in the lower tract with the low DM diet, while in the high DM diet, 73 percent occured in the lower tract. Interestingly the digestibility of the the dry matter which reached the lower tract was not different from between the two diets (31%).

The flow and digestion coefficients for organic matter (Table 1.3) are very similar to the dry matter data. Intake for the two diets is very similar (8513, 8795 g/day). The flow of organic matter to the duodenum, however, was lower in the low DM diet (4248, 7562 g/day) than in the high DM diet. Fecal flow was also lower in the low DM diet than in the high DM diet (3088, 5740 g/day).

TABLE 1.3. Intake,Flow and Site and Extent of Digestion of Organic Matter (Experiment 1)

Item	[--- Haylage ---] 30% DM	60% DM	SEM	Sig.
Intake (g/day)	8513	8795		
Duodenal Flow (g/day)	4248	7560		
Fecal Flow (g/day)	3088	5740		
Digestibility (% of Intake)				
Total Tract	64.11	37.00	0.32	P<.05
Ruminal	51.04	15.63	0.51	P<.05
Lower Tract	13.07	21.37	0.19	P<.05
Digestibility (% of Total Tract Digestion)				
Ruminal	78.70	43.39	0.62	P<.05
Lower Tract	21.29	56.60	0.62	P<.05
Digestibility of Component Reaching Lower Tract	24.15	24.22	0.01	NS

Total tract digestion (as a percent of intake), of organic matter, was significantly higher for the low DM haylage (64%) than for the high DM haylage (37%). Ruminal digestion (as a percent of intake) was also higher for the low DM haylage (51%) than for the high DM (16%). The proportion of the total tract digestion occuring in the rumen was significantly (P<.05) higher for the low DM haylage diet (79%) than for the high DM haylage diet (43%). The digestibility of the organic matter which reached the lower tract was not significantly different between diets (24%).

The nitrogen, intake, flow and digestion values are found in Table 1.4. Intake of nitrogen tended to be lower in the low DM haylage diets (201.1g/day) than in the high DM

haylage diets (227.2g/day). This was the result of a higher nitrogen content in the high DM haylage. The reason for this higher nitrogen content is not clear but could be due to lower seepage from the silo since it was ensiled at a higher percent dry matter. However, higher field losses of the leaf portion of the plant (higher in nitrogen than the rest of the plant) generally occur with higher DM haylages. This loss of a high nitrogen component of the plant would tend to lower nitrogen values in the high DM haylages, which was not the case in this study.

Fecal flow of nitrogen was lower for the low DM haylage (89.0 g/day) than for the high DM (133.3 g/day). This follows from the significantly ($P<.05$) higher digestibility of the low DM haylage diet (67%) than the high DM diet (46%). In both diets the intake of nitrogen was lower than the flow of nitrogen to the duodenum (intake : duodenum flow)(201.1:204.0, 227.2 : 368.6 g/day).

TABLE 1.4. Intake,Flow and Site and Extent of Digestion of Nitrogen (Experiment 1)

Item	[--- Haylage ----] 30% DM	60% DM	SEM	Sig.
Intake (g/day)	201.1	227.2		
Duodenal Flow (g/day)	204.0	368.6		
Fecal Flow (g/day)	89.0	133.3		
Digestibility (% of Intake)				
Total Tract	66.91	45.19	0.24	P<.05
Ruminal	-1.67	-42.26	0.01	P<.05
Lower Tract	65.25	87.45	0.24	P<.05
Digestibility (% of Total Tract Digestion)				
Ruminal	-1.28	-99.04	0.09	P<.05
Lower Tract	101.28	199.04	0.09	P<.05
Digestibility of Component Reaching Lower Tract	64.79	61.06	0.14	NS

The flow of nitrogen to the lower tract represents 102 percent of the intake for the low DM haylage diet. This indicates that no net movement of nitrogen from the rumen to the blood stream or visa versa occured. However, in the high DM haylage diet, the nitrogen flowing to the lower tract represents 145 percent of the intake nitrogen. The implication is that a net movement of nitrogen ocured from the blood stream and saliva to the rumen. This has been shown to occur in low nitrogen diets (Houpt and Houpt, 1968). These diets would not be considered to be low nitrogen diets (6% N). However, the low N digestibility of the high DM haylage, may have reduced the available nitrogen to a level such that the 60 % DM haylage could be considered a low nitrogen feed. As with the previous components of the

diet, the digestibility of the nitrogen which reached the lower tract was not different between the two haylage diets.

Intake, flow and site of digestion of acid detergent fiber is represented in Table 1.5. Intake was slightly higher with the low DM haylage than the high DM (4471, 4390 g/day). However, duodenal (1214, 1966 g/day) and fecal flows (1794,3069 g/day) were lower with the low DM haylage versus the high.

TABLE 1.5. Intake,Flow and Site and Extent of Digestion of Acid Detergent Fiber (Experiment 1)

Item	[--- Haylage ----] 30% DM	60% DM	SEM	Sig.
Intake (g/day)	4471	4390		
Duodenal Flow (g/day)	1214	1966		
Fecal Flow (g/day)	1794	3069		
Digestibility (% of Intake)				
Total Tract	60.16	32.42	0.28	P<.05
Ruminal	73.10	51.50	0.50	P<.05
Lower Tract	-12.94	-19.08	0.23	P<.05
Digestibility (% of Total Tract Digestion)				
Ruminal	121.66	157.78	0.71	P<.05
Lower Tract	-21.66	-57.78	0.71	P<.05
Digestibility of Component Reaching Lower Tract	< 0.0	< 0.0	< 0.0	*

Extent of ADF digestion, of the two haylages, was considerably different. Total tract digestion (60.16, 32.42 as a % of intake) was significantly higher(P<.05) with the low DM haylage than the high DM. Examination of the other

digestion coefficients suggests that ADF was synthesized in the lower tract. This results in the ruminal digestion representing greater than 100% (121.66, 157.78) of the total tract digestion of ADF. Since, it is very unlikely that ADF was synthesized in the lower gut, either the estimate of intake was too low and fecal outflow was too high or the duodenal flow of ADF was underestimated. A further discussion of this will follow in later sections.

Intake, flow and site and extent of digestion of neutral detergent fiber is presented in Table 1.6. Intake of NDF (5529, 5275 g/day) was higher with the low DM haylage diets than with the high DM diets. Following the same general pattern as for ADF, duodenal (1725, 2676 g/day) and fecal (2071, 3817 g/day) flow were lower with the low DM haylage than with the high DM haylage.

TABLE 1.6. Intake, Flow and Site and Extent of Digestion of Neutral Detergent Fiber (Experiment 1)

Item	[--- Haylage ----] 30% DM	60% DM	SEM	Sig.
Intake (g/day)	5529.0	5257.0		
Duodenal Flow (g/day)	1725.0	2676.0		
Fecal Flow (g/day)	2071.0	3817.0		
Digestibility (% of Intake)				
Total Tract	62.70	30.05	0.35	P<.05
Ruminal	68.98	43.11	0.72	P<.05
Lower Tract	-6.29	-13.06	0.34	P<.05
Digestibility (% of Total Tract Digestion)				
Ruminal	110.03	143.51	1.10	P<.05
Lower Tract	-10.03	-43.51	1.10	P<.05
Digestibility of Component Reaching Lower Tract	-27.70	-27.82	1.20	NS

Total tract NDF digestibility (62.7, 30.05), as a percent of intake, was significantly (P<.05) higher in the animals fed the low DM haylage diets. As with ADF, site of digestion estimates seem to be unrealistic. Based on the values obtained in the study NDF was produced in the lower gut. This resulted in ruminal digestion (110.03, 143.51 percent of total tract digestion) accounting for more than 100% of the total tract digestion. Underestimates of intake and overestimates of fecal outflow or underestimates of duodenal flow could account for this discrepancy.

Liquid turnover rates for the two diets are presented in Table 1.7. These were determined by a least squares linear regression. The natural log of the concentration of Cr:EDTA, in the duodenal fluid, was the dependent variable and time

after withdrawl of the marker was the independent variable. The dependent and independent variables were used to fit a line like equation 1,

$$1.\ y = b + mx$$

where y is the concentration variable and x is the time variable. The slope (m) of the line is the turnover rate in percent per hour.

TABLE 1.7. Rumen Liquid Turnover Rates. (Experiment 1)

	[--- Haylage ----]			
Item	30% DM	60% DM	SEM	Sig.
%/hr	3.21	4.34	.123	P<.10

The animals fed the low DM haylage diet had a significantly (P<.10) lower rumen liquid turnover rate than those on the high DM haylage. Rumen turnover rate has been shown to be related to rumen availability of dietary components (Bull et.al., 1979). In this study digestibility, of all components, of the high DM haylage were lower and its liquid turnover rate was higher. Whether, lower digestibility resulted in higher liquid turnover or some factor in the haylage resulted in a higher turnover rate cannot be determined. A higher liquid turnover could also help explain the higher nitrogen recovery at the duodenum,

with the high DM haylage diets. If movement across the rumen wall is an equilibrium phenomenon, as liquid movement out of the rumen increases more nitrogen would move into the rumen, to maintain this equilibrium.

EXPERIMENT 2

The results of the first experiment suggested that there were some problems with the site and extent of digestion estimates we had obtained. The total tract digestion estimates obtained were very close to those found in the literature (Hawkins et.al., 1970, Merchen and Satter, 1983). However, when digestion was partitioned into the various sites of digestion, some of the results made little sense. For example, ruminal digestion of fiber (both ADF and NDF) accounted for more than 100 % of the total tract fiber digestion. Therefore, the second experiment was designed to repeat the first experiment, to provide some realistic estimates of site and extent of digestion and hopefully identify some of the problems encountered with the type of study presented in experiment one.

MATERIALS AND METHODS

Animals and Diets

Four Holstein steers were used in this study. The steers weighed, at the begining of the study, approximately 350 kg. They gained approximately 100 kg during the experiment. Each steer was fitted with a T - type cannula

in the duodenum and ileum and a small infusion cannula in the abomasum. They were housed in the metabolism room facility described earlier. Each animal was fed twice daily. Markers were included in the diet, at each feeding, for 10 days prior to the collection period.

The two alfalfa haylages were prepared as described in the first experiment. However, the high dry matter haylage contained approximately 45 % dry matter instead of 60 % as in the first experiment. The two haylages were fed alone or mixed with high moisture corn (HMC). The high moisture corn was added at 30 percent of the total dry matter. All diets were supplemented with salt at 0.25% of the diet dry matter. As in the previous experiment, animals were adapted to their diets for at least 21 days prior to the collection period. Feed was provided at levels in excess of ad libitum intake until 5 days prior to the collection period. At this time intake was reduced to aproximately 90 percent of ad libidum. Dry matter content of the haylages and the high moisture corn varied slightly between the periods. This was the result of the inherent variation in the composition, of high moisture feedstuffs, present at different levels in a silo. As feed is removed from a silo this variation results in slight differences in the composition of the diet. Table 2.1 presents the average analysis of the diets across periods.

TABLE 2.1. Composition of Diets
(Experiment 2)

Component	Haylage 30% DM w/o HMC	Haylage 30% DM +HMC	Haylage 45% DM w/o HMC	Haylage 45% DM +HMC
Dry Matter (%)	31.33	39.75	39.61	48.92
Organic Matter (% of DM)	90.72	93.74	90.77	94.21
Nitrogen (% of DM)	2.00	1.85	2.29	2.00
Acid Detergent Fiber (% of DM)	42.53	26.21	42.79	23.99
Acid Detergent Lignin (% of DM)	6.09	3.76	6.16	3.42

Experimental Design and Analysis

The design used to evaluate the different diets consisted of a 4 X 4 latin square (Figure 2.1). During period 3 animal 2 stopped eating and was taken off of the collection schedule for that period. This resulted in the loss of one cell of the experiment.

Period 1	
An 1 / Diet 1	An 3 / Diet 3
An 2 / Diet 2	An 4 / Diet 4

Period 2	
An 1 / Diet 2	An 3 / Diet 4
An 2 / Diet 3	An 4 / Diet 1

Period 3	
An 1 / Diet 3	An 3 / Diet 1
An 2 / Diet 4	An 4 / Diet 2

Period 4	
An 1 / Diet 4	An 3 / Diet 2
An 2 / Diet 1	An 4 / Diet 3

FIGURE 2.1. Experimental Design
(Experiment 2)

Statistical analysis of the experiment was done by the Genstat statistical analysis package. The model used allowed separation of period, animal and diet effects. This resulted in the analysis of varience table found in Figure 2.2. The residual (error) degrees of freedom were reduced by one due to the loss of one cell and the generation of the missing value.

Source of Variation	Df	SS	MS	f Ratio
Period	3			
Animal	3			
Diet	3			
Residual	5 (1)			
Total	8			
Grand Total	14			

FIGURE 2.2. Analysis of Varience Table (Experiment 2)

Sample Handling and Analysis

Samples were collected on the four day collection schedual described in experiment one. All samples were immediately frozen after collection. Fecal and duodenal samples were handled and composited as described in the previous experiment. In addition, ileal samples were handled like the duodenal samples.

Dry matter,ash, nitrogen and acid detergent fiber were

determined as described in experiment 1. Acid detergent lignin was determined by the method outlined by Georing and Van Soest (1970). The nitrogen content of the residue after an ADF determination was designated as acid detergent insoluble nitrogen (Yu, 1976). Chromium and Ytterbium analysis was preformed by atomic emission spectrophotometry analysis following wet ashing of the freeze dried sample.

RESULTS

Tables 2.2 - 2.7 present the average daily intakes, the duodenal, ileal and fecal flow for dry matter (DM), organic matter (OM), nitrogen (N), acid detergent fiber (ADF), acid detergent lignin (ADL) and acid detergent insoluble nitrogen (ADIN) respectively. Included in these tables are the estimates of the sites of digestion for each of the components. These digestibility estimates are expressed as a percent of intake, percent of total tract digestion and as a percent of the component which reached the lower tract.

The sites of digestion were calculated by difference from the daily flow estimates using Yb as a digestion marker as explained in the materials and methods section of experiment 1. Total tract digestion (TTD) is 100 % minus the difference between intake and fecal flow. Rumen digestibility (RD) is the 100 % minus the difference between intake and duodenal flow. Lower tract digestion (LTD) is the difference between total tract digestion and rumen digestion. Small

intestine digestion (SID) is 100 % minus the difference between duodenal and ileal flow. Large intestine digestion (LID) is the difference between lower tract and small intestinal digestion.

Table 2.2 presents the dry matter intake, flow and digestion coefficients for the four diets. Dry matter intakes were 5690, 6601, 5560 and 5807 g/day for the low DM haylage, low DM haylage +HMC, high DM haylage and the high DM haylage + HMC diets respectively (note unless stated otherwise all subsequent reference to specific values will be listed in order as follows - low DM haylage, low DM haylage + HMC, high DM haylage and the high DM haylage + HMC diets). Duodenal DM flows, as a percent of intake, (55.22, 51.63, 54.04, 56.3) were similar across diets, this was also true for ileal DM flows (41.31, 39.85, 40.91, 40.39). Fecal flow (as a % of intake), with the two haylages fed alone, were were similar (low DM = 40.86, high DM = 38.26). The diets with HMC added tended (not significant) to have a lower fecal flow, as a percent of intake, than the haylages fed alone (40.86 vs 34.16 and 38.26 vs 34.29). Organic matter flows (Table 2.3) followed the same trends dry as matter flow. Fecal and ileal nitrogen flows (as a % of intake)(Table 2.4) were similar across diets (fecal : 32.67, 33.98, 33.98, 40.79; ileal: 44.65, 45.42, 44.00, 48.17). Diets with the HMC added tended to have higher duodenal nitrogen flow (as a % of intake) than haylage fed alone (65.57 vs. 81.63, 68.99 vs. 86.16). ADF (Table 2.5) flows were not significantly

different between diets at any sampling site, but the diets with the HMC added tended to have a greater flow of ADF, as a proportion of intake ADF, at the ileum and feces. ADL (Table 2.6) flows also were not different, between the four diets at the duodenum (86.31, 76.13, 81.90, 100.22), ileum (76.08, 58.66, 80.46, 109.62) or the feces (77.59, 86.59, 77.22, 98.50).

There were no significant differences in digestion coefficients between the diets in this study. This indicates that moisture level of the haylage at ensiling time or the addition of high moisture corn did not have a statistically significant affect on the site or extent of digestion of the alfalfa haylage used in this study. However, the variation and the loss of one degree of freedom, due to a missing cell, reduced our ability to demonstrate significant differences between the diet digestion coefficients in this study. There appears to be some trends in the data which may be of interest and may provide some insight into the effect of moisture level, at ensiling time and the addition of high moisture corn to the diet, on the site and extent of digestion of alfalfa haylage. With this in mind, the following discussion will utilize trend in the data to present some pertinent observations. In addition, no correction was made for endogenous or bacterial components, which may be present at the various sites in the digestive tract, so all digestion coefficients in this study are apparent digestion coefficients.

TABLE 2.2. Intake, Flow and Site and Extent of Digestion of Dry Matter. (Experiment 2)

Item	30% DM Haylage w/o HMC	30% DM Haylage +HMC	60% DM Haylage w/o HMC	60% DM Haylage +HMC	SEM
Intake (g/day)	5690.0	6601.0	5560.0	5807.0	
% of Intake					
Duod Flow	55.2	51.6	54.1	56.3	
Ileal Flow	41.3	39.9	40.9	40.4	
Fecal Flow	40.9	34.2	34.3	34.3	
Digestibility (% of intake)					
Total Tract	59.1	65.8	61.7	68.4	3.5
Rumen	44.8	48.4	46.0	45.9	6.2
Lower Tract (SI+LI)	14.4	17.5	15.8	22.5	4.4
Small Intest.	13.9	11.7	13.1	17.7	6.7
Large Intest.	0.4	5.8	2.7	4.8	3.8
Digestibility (% of total tract dig.)					
Rumen	75.4	74.5	73.3	65.6	8.4
Lower Tract (SI+LI)	24.6	25.5	26.7	34.3	8.4
Small Intest.	24.4	16.8	21.9	28.6	8.7
Large Intest.	0.2	8.7	4.8	5.7	8.7
Digestibility (% of DM reaching lower tract)					
Total	26.1	34.1	29.3	42.5	3.3
Small Intest.	24.8	22.5	24.3	33.4	11.1
Large Intest.	1.3	11.7	5.0	9.1	12.1

SI - Small Intestine
LI - Large Intestine
DM - Dry Matter
HMC - High Moisture Corn

TABLE 2.3. Intake, Flow and Site and Extent of Digestion of Organic Matter. (Experiment 2)

Item	30% DM Haylage w/o HMC	30% DM Haylage +HMC	60% DM Haylage w/o HMC	60% DM Haylage +HMC	SEM
Intake (g/day)	5162.0	6188.0	5047.0	5471.0	
(% of Intake)					
Duod Flow	42.8	40.7	41.9	47.0	
Ileal Flow	37.9	35.8	37.5	36.6	
Fecal Flow	41.1	33.8	38.8	33.9	
Digestibility (% of intake)					
Total Tract	58.9	66.2	61.2	68.8	2.0
Rumen	57.2	59.3	58.2	55.8	5.7
Lower Tract (SI+LI)	1.6	6.9	3.0	13.0	2.2
Small Intest.	4.9	4.9	4.4	11.1	3.6
Large Intest.	3.3	2.0	-1.4	1.9	5.0
Digestibility (% of total tract dig.)					
Rumen	97.5	91.2	95.1	80.8	4.2
Lower Tract (SI+LI)	2.5	8.8	4.9	19.2	4.2
Small Intest.	8.9	5.9	7.4	17.6	5.8
Large Intest.	-5.3	2.9	-2.5	1.6	5.8
Digestibility (% of OM reaching lower tract)					
Total	3.1	16.6	7.3	29.6	4.4
Small Intest.	11.6	11.4	10.6	25.1	8.8
Large Intest.	-8.5	5.2	-3.3	4.4	11.7

SI - Small Intestine
LI - Large Intestine
OM - Organic Matter
DM - Dry Matter
HMC - High Moisture Corn

Apparent total tract digestibility of dry matter (Table 2.2) and organic matter (Table 2.3) show no general tendencies for differences between diets. Ruminal digestion, as a percent of total tract digestion, represented between 65 and 75 percent of the dry matter digestion and between 80 and 97 percent of the organic matter digestion. This indicates that digestion of the dry matter reaching the small intestine was not high (26 - 42 %). Organic matter reaching the lower tract was also not digestible (3 - 30 %). However, in the diets with added corn the digestion (16.6 and 29.6) of the organic matter reaching the lower gut was much higher than diets without added corn (3.1 and 7.3).

Nitrogen digestion (Table 2.4),total tract, demonstrated no discernible differences between diets. However, there appears to be an effect of adding HMC to the diet, on the site of digestion. A larger proportion of the daily intake of nitrogen disappeared in the rumen when the haylage was fed alone. The addition of HMC to the diet tended to shift the site of digestion of the nitrogenous components in the diet. Thus, more of the dietary nitrogen was made available to the lower gut (65.57 vs 81.63 and 68.99 vs 86.16 %) with both haylages when HMC was added to the ration. Not only was more of the dietary nitrogen reaching the lower gut but it tended to have a higher digestibility in the lower tract, (50.3 vs 58.6 and 50.6 vs 54.6) when the HMC was added.

Intake, flow and site and extent of digestion of acid

detergent insoluble nitrogen (ADIN) is presented in Table 2.5. ADIN is thought to be an indigestible nitrogen fraction in feedstuffs (Yu, 1976). It consituted 13.79 percent of the nitrogen in the low DM haylage and 16.46 percent in the high DM haylage. This resulted in higher ADIN intakes with the high DM haylage diets even though the low DM haylage diets contained more nitrogen (Table 2.4). In addition, there was no detectable ADIN in the HMC. Therefore, the diets with HMC added had lower ADIN intakes (15.72, 12.53, 21.00, 12.83 g/day). Duodenal and fecal flows of ADIN were also lower with the low DM haylage diets than the high DM diets. There was only slight total tract digestion of the ADIN in the haylage alone diets (low DM = -2.78, high DM = 5.89). The low DM + HMC diet indicated slight digestion (15.26%). The high DM + HMC had higher ADIN fecal flow than intake which resulted in a negative digestion coefficient (-33.23). With the high standard error, of these estimates, (11.6) these are probably not different from zero. Duodenal ADIN flow was also higher than fecal flow and intake which resulted in negative digestion coefficients (-21.92, -33.95, -6.65, -60.89%). Again with the large standard error (20.16) these coefficients are probably not different from zero. The higher flows of ADIN at the duodenum indicate an over estimate of the flow of duodenal digesta the final section of this paper will contain a discussion of the possible reasons for this.

The site and extent of acid detergent fiber digestion

(Table 2.6) tended to be altered by the addition of HMC to the diet. Total tract digestion of ADF (as a percent of daily ADF intake) tended to be reduced with the addition of HMC (64.0 vs. 57.3 and 67.0 vs. 60.3). In addition, the contribution of ruminal digestion, to the total tract digestion, of ADF was increased (91.0 vs. 104.3 and 93.7 vs. 103.7) when the HMC was added to the diet. With the haylage alone almost 10 % of the total ADF digestion occured in the lower tract but when HMC was added ruminal digestion of ADF constituted virtually all of the ADF digestion that occured.

The digestion of acid detergent lignin (Table 2.7) is somewhat difficult to explain. As discussed earlier, lignin represents a supposed indigestible component of a feedstuff (VanSoest, 1982). Therefore, daily fecal output of lignin should represent 100% of the daily intake. In this study, the fecal flow of ADL was generally less than 100% of intake (77.59, 86.59, 77.22, 98.5). The diets with the added HMC tended to have a higher recovery. VanSoest (1982) suggested that young forages may have digestible lignin. Lignin recovery tends to be variable. Some workers report good recoveries (100%) (Kennedy,1982) and others report low recoveries (50 -75 %)(Galyean et.al.,1979) of lignin. Some work has shown lignin incrases from feed to feces, resulting in recoveries of up to 150 % (Fahey et.al.,1979).

TABLE 2.4. Intake, Flow and Site and Extent of Digestion of Nitrogen. (Experiment 2)

Item	30% DM Haylage w/o HMC	30% DM Haylage +HMC	60% DM Haylage w/o HMC	60% DM Haylage +HMC	SEM
Intake (g/day)	114.0	122.1	127.6	116.1	
(% of Intake)					
Duod Flow	65.6	81.6	67.0	86.2	
Ileal Flow	44.7	45.4	44.0	48.2	
Fecal Flow	32.7	34.0	34.0	40.8	
Digestibility (% of intake)					
Total Tract	67.3	66.0	66.0	62.2	4.3
Rumen	34.4	18.4	31.0	17.2	9.5
Lower Tract (SI+LI)	32.9	47.7	35.0	45.0	6.0
Small Intest.	20.9	36.2	25.0	40.6	5.8
Large Intest.	12.0	11.4	10.0	4.4	4.6
Digestibility (% of total tract dig.)					
Rumen	50.5	27.5	46.9	22.3	14.3
Lower Tract (SI+LI)	49.5	72.5	53.1	77.7	14.3
Small Intest.	30.9	55.5	37.4	70.7	10.9
Large Intest.	18.6	17.0	15.7	7.0	12.6
Digestibility (% of N reaching lower tract)					
Total	50.3	58.6	50.6	54.6	2.5
Small Intest.	32.9	44.0	36.7	50.3	7.4
Large Intest.	17.5	14.6	13.9	4.2	6.2

SI - Small Intestine
LI - Large Intestine
N - Nitrogen
DM - Dry Matter
HMC - High Moisture Corn

TABLE 2.5. Intake, Flow and Site and Extent of Digestion of Acid Detergent Insoluble Nitrogen. (Experiment 2)

Item	30% DM Haylage w/o HMC	30% DM Haylage +HMC	60% DM Haylage w/o HMC	60% DM Haylage +HMC	SEM
Intake (g/day)	15.72	12.53	21.00	12.83	
Duod Flow (g/day)	18.75	16.60	22.43	19.98	
Fecal Flow (g/day)	15.95	13.22	20.02	17.76	
Digestibility (% of intake)					
Total Tract	2.78	15.26	5.89	-33.73	11.60
Rumen	21.92	-33.95	-6.65	-60.89	20.16
Lower Tract (SI+LI)	24.71	18.70	12.54	27.14	13.60

SI - Small Intestine
LI - Large Intestine
ADIN - Acid Detergent Insoluble Nitrogen
DM - Dry Matter
HMC - High Moisture Corn

TABLE 2.6 Intake, Flow and Site and Extent of Digestion of Acid Detergent Fiber. (Experiment 2)

Item	30% DM Haylage w/o HMC	30% DM Haylage +HMC	60% DM Haylage w/o HMC	60% DM Haylage +HMC	SEM
Intake (g/day)	2420.0	1730.0	2379.0	1393.0	
(% of Intake)					
Duod Flow	41.7	41.4	37.0	42.2	
Ileal Flow	35.2	42.9	33.6	49.1	
Fecal Flow	36.0	42.7	33.0	43.2	
Digestibility (% of intake)					
Total Tract	64.0	57.3	67.0	60.3	5.0
Rumen	58.3	59.3	63.0	61.8	5.6
Lower Tract (SI+LI)	5.6	-2.0	3.9	-1.5	3.9
Small Intest.	6.5	-2.2	3.4	-8.0	10.4
Large Intest.	-0.8	0.2	0.6	6.6	7.0
Digestibility (% of total tract dig.)					
Rumen	91.0	104.3	93.7	103.9	7.2
Lower Tract (SI+LI)	9.0	-4.3	6.3	-3.9	7.2
Small Intest.	10.5	-5.6	5.9	-12.9	18.0
Large Intest.	-1.5	-0.7	0.4	-9.0	18.0
Digestibility (% of ADF reaching lower tract)					
Total	13.4	-5.3	8.2	-1.2	9.3
Small Intest.	14.6	-5.6	3.8	19.9	26.5
Large Intest.	-1.2	0.3	4.4	18.7	18.5

SI - Small Intestine
LI - Large Intestine
ADF - Acid Detergent Fiber
DM - Dry Matter
HMC - High Moisture Corn

TABLE 2.7. Intake, Flow and Site and Extent of Digestion of Acid Detergent Lignin. (Experiment 2)

Item	30% DM Haylage w/o HMC	30% DM Haylage +HMC	60% DM Haylage w/o HMC	60% DM Haylage +HMC	SEM
Intake (g/day)	346.7	248.2	342.6	198.8	
(% of Intake)					
Duod Flow	86.3	76.1	81.9	100.2	
Ileal Flow	76.1	58.7	80.5	109.6	
Fecal Flow	77.6	86.6	77.2	98.5	
Digestibility (% of intake)					
Total Tract	22.4	13.4	22.8	9.2	6.2
Rumen	13.7	23.9	18.1	5.1	9.3
Lower Tract (SI+LI)	8.7	4.8	4.7	2.5	4.2
Digestibility (% of ADL reaching lower tract)	10.8	-14.1	-7.8	-2.5	

SI - Small Intestine
LI - Large Intestine
ADL - Acid Detergent Lignin
DM - Dry Matter
HMC - High Moisture Corn

DISCUSSION
(Experiments 1 and 2)

The effect of moisture level of alfalfa at ensiling time on site and extent of digestion was examined in two separate experiments. In the first experiment, the haylages were ensiled at 30 % DM and 60 % DM. These haylages were fed alone, to four duodenally cannulated Holstein steers. The experimental design, of experiment 1, was a replicated 2 X 2 latin square.

The haylages in the second experiment were ensiled at 30 and 45 percent dry matter. The experimental design was a 4 X 4 latin square using four Holstein steers, fitted with duodenal and ileal cannulas, and four diets. The diets consisted of the two alfalfa haylages fed alone or in combination with HMC. The HMC was included in the diet at approximately 30 percent of the total diet DM.

The high moisture haylage tended to have a more appealing appearance and odor. In the first study, the high DM haylage exhibited a brown color and had a caramel like smell. The low DM and high DM haylages in the second study both had good appearence and odor. The dry matter intakes were controlled, in both studies. This was accomplished by feeding all animals at the level of the

animal which ate the least feed. However, it appeared that the low DM haylages had greater palatability since the diets which gave the lowest intakes during the adaptation period were the high DM haylages in both studies. In the second study, the addition of HMC tended to increase acceptability of the diets.

After ensiling, the composition of the two haylages were somewhat different between experiments. The effect of the ensiling process cannot be ascertained since no samples of the fresh forage were obtained in either study and the haylages were not charaterized (lactate, pH, VFA, etc.). The low DM haylages were similar in dry matter (Exp.1= 29.15, Exp.2= 31.33 %), nitrogen (2.06, 2.00 % of DM) and acid detergent fiber (42.53, 45.86 % of DM) content. The high DM haylages were considerably different in dry matter content (Exp.1= 60.70, Exp.2= 39.61 %) but were similar in nitrogen (2.36, 2.29 % of DM) and acid detergent fiber (45.56, 42.79 % of DM). In the second experiment, the ADIN level in the low DM (13.79 % of N) was lower than in the high DM (16.46 % of N).

Site and extent of digestion estimates, of dry matter, were similar between the two studies, for the low DM haylages. Total tract digestion was nearly the same (Exp.1= 62.13, Exp.2= 59.1). Ruminal digestion constituted about the same proportion of the total tract digestion in the two studies (69.84, 75.4 % of total tract digestion). This data is consistant with other workers. Merchen and Satter (1983)

reported total tract digestibilies in 29% DM haylages of 69.8 % with 70.9% of the total tract digestion occuring in the rumen of Holstein cows. Sutton and Vetter (1971) reported somewhat lower (59.5 %) but Hawkins et.al. (1970) reported similar (60 %) total tract digestion, with sheep.

Nitrogen digestion of the low DM haylages, from the two studies were similar. Total tract digestibility was 62.13 % in the first study and 67.3 % in the second. Ruminal disappearance accounted for more than half of total tract digestion in both studies (69.84, 50.5 % of total tract digestion). The total tract nitrogen digestibilies, in these studies, were slightly lower than the value (72.3 %) reported by Merchen and Satter (1983). Merchen and Satter also reported higher values for the ruminal contribution (73.3) to the total tract digestion. Other workers have reported higher total tract nitrogen digestion (Sutton and Vetter (1971) (72.8 %) and Hawkins et.al.(1970) (72 %)) than were obtained in our two studies.

The acid detergent fiber digestion, in our two studies, was similar for the low DM haylages. Total tract digestion was 64.11 % in the first study and 64.0 % in the second. Ruminal contribution, to the total tract digestion, was lower in the first study (78.7 %) than in the second study (91.0). Merchen and Satter (1983) reported lower total tract ADF digestion (52.6 %) but similar ruminal availability (97.9 %). Both of our studies indicate the majority of the ADF

digestion occures in the rumen, which is to be expected, but sugests that the lower gut may contribute 10% or more of the total tract digestion of fiber. This observation has been supported by Dixon and Nolan (1982) and Putnam and Davies (1965).

The high DM haylages appear to be entirely different between the the two studies. Total tract dry matter digestibility, of the high DM haylage, in the first study was low (37.0 %), but the in the second study the total tract digestibility , of the high DM haylage (61.7), was similar to the low DM haylage (59.1%). Ruminal digestion consituted only 26.5 % of total tract digestion, in the first study, but accounted for 73.3 % in the second. ADF digestion followed a similar pattern. Total tract digestion was 67.0, in the second study, but only 32.42 in the first study. Ruminal digestion accounted for 93.7 % of the total tract ADF digestion, in the second study, but acccounted for over 100 % in the first study. Nitrogen digestion with the high DM haylage diets, in the second study, was also similar to the low DM haylage and greatly different from the high DM haylage in the first study. Total tract nitrogen digestion was 45.19 % in the first study and was 66.0 % in the second.

The high DM haylage, in the second study, generally was not different from the low DM in composition (except % DM) and was similar in the site and extent of digestion of the various components. This can supported by other work. Merchen and Satter (1983) reported little difference

between haylages ensiled at 30% and 40% DM in site or extent of dry matter, nitrogen and acid detergent fiber digestion. However, they reported a marked depression in the digestibility of nitrogen in haylages ensiled at 60% dry matter. This has been supported by other workers (Thomas et.al,1972, Beever et.al,1976). The high DM haylage in the first study was clearly a lower quality forage. The digestibility of dry matter, organic matter, nitrogen and acid detergent fiber was lower than the low DM haylage in the first study and both haylages in the second study. Several workers (Yu, 1977, Thomas et.al. 1972, Merchen and Satter, 1983) have suggested that excessive heating, which occurs during the ensiling process, can reduce the availability of nitrogen in the forage. A similar observation for fiber availability has not been found by this author. However, it could be argued that, the low nitrogen availability of the heat damaged forage could inhibit microbial activity in the rumen and thereby reduce the digestion of the fiberous components of the forage.

EXPERIMENT 3.
Duodenal Digesta Flow Study

The data obtained from the first study resulted in values for duodenal flow and composition which when used to generate site of digestion estimates gave some unrealistic values. Fiber (both NDF and ADF) was generated in the rumen and nitrogen flow through the duodenum was 145 % of that fed to the animals. Data reported by Weber (1984) suggested further problems with marker ratio, site and extent of digestion studies. Weber reported that if more than one indigestible marker, was added to a diet and then each marker was used, to calculate site of digestion estimates, the results obtained, for each marker, could be significantly different. This should not be possible if the basic assumptions of a marker ratio study are met. The assumptions are: 1. nonabsorbable markers; 2. representative sampling; 3. steady state conditions. As discussed in earlier sections, the assumption which was probably not being met was steady state.

The third experiment was conducted, to determine to what extent the steady state assumption was being met in these haylage digestion studies. Continuous infusion of a marker, into the abomasum, was used to monitor the flow of digesta,

in the duodenum and determine the flow rate and composition of the digesta. If flow and composition are shown to be variable, potentially, the data obtained could be used to demonstrate why some of the values obtained, in marker ratio types of studies, are inconsistant and unrealistic.

MATERIALS AND METHODS

This experiment was added on to the second experiment during the second block and continued through the fourth block. It was felt that adding one more marker to the study would not appreciably affect the results of the second haylage study. Thus, animals, diets, markers, analysis and sample collection procedures for experiment 2 apply to this study. Additional procedures, described below include separate handling of the duodenal samples and PEG infusion and analysis.

Infusion Procedure

In this study an infusion system was used to estimate the digesta flow passing through the duodenum. The method used was based on down stream dilution of a tracer in a closed system. This involves the infusion of a non absorbable marker, into a fluid stream, at a constant rate and measurement of the concentration of the marker down stream. The dilution of the marker can be used to estimate the rate

of flow of the fluid (Figure 3.1).

Infusion Rate = INFRT = 0.180 l/hr
Concentration of Infusate = CNCINF = 10 g/l
Marker Concentration In Liquid = MKRCNC = 0.30 g/l

$$\text{Liquid Flow Rate (l/hr)} = \frac{\text{INFRT(l/hr)} * \text{CNCINF(g/l)}}{\text{MKRCNC(g/l)}}$$

$$\frac{0.180 \text{ l/hr} * 10 \text{ g/l}}{0.30 \text{ g/l}} = 6.0 \text{ l/hr}$$

FIGURE 3.1. Flow Rate Calculations by Marker Dilution.

The four animals were infused simultaneously, by means of a Harvard peristaltic pump, through a small cannula placed in the abomasum. The infusate was a concentrated solution of polyethlene glycol (PEG)(75.0 - 150.0 g/l). It was delivered at approximately 3.0 ml/min. The infusion was begun at least 24 hours before the begining of the four day collection period and continued until after collection of the final sample.

Infusion rate was monitored throughout the infusion period. This was accomplished by weighing the infusion reservoirs. The infusion rate is calculated by the difference in weight from time one to two, divided by the time elapsed. A separate reservoir was provided for each animal, thereby, allowing the calculation of separate infusion rates for each animal and between sampling times.

Actual infusion rates varied from 2.50 to 3.00 ml /min. with most of the variation coming between infusion ports. Fluctuations in the infusion rate, to one animal, generally was less than 10 percent.

After determination of the concentration of PEG in the samples the flow of liquid, whole digesta and dry matter was calculated. Since PEG is a liquid phase marker, it can be used to calculate the liquid flow rate in the duodenum (Figure 3.1). Once the liquid flow rate is known the flow of whole digesta and dry matter can be calculated (Figure 3.2). Dry matter flow is then used to calculate the flow of the other components of interest. This is accomplished by multiplication of the amount of the component in the dry matter by the dry matter flow rate for that time period.

Liquid Flow = LIQFLW = 6.0 l/hr (assume 1 l = 1 kg)

% Dry Matter in Whole Digesta = DIGDM = 5.0

$$\text{Whole Digesta Flow} = \frac{\text{LIGFLW (kg/hr)}}{\text{1.00 - DIGDM (\%)}} =$$

$$= \frac{\text{6.0 kg/hr}}{\text{1.00 - 0.05}} = 6.32 \text{ kg/hr}$$

FIGURE 3.2. Digesta and Dry Matter Flow Calculations

Sample Handling and Analysis

Samples were collected on a four day collection schedule as described previously. All samples were immediately frozen after collection. The duodenal samples were thawed and homogenized. Two 50 ml aliquots were oven dried at 100 C for 24 hours to determine the dry matter content of the digesta. In addition, two 25 ml aliquots were placed into 25 ml corex centrifuge tubes and spun at 45,000 X g for 30 min. Each duodenal sample was then indiviually freeze dried and ground through a 1 mm screen for subsequent analysis.

Dry matter, ash, nitrogen and acid detergent fiber were determined as described previously. Acid detergent lignin was determined by the method outlined by Georing and Van Soest (1968). PEG analysis was performed on the supernatant from the centrifuged subsample. Chromium and ytterbium analysis was performed by atomic emission spectrophotometry following wet ashing of the freeze dried sample.

As mentioned earlier, samples were frozen immediately after collection. In addition, a sample of the infusate was obtained for each infusion period - animal combination. The infusate samples were frozen and analyzed for PEG at the same time as the rest of the samples. The analysis of PEG which was used was an adaptation of the method originally described by Hyden et al (1955). The actual procedure used was as outlined by Malawar and Powell (1967) and involved the use of

an emulsifier (gum arabic). The emulsifier helps to stablize the emulsion formed between trichloroacidic acid (TCA) and PEG. The samples were first thawed, then centrifuged at 45,000 x g for 30 min. Soluble proteins were removed by adding $Zn SO_4$ to the supernatant and then filtering the precipitate through two layers of Whatman #52 filter paper. The emulsion was then formed by adding concentrated TCA to the filtrate. This emulsion was then read, at 350 nm, on a spectrophotometer after allowing the emulsion to stabilize for 45 - 90 min.

RESULTS AND DISCUSSION

The objective of this study was to examine the steady state assumption made when conducting a passage study as described in experiment 1. Steady state, as it relates to the rumen, is a condition in which the rumen outflow rate remains constant through the collection period. This is a critical assumption due to the method of compositing digesta samples to achieve average daily digesta. As discussed in previous sections, nonsteady state may result in a composite sample which is not representative of average daily flow, when compositing on an equal proportion basis.

Tables 3.1 - 3.4 contain the duodenal dry matter flow rates for the final three block of experiment 2. Flow rates are presented, as the hourly flow, as a percent of the total daily flow. These were determined by the constant infusion PEG dilution technique described in the material and methods section. Individual flow rates for each animal and during each block are contained in the appendix. Table 3.1 contains the grand average for all observations of flow rates and the standard deviation at each sampling time. Figure 3.3 presents this average flow rate in a graphic manner. Each point represents 11 observations. Average hourly flow varied from 3.06 to 4.8 % of the total daily flow. High flow

TABLE 3.1. Duodenal Dry Matter Flow Rate
Average of All Observations

Sampling Time	Hourly Flow Rate (% of Daily Flow)	Standard Deviation
7:00 am	4.50	1.01
9:00 am	4.80	0.65
11:00 am	3.77	0.66
1:00 pm	4.06	1.13
3:00 pm	3.91	0.50
5:00 pm	3.99	0.69
7:00 pm	4.63	0.83
9:00 pm	4.04	0.39
11:00 pm	3.88	0.82
1:00 am	4.53	1.07
3:00 am	4.42	1.44
5:00 am	3.06	0.80

TABLE 3.2. Duodenal Dry Matter Flow
Averaged by Block

Sampling Time	Hourly Flow Rate (% of Daily Flow) Block 2	Block 3	Block 4
7:00 am	4.11	4.52	4.87
9:00 am	4.64	5.48	4.44
11:00 am	3.97	4.08	3.34
1:00 pm	4.14	3.15	4.67
3:00 pm	4.10	3.68	3.90
5:00 pm	3.71	4.82	3.64
7:00 pm	5.03	4.13	4.59
9:00 pm	3.93	3.92	4.26
11:00 pm	3.50	4.69	3.66
1:00 am	4.99	4.22	4.30
3:00 am	5.19	3.74	4.17
5:00 am	2.68	3.56	*

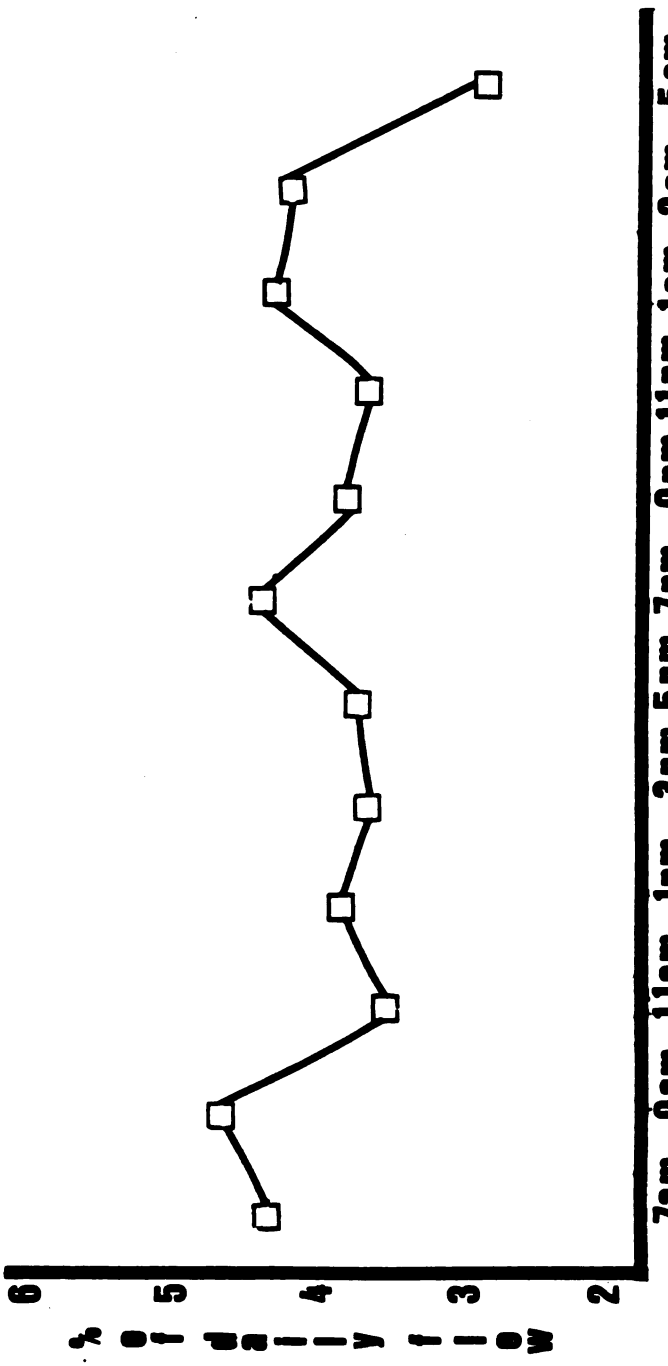


FIGURE 3.3. Duodenal Dry Matter Flow Pattern.
Average of All Observations

times were 9am, 7pm and 1am and low flow occured at 11am, 11pm and 5am. Low flow periods occured about 3 hours after feeding which is typically considered to be a high volatile fatty acid production period. The lowest flow of the day occured at 5am. This may have been the result of low activity of the animals. High flow periods tended to be in the early morning prior to feeding and between 1am and 3am.

Tables 3.2, 3.3 and 3.4 represent the duodenal dry matter flows averaged by block, diet and animal respectively. Figures 3.4 - 3.6 graphically depict these values. The observations are grouped in this manner in an attempt to examine the influence of time period (block), diets and animals on rumen emptying and the resulting duodenal digesta flow.

The effect of time of the year is not clear in this study. Collections for the each block were conducted during the following time periods: block 2, third week of March; block 3, first week in June; block 4, third week of July. The flow for each block (Figure 3.4) (each point the average of 4 values, one from each diet) follows the same general pattern with shifts in the phase and amplitude of the curves. Blocks two and three seem to have similar flow patterns. Block four seems to be out of phase by one collection period. The flow in block two is much more constant than in the other blocks, during most of the day, but is very high from 1 - 5 am. What creates these shifts or if these shifts are even real is difficult to access. Corbet and Pickering (1983)

TABLE 3.3. Duodenal Dry Matter Flow Averaged By Diet

Sampling Time	Hourly Flow Rate (% of Daily Flow) 30% DM Haylage w/o HMC	30% DM Haylage +HMC	45% DM Haylage w/o HMC	45% DM Haylage +HMC
7:00 am	4.23	4.00	4.48	5.66
9:00 am	5.03	4.72	4.92	4.39
11:00 am	4.16	3.31	3.77	3.87
1:00 pm	3.59	4.50	3.44	5.06
3:00 pm	4.00	4.08	4.06	3.32
5:00 pm	4.26	4.15	4.21	3.00
7:00 pm	4.25	4.68	4.66	5.06
9:00 pm	3.58	4.14	4.15	4.44
11:00 pm	4.46	3.96	4.09	2.60
1:00 am	4.04	4.38	5.66	3.79
3:00 am	4.84	4.36	3.38	5.47
5:00 am	3.26	2.49	2.68	2.57

TABLE 3.4. Dry Matter Flow Rates Averaged by Animal

Sampling Time	Hourly Flow Rate (% of Daily Flow) An Number 1	2	3	4
7:00 am	4.63	4.69	3.78	4.65
9:00 am	4.48	5.27	5.00	4.51
11:00 am	3.56	3.61	3.89	4.06
1:00 pm	4.22	4.64	3.06	4.00
3:00 pm	3.64	3.62	4.40	4.15
5:00 pm	4.17	3.60	4.10	4.12
7:00 pm	3.94	5.22	5.11	4.40
9:00 pm	4.26	4.16	3.88	4.34
11:00 pm	3.57	3.53	4.48	4.15
1:00 am	5.00	3.85	5.69	3.98
3:00 am	5.00	3.94	3.09	5.23
5:00 am	3.24	3.72	2.93	2.29

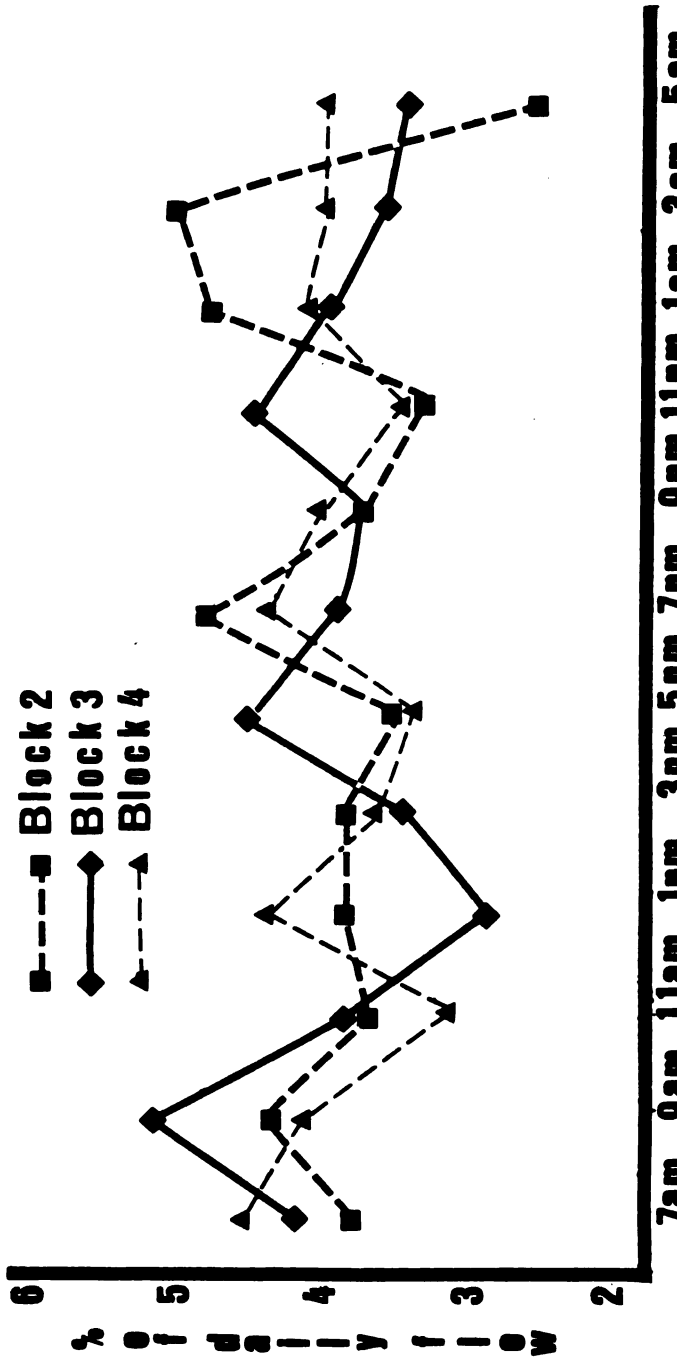


FIGURE 3.4. Duodenal Dry Matter Flow Pattern. Averaged by Block

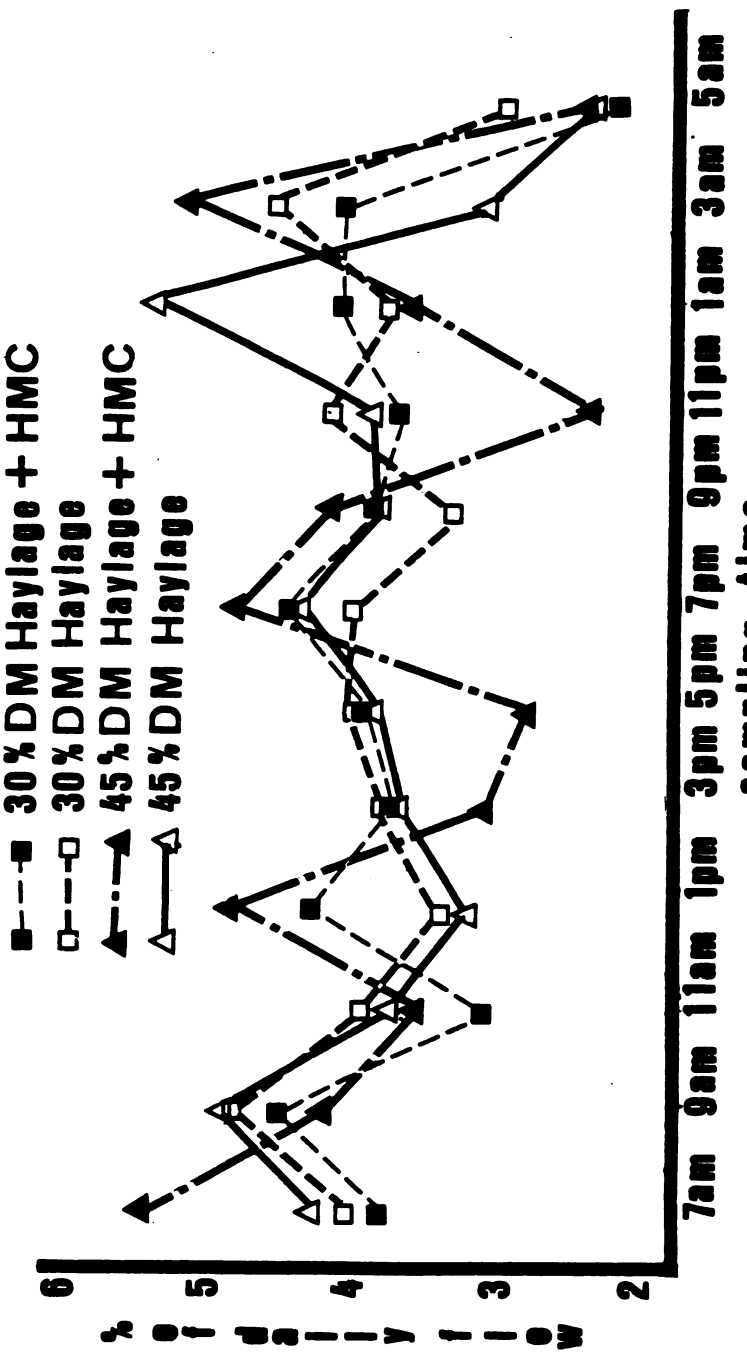


FIGURE 3.5. Duodenal Dry Matter Flow Pattern. Averaged by Diet

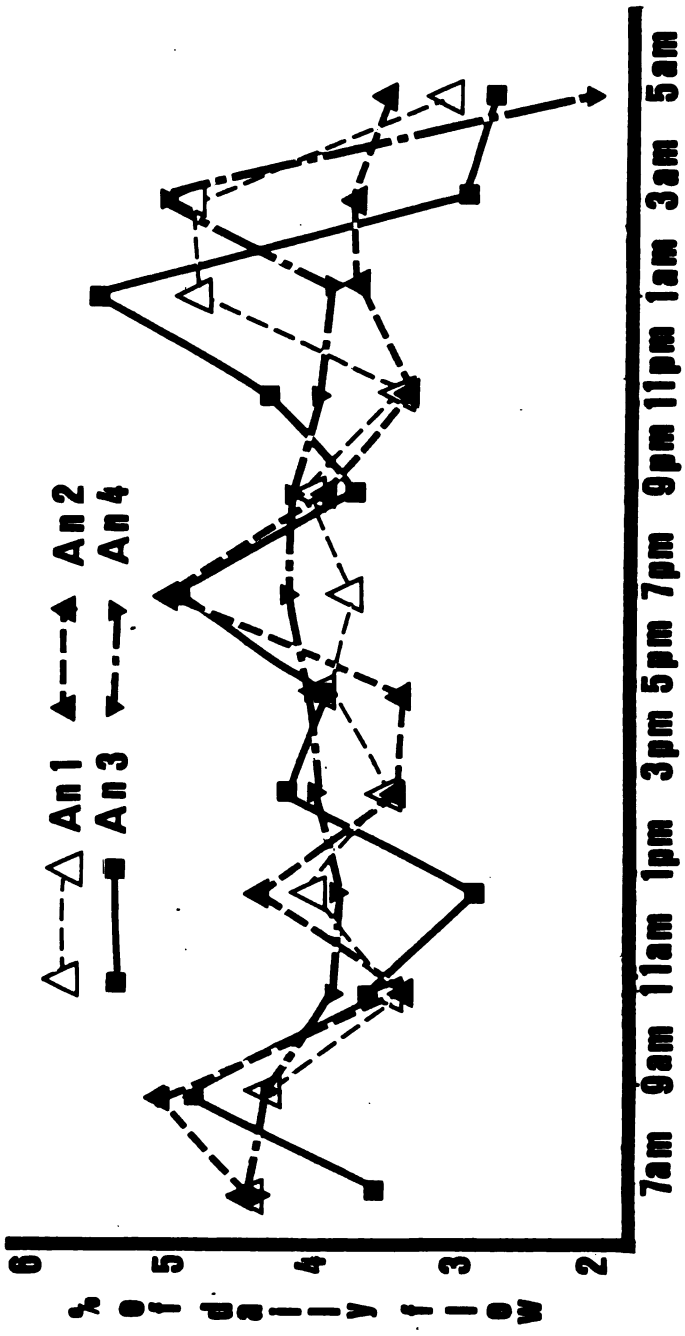


FIGURE 3.6. Duodenal Dry Matter Flow Pattern. Averaged by Animal

reported a marked effect of season and attributed it to light dark cycles. Another factor to consider is environmental conditions. Temperature has been shown to effect intake and digestibility in sheep (Kennedy and Milligan, 1978) and the animals were certainly exposed to different temperatures in March than in July.

Figure 3.5 represents the flow averaged by diet. Each point represents three observations (one from each block). The diets with the added corn seem to flow in a similar pattern as do the two haylage only diets. The corn added diets tend to be more variable (especially the 45% +HMC). The similarities between the corn diets and between the haylage diets could be a response to metabolites produced in the rumen, feeding behavior, drinking behavior, bulk density of the diet, any combination of these or none of these factors. This is difficult to determine since there is no data (to this authors knowlege) available on the affect of these factors on rumen outflow patterns.

The flow averaged by animal (each point represents three observations) is found in Figure 3.6. The four animals seemed to follow the same general trend. They exhibited high flow at 9am, 7am and between 1 -3 am and low flow at 11am, 11pm and between 3 - 5 am. The flow patterns seemed to be more consistant between animal than between block or diets. This may indicate that the factors creating differences in flow patterns may not be related to individual animals but to external factors such as diet or environment.

A number of other workers have reported this type of variation in flow. Early work by Harris and Phillipson (1962), utilizing reentrant cannulated sheep, demonstrated a definate periodicity to flow. Subsequent work in sheep (Phillips and Dyck, 1964, Topps et.al., 1968, Thompson and Lamming, 1972) and cattle (Topps et.al.,1968, Weber, 1983) demonstrated similar results. It is difficult to speculate as to what factors regulate rumen outflow. Ruckebusch (1981) provides an excellent review of intestinal motility and factors regulating it. However, no clear understanding of the factors regulating rumen outflow has been achieved. Several factors, which may be related to rumen outflow, have been investigated. Rumination has been shown to vary in intensity througout the day (Gordon and McAllister, 1970, Deswysen and Ehrlein, 1981) and does not seem to be related to feeding behavior. Gordon and McAllister (1970) demonstrated a circadian rhythm to rumination. Corbett and Pickering, (1983) presented a compilation of several studies and attempted to discern the effect of feeding, season and light dark cycles. They concluded, rumen outflow was more related to season and light dark cycles then feeding. Ruckebusch (1981) observed gut motility was highly related to endogenous blood glucose levels, hormonal levels, (etc). Many such endogenous factors demonstrate cyclic patterns and may be related to digesta flow.

Digesta Composition

The previous section demonstrated variation in flow. Another important consideration is the composition of the duodenal digesta. If the digesta composition remains constant throughout the day the importance of nonsteady flow diminishes. In fact, if composition is constant one sample or possibly only a few samples could be used to represent daily flow. In an attempt to evaluate this problem, all samples, from each sampling time, obtained during the second block were analyzed for chromium (Cr), ytterbium (Yb), nitrogen, acid detergent fiber (ADF) and ash. Table 3.5 - 3.8 represent the analysis for each animal, which were on one of the four diets. Figures 3.8 - 3.10 present the Cr, Yb and nitrogen composition in block diagram format.

The whole digesta (DM + liquid) flow and DM flow, for each of the four sets of samples, is presented in Figure 3.7. Each animal demonstrates a slighly different flow pattern. All animals, however, demonstrated low flows early in the morning and 3 - 5 hours after feeding (feeding indicated by the arrows). High flow periods existed shortly after midnight and around feeding time. Digesta flow (DM + liquid), indicated by the solid line and dry matter flow (broken line), followed similar but not identical patterns. This is due to the fluctuation in the dry matter content of the duodenal fluid. It is apparent that none of the animals exhibited steady flow.

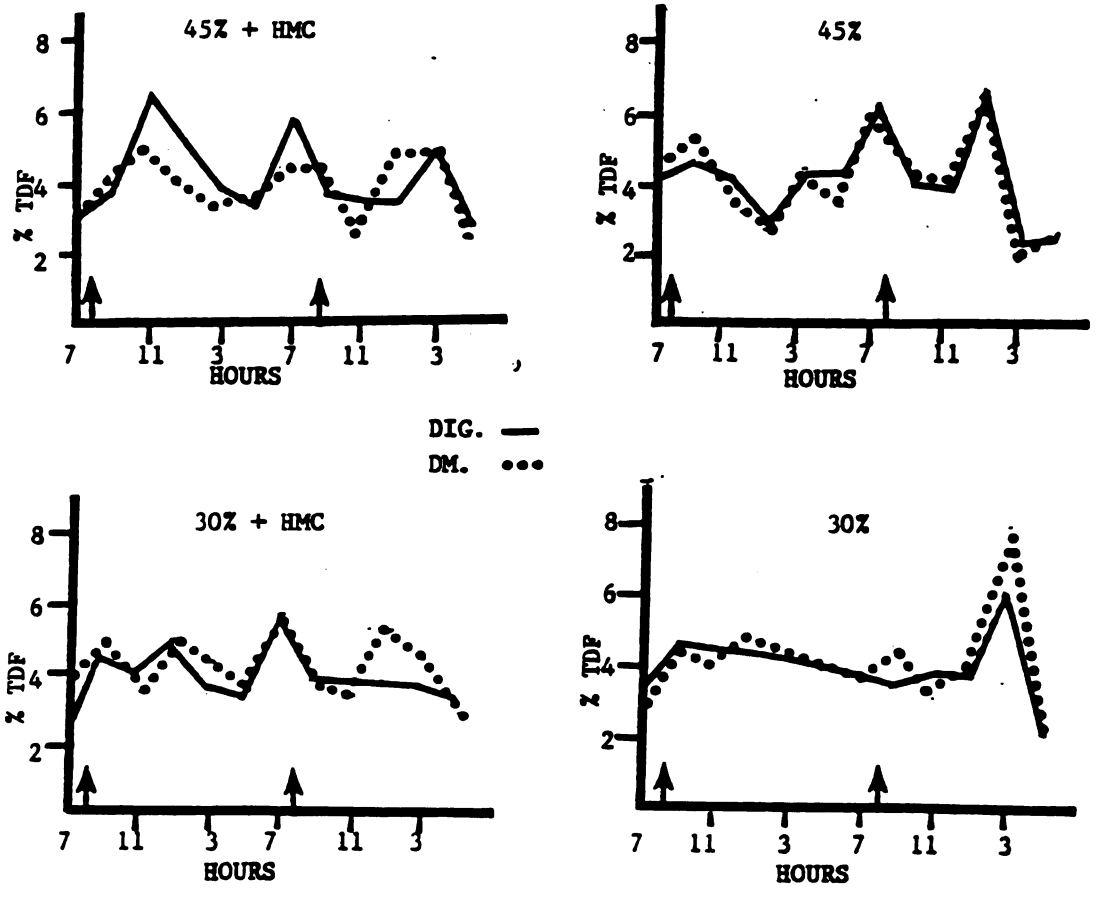


FIGURE 3.7. Duodenal Dry Matter Flow Pattern. Averaged by Animal

Figures 3.8 - 3.10 show the Cr, Yb and nitrogen content in duodenal digesta, respectively. The concentration of each of these components varied throughout the day. However, nitrogen tended to be more consistant, in the 30% haylage diets, than any other component in any of the other diets. Attempting to related the flow at any one time to composition, results in no consistant pattern. The two indigestible components (Yb and Cr:EDTA) seemed to behave differently. Cr tended to be high when flow was high and Yb tended to be low. The opposite seemed to be true as well, when flow was low Yb tended to be high and Cr low. Nitrogen tended to follow flow. Thus, when flow was high nitrogen concentration tended to be high and when flow was low nitrogen concentration was low. Cr (a liquid phase marker) tended to behave like nitrogen suggesting the nitrogen in these diets may be flowing with the liquid phase. This observation is conceivable, since bacterial nitrogen would tend to flow with the liquid phase and any soluble protein or nonprotein nitrogen would also follow the liquid phase. In forage diets, these sources of nitrogen (bacterial,soluble protein, NPN) would tend to be a major portion of the nitrogen flowing out of the rumen.

TABLE 3.5. Composition of Duodenal Digesta Dry Matter. Animal 7375(4), Diet 30% DM Haylage

Sampling Time	Cr Conc. (ug/g)	Yb Conc. (ug/g)	N Conc. (g/100g)	ADF Conc (g/100g)	Ash Conc (g/100g)
7:00 am	24.02	622.4	2.38	30.33	19.92
9:00 am	22.57	552.7	2.26	31.97	19.45
11:00 am	24.39	552.8	2.16	33.20	16.73
1:00 pm	25.36	468.0	2.06	35.61	13.46
3:00 pm	27.34	474.1	2.00	35.61	14.25
5:00 pm	23.45	530.9	2.20	31.66	15.14
7:00 pm	23.81	569.9	2.13	34.24	15.66
9:00 pm	23.45	530.9	2.20	31.66	15.14
11:00 pm	25.89	540.0	2.16	33.09	15.80
1:00 am	27.79	538.6	2.28	32.78	17.51
3:00 am	26.68	536.0	2.20	34.33	15.23
5:00 am	26.32	557.3	2.28	33.60	14.65

TABLE 3.6. Composition of Duodenal Digesta Dry Matter. Animal 7373(3), Diet 30% DM Haylage + HMC

Sampling Time	Cr Conc. (ug/g)	Yb Conc. (ug/g)	N Conc. (g/100g)	ADF Conc (g/100g)	Ash Conc (g/100g)
7:00 am	24.04	563.4	2.56	19.33	13.88
9:00 am	22.65	404.7	2.60	17.39	13.63
11:00 am	30.22	445.6	2.72	21.24	16.94
1:00 pm	29.93	420.5	2.44	22.92	15.38
3:00 pm	29.63	397.5	2.38	21.71	11.98
5:00 pm	23.82	424.6	2.56	20.77	13.06
7:00 pm	22.67	443.8	2.92	18.97	15.40
9:00 pm	22.19	402.8	3.16	16.61	13.93
11:00 pm	32.91	475.1	3.34	21.83	14.13
1:00 am	35.72	395.1	2.88	16.59	16.82
3:00 am	29.03	437.2	2.24	20.62	11.17
5:00 am	26.34	689.0	2.60	23.33	12.90

TABLE 3.7 Composition of Duodenal Digesta Dry Matter.
Animal 7372(2), Diet 45% DM Haylage

Sampling Time	Cr Conc. (ug/g)	Yb Conc. (ug/g)	N Conc. (g/100g)	ADF Conc (g/100g)	Ash Conc (g/100g)
7:00 am	23.58	755.2	2.97	28.34	18.71
9:00 am	26.65	644.4	2.46	33.31	18.44
11:00 am	29.80	625.4	2.84	27.70	15.20
1:00 pm	30.58	677.5	2.92	26.73	18.61
3:00 pm	27.75	654.5	2.90	25.08	15.16
5:00 pm	28.60	733.8	3.10	24.11	16.75
7:00 pm	21.40	676.6	2.96	27.77	16.46
9:00 pm	24.30	601.8	2.70	28.95	14.39
11:00 pm	24.76	679.1	2.68	30.40	14.53
1:00 am	31.76	536.1	2.78	29.27	15.58
3:00 am	30.04	593.4	2.61	25.13	14.35
5:00 am	27.02	493.5	2.39	27.77	21.32

TABLE 3.8. Composition of Duodenal Haylage Dry Matter.
Animal 7371(1), Diet 45% DM Haylage + HMC

Sampling Time	Cr Conc. (ug/g)	Yb Conc. (ug/g)	N Conc. (g/100g)	ADF Conc (g/100g)	Ash Conc (g/100g)
7:00 am	23.58	554.00	3.10	15.17	13.66
9:00 am	26.65	546.90	3.06	22.00	12.97
11:00 am	29.80	425.00	1.85	9.97	8.06
1:00 pm	30.58	308.20	1.85	10.99	6.94
3:00 pm	27.75	563.26	3.33	10.60	16.98
5:00 pm	28.60	563.30	3.12	18.03	13.25
7:00 pm	21.40	575.00	3.66	8.18	17.66
9:00 pm	24.30	467.80	2.78	16.03	11.66
11:00 pm	24.76	547.80	3.24	12.51	16.40
1:00 am	31.76	399.00	2.37	16.60	9.63
3:00 am	30.04	378.70	2.35	16.39	8.65
5:00 am	27.02	537.00	2.88	18.34	13.30

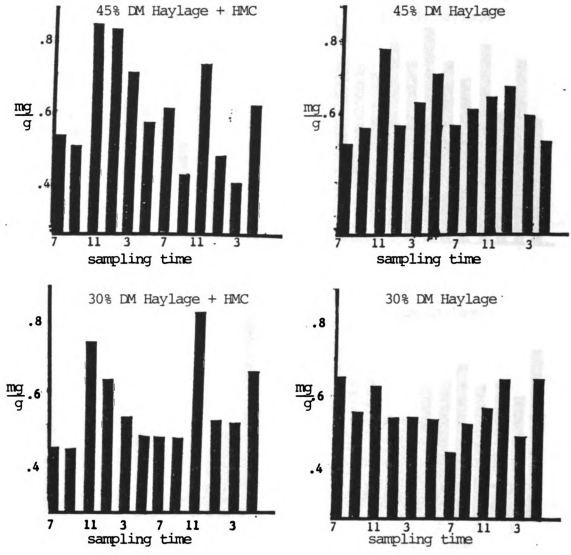


FIGURE. 3.8. Chromium Concentration in Duodenal Digesta Dry Matter. (Second Block)

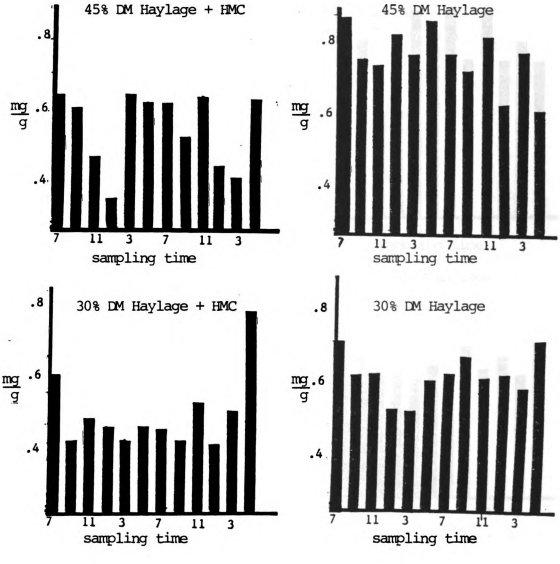


FIGURE 3.9. Ytterbium Concentration in Duodenal Digesta Dry Matter. (Second Block)

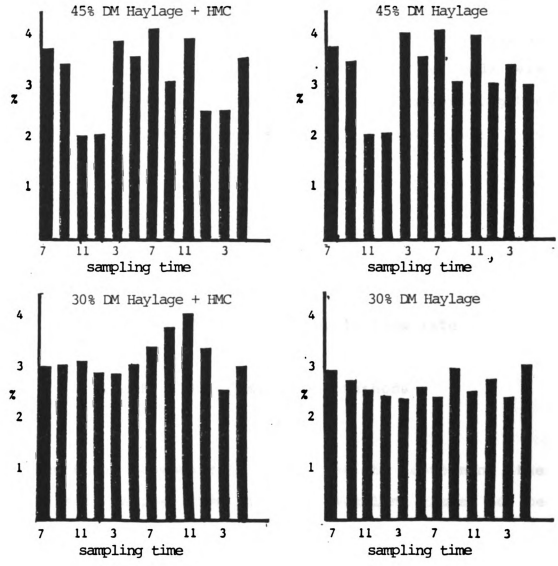


FIGURE. 3.10. Nitrogen Concentration in Duodenal Digesta Dry Matter. (Second Block)

Effect of Compositing Method

The data and discussion, presented above certainly, suggest that duodenal digesta flow and composition varies thoughout the day. In the literature review, it was suggested that, if flow and composition vary thoughout the day, the method used to composite samples could have an effect on the results obtained. To furthur investigate this contention, all samples obtained in the final three blocks of the study were analyzed for Yb and Cr. Several methods of compositing the samples were then attempted mathematically (Figure 3.11)

1. Average Dry Matter:
   - add equal amounts of dry sample
2. Average Digesta:
   - add equal amounts of whole digesta
3. Flow:
   - add amounts adjusted for hourly flow rate

FIGURE 3.11. Compositing Methods

Mathematical compositing was used to determine the amount of each sample (from different sampling times) to be added to a theoretical composite. Since the concentration of Yb and Cr was determined for each sample by weighted averaging the concentration of Yb and Cr in the composite was estimated. The marker ratio technique described in the material and methods section of experiment 1 estimates of

apparent ruminal dry matter digestibility were calculated (Table 3.9). Statistical analysis of the results was performed by treating all observations as a completly randomized design.

TABLE 3.9. Effect of Compositing Technique on Apparent Ruminal Dry Matter Digestibility Based on Yb or Cr as Markers

Marker	Composite Method	[-- Ruminal DM Digestibility --] 30% DM Haylage w/o HMC	+HMC	45% DM Haylage w/o HMC	+HMC
Yb	Ave. DM	41	40	43	35
	Ave. Dig	41	39	43	33
	Flow	41	39	42	32
Cr	Ave. DM	41	49	42	44
	Ave. Dig	39	48	40	43
	Flow	39	48	41	44

No statistically significant difference was seen between compositing methods. However, the two markers did give different digestibility estimates for the diets with added HMC. This indicates that compositing by using the individual flow rates as a weighting factor did not improve the estimate of digestibility for DM. A possible explanation for the lack of difference between the equal compositing method and the weighting method is the lack of precision of the flow measurement. If the scalars used to create a weighted average are not accurate a weighted average is no better than not weighting. Other methods of compositing the samples are

being investigated and hopefully will be more usefull.

In summary, it is evident that steady flow probably was not occuring and that the composition of the flow, out of the rumen changes thoughout the day. If this is a real phenomenon than some sort of weighting scheme is necessary. However, this study indicates that simply applying the flow rate to a weighting scheme may not be appropriate and give unsatisfactory results.

CONCLUSIONS

1. In experiment 1 haylage ensiled at 60 percent dry matter demonstrated signigicantly lower digestibiliy of dry matter, organic matter, nitrogen, acid detergent fiber and neutral detergent fiber than the haylage ensiled at 30 percent dry matter.

2. In experiment 2 haylages ensiled at different dry matter levels (30 and 45 percent) were not significantly different in digestibiliy for any of the components measured (DM, OM, N, ADF, ADIN). The addition of high moisture corn also did not significantly alter the digestibility of any of the components measured. However, it appeared (not significant) that the addition of high moisture corn did alter the site of digestion of the components in the diet.

3. In experiment 3 it was demonstrated that doudenal digesta flow was not in steady state in Holsteins fed alfalfa haylage diets two times per day. However, no difference in the estimate of digestion obtained by the marker ratio could demonstrated when compositing by average digesta or by weighting the composite based on flow rate.

APPENDIX

APPENDIX TABLE 1
Composition of Feed, Duodenal and Fecal Samples
(Block 1, Experiment 1)

SAMPLE #	% ASH	Yb Cnc.	% Nit.	% ADF	% NDF
30% DM A.H.	12.90	0.00	2.00	46.26	59.50
60% DM A.H.	14.91	0.00	2.39	48.46	56.10
Anim.-Diet		Duodenal Samples			
#2 - B	18.40	263.74	3.27	26.67	34.90
#3 - A	21.60	322.80	3.57	23.26	31.50
#4 - A	17.70	321.13	3.26	29.96	40.50
		Fecal Samples			
#2 - B	9.40	383.90	2.07	49.98	61.00
#3 - A	12.10	399.60	1.72	52.94	61.20
#4 - A	12.40	430.40	1.81	52.53	60.00

Diet A is 30 % DM haylage. Yb intake = 1.162g/day
Diet B is 60 % DM haylage. Yb intake = 1.972g/day

APPENDIX TABLE 2
Composition of Feed, Duodenal and Fecal Samples
(Block 2, Experiment 1)

SAMPLE #	% ASH	Yb Cnc.	% Nit.	% ADF	% NDF
30% DM A.H.	13.26	0.00	2.12	45.50	54.30
60 DM A.H.	14.59	0.00	2.33	43.40	53.40
Anim.-Diet		Duodenal Samples			
#1 - A	18.70	125.90	3.99	21.20	33.10
#2 - A	22.50	138.08	4.11	20.48	27.80
#3 - B	22.50	172.80	4.27	15.11	20.10
#4 - B	17.30	171.10	4.06	22.59	32.20
		Fecal Samples			
#1 - A	11.10	223.50	2.08	50.70	58.56
#2 - A	10.70	221.60	2.04	50.76	58.77
#3 - B	9.50	262.40	2.05	48.43	60.50
#4 - B	10.40	247.90	2.16	46.77	58.77

Diet A is 30 % DM haylage. Yb intake = .927g/day
Diet B is 60 % DM haylage. Yb intake = 1.779g/day

APPENDIX TABLE 3
Composition of Feed, Duodenal, Ileal and Fecal Samples. (Block 1, Experiment 2)

SAMPLE #	% ASH	Yb Cnc.	% Nit.	% ADF	% ADL	% ADIN
30% DM A.H.	10.37	0.00	1.96	43.42	5.47	0.00
45% DM A.H.	9.20	0.00	2.44	40.86	5.08	0.00
HMC	1.47	0.00	1.65	0.00	0.00	0.00
Animal-Diet		Duodenal Composite Samples				
1-A	20.91	931.18	2.55	38.11	9.94	0.00
2-B	16.56	863.12	3.17	19.44	5.20	0.00
3-C	20.84	976.46	3.80	25.91	10.00	0.00
4-D	13.35	1019.08	3.55	22.66	7.71	0.00
		Ileal Composite Samples				
1-A	21.05	910.31	2.60	26.93	8.19	0.00
2-B	15.81	1262.53	2.65	27.53	7.95	0.00
3-C	13.84	1160.13	2.56	40.27	16.77	0.00
4-D	13.28	1597.19	2.66	32.30	11.68	0.00
		Fecal Composite Samples				
1-A	10.49	1256.86	1.79	43.80	13.32	0.00
2-B	9.30	1605.61	2.37	36.46	11.59	0.00
3-C	8.65	1406.37	2.88	41.37	17.20	0.00
4-D	7.97	1772.49	3.16	36.43	14.50	0.00

DietA= 30%DM Haylage. Yb Intake=2.477 g/day
DietB= 30%DM Haylage + HMC. Yb Intake=2.477 g/day
DietC= 45%DM Haylage. Yb Intake=2.444 g/day
DietD= 45%DM Haylage + HMC. Yb Intake=2.444 g/day

APPENDIX TABLE 4.
Composition of Feed, Duodenal,Ileal and Fecal Samples. (Block 2, Experiment 2)

SAMPLE #	% ASH	Yb Cnc.	% Nit.	% ADF	% ADL	% ADIN
30% DM A.H.	9.18	0.00	1.93	42.76	6.23	0.00
45% DM A.H.	9.30	0.00	2.36	42.69	6.07	0.00
HMC	1.42	0.00	1.57	0.00	0.00	0.00
Animal-Diet	Duodenal Composite Samples					
1-D	15.34	745.20	2.97	15.34	4.04	0.00
2-C	29.51	942.16	2.99	29.51	9.06	0.00
3-B	22.00	895.33	2.84	22.00	5.91	0.00
4-A	30.72	857.02	2.27	30.72	8.57	0.00
	Ileal Composite Samples					
1-D	15.94	793.42	2.00	26.82	7.97	0.00
2-C	12.93	1045.75	2.09	29.79	8.97	0.00
3-B	12.01	1423.38	1.89	26.48	7.56	0.00
4-A	12.59	1076.30	1.60	34.36	10.42	0.00
	Fecal Composite Samples					
1-D	6.01	1375.80	2.23	29.04	8.50	0.00
2-C	6.46	1280.68	2.07	33.60	11.40	0.00
3-B	7.22	1469.24	1.82	32.14	9.25	0.00
4-A	7.98	1224.62	1.55	37.77	11.37	0.00

DietA= 30%DM Haylage. Yb Intake=2.934 g/day
DietB= 30%DM Haylage + HMC. Yb Intake=2.934 g/day
DietC= 45%DM Haylage. Yb Intake=2.509 g/day
DietD= 45%DM Haylage + HMC. Yb Intake=2.509 g/day
PEG Infusion Animal 1 = 445.10 g/day
PEG Infusion Animal 2 = 463.92 g/day
PEG Infusion Animal 3 = 468.00 g/day
PEG Infusion Animal 4 = 459.95 g/day

APPENDIX TABLE 5.
Composition of Feed, Duodenal, Ileal and Fecal Samples. (Block 3, Experiment 2)

SAMPLE #	% ASH	Yb Cnc.	% Nit.	% ADF	% ADL	% ADIN
30% DM A.H.	8.73	0.00	2.06	41.76	6.16	0.00
45% DM A.H.	8.96	0.00	2.26	41.82	6.06	0.00
HMC	1.27	0.00	1.63	0.00	0.00	0.00
Animal-Diet		Duodenal Composite Samples				
2-A	17.70	913.61	2.32	30.78	9.91	0.00
3-B	15.52	763.61	2.71	18.98	4.62	0.00
4-C	17.73	796.39	3.05	28.07	8.66	0.00
		Ileal Composite Samples				
2-A	12.05	1073.36	1.48	34.61	10.93	0.00
3-B	16.61	717.10	1.60	18.98	4.76	0.00
4-C	11.70	1013.30	1.83	34.19	11.14	0.00
		Fecal Composite Samples				
2-A	7.51	1273.25	1.52	38.04	11.09	0.00
3-B	6.18	1299.35	1.62	33.85	8.95	0.00
4-C	7.45	1161.28	1.97	37.87	11.90	0.00

DietA= 30%DM Haylage. Yb Intake=2.860 g/day
DietB= 30%DM Haylage + HMC. Yb Intake=2.860 g/day
DietC= 45%DM Haylage. Yb Intake=2.558 g/day
PEG Infusion Animal 2 = 483.64 g/day
PEG Infusion Animal 3 = 528.58 g/day
PEG Infusion Animal 4 = 478.86 g/day

APPENDIX TABLE 6.
Composition of Feed, Duodenal, Ileal and Fecal Samples. (Block 4, Experiment 2)

SAMPLE #	% ASH	Yb Cnc.	% Nit.	% ADF	% ADL	% ADIN
30%DM Haylg.	9.14	0.00	2.04	42.48	6.43	0.00
45%Dm Haylg.	9.53	0.00	2.09	46.51	7.77	0.00
HMC	1.37	0.00	1.56	0.00	0.00	0.00
Animal-Diet		Duodenal Composite Samples				
1-B	15.26	726.20	2.95	23.13	6.13	0.00
2-C	14.88	699.14	2.23	33.30	10.41	0.00
3-D	14.11	691.21	2.72	15.75	6.52	0.00
4-A	16.44	851.16	2.33	29.50	9.62	0.00
		Ileal Composite Samples				
1-B	11.44	646.31	1.63	27.72	7.32	0.00
2-C	17.83	724.56	3.08	23.24	7.28	0.00
3-D	10.23	897.57	1.95	20.98	6.94	0.00
4-A	11.59	1052.89	1.55	30.89	9.42	0.00
		Fecal Composite Samples				
1-B	6.48	807.22	1.68	30.21	8.68	0.00
2-C	8.51	982.56	1.56	37.47	10.93	0.00
3-D	5.93	1013.91	2.00	25.22	7.98	0.00
4-A	8.21	1064.78	1.51	32.01	10.15	0.00

DietA= 30%DM Haylage. Yb Intake=2.720 g/day
DietB= 30%DM Haylage + HMC. Yb Intake=2.720 g/day
DietC= 45%DM Haylage. Yb Intake=2.410 g/day
DietD= 45%DM Haylage + HMC. Yb Intake=2.410 g/day
PEG Infusion Animal 1 = 463.47 g/day
PEG Infusion Animal 2 = 488.06 g/day
PEG Infusion Animal 3 = 278.29 g/day
PEG Infusion Animal 4 = 579.17 g/day

APPENDIX TABLE 7.
Doudenal Digesta Composition. Animal 1, Block 2
(Experiment 3)

Sample Time	PEG Cnc. (mg/100ml)	I.R (ml/min)	Dig.DM (%)	DM (%)	Yb Cnc. (mg/g)	Cr Cnc. (mg/g)	N Cnc. (%)	ADF Cnc. (%)	ADL Cnc. (%)	ASH Cnc. (%)
7:00am	248.00	2.70	4.73	96.30	554.00	23.58	3.10	15.17	5.03	13.66
9:00am	437.55	2.76	5.45	97.81	546.90	26.65	3.06	22.00	6.77	12.97
11:00am	240.30	2.70	3.61	97.65	425.00	29.80	1.85	9.97	4.59	8.06
1:00pm	351.60	3.01	3.86	96.66	308.20	30.58	1.85	10.99	3.21	6.94
3:00pm	422.40	2.76	4.12	96.07	563.26	27.75	3.33	10.60	3.83	16.98
5:00pm	488.10	2.68	5.30	96.80	563.30	28.60	3.12	18.03	5.72	13.25
7:00pm	273.20	2.70	3.63	97.17	575.00	21.40	3.66	8.18	3.68	17.66
9:00pm	454.70	2.76	5.92	98.03	467.80	24.30	2.78	16.03	5.22	11.66
11:00pm	472.90	2.76	3.67	96.56	547.80	24.76	3.24	12.51	4.48	16.40
1:00am	467.90	2.68	6.84	96.55	399.00	31.76	2.37	16.60	4.76	9.63
3:00am	346.50	2.70	7.38	96.72	378.70	30.04	2.35	16.39	4.48	8.65
5:00am	599.40	2.76	4.73	95.72	537.00	27.02	2.88	18.34	6.06	13.30

Concentration of Infusate = 112.50 g/l

APPENDIX TABLE 8.
Doudenal Digesta Composition. Animal 2, Block 2
(Experiment 3)

Sample Time	PEG Cnc. (mg/100ml)	I.R (ml/min)	Dig.DM (%)	DM (%)	Yb Cnc. (mg/g)	Cr Cnc. (mg/g)	N Cnc. (%)	ADF Cnc. (%)	ASH Cnc. (%)
7:00am	643.50	2.56	5.25	96.17	755.20	26.03	2.97	28.34	18.71
9:00am	508.20	2.77	5.82	94.09	644.40	31.95	2.46	33.31	18.44
11:00am	503.40	2.60	4.23	96.64	625.40	30.77	2.84	27.70	15.20
1:00pm	788.40	2.70	5.27	96.46	677.50	26.94	2.92	26.73	18.61
3:00pm	525.60	2.77	4.99	95.89	654.50	29.82	2.90	25.08	15.16
5:00pm	500.50	2.60	4.57	96.07	733.80	30.68	3.10	24.11	16.75
7:00pm	339.10	2.60	4.74	96.75	676.60	26.01	2.96	27.77	16.46
9:00pm	560.40	2.77	5.08	95.74	601.80	30.17	2.70	28.95	14.39
11:00pm	566.20	2.77	5.09	95.66	679.10	31.46	2.68	30.70	14.53
1:00am	324.60	2.70	4.62	95.47	536.10	30.52	2.78	29.27	15.58
3:00am	923.70	2.62	4.91	96.38	593.40	25.47	2.61	25.13	14.35
5:00am	923.70	2.77	5.85	97.08	493.50	27.44	2.39	27.77	21.32

Concentration of Infusate = 119.95 g/l

APPENDIX TABLE 9.

Doudenal Digesta Composition. Animal 3, Block 2
(Experiment 3)

Sample Time	PEG Cnc. (mg/100ml)	I.R (ml/min)	Dig.DM (%)	DM (%)	Yb Cnc. (mg/g)	Cr Cnc. (mg/g)	N Cnc. (%)	ADF Cnc. (%)	ASH Cnc. (%)
7:00am	582.50	2.73	5.60	97.22	563.40	24.04	2.56	19.53	13.88
9:00am	431.50	2.87	5.30	95.40	404.70	22.65	2.60	17.39	13.63
11:00am	442.10	2.70	4.29	95.33	445.60	30.22	2.72	21.24	16.94
1:00pm	406.20	2.98	4.87	95.08	420.50	29.93	2.44	22.92	15.38
3:00pm	529.70	2.87	5.80	95.19	397.50	29.63	2.38	21.71	11.98
5:00pm	545.60	2.73	5.24	95.04	424.60	23.82	2.56	20.77	13.06
7:00pm	355.50	2.98	4.93	96.11	443.80	22.67	2.92	18.97	15.40
9:00pm	492.80	2.87	4.99	94.90	402.80	22.19	3.16	16.61	13.93
11:00pm	503.30	2.87	4.31	95.45	475.10	32.91	3.34	21.83	14.13
1:00am	507.00	2.80	6.72	94.85	395.10	35.72	2.88	16.59	16.82
3:00am	511.80	2.78	5.81	95.25	437.20	29.03	2.24	20.62	11.17
5:00am	540.30	2.87	4.29	95.32	689.00	26.34	2.60	23.33	12.90

Concentration of Infusate = 114.55 g/l

APPENDIX TABLE 10.
Doudenal Digesta Composition. Animal 4, Block 2
(Experiment 3)

Sample Time	PEG Cnc. (mg/100ml)	I.R (ml/min)	Dig.DM (%)	DM (%)	Yb Cnc. (mg/g)	Cr Cnc. (mg/g)	N Cnc. (%)	ADF Cnc. (%)	ASH Cnc. (%)
7:00am	443.10	2.63	3.98	95.27	622.40	24.02	2.38	60.66	19.92
9:00am	401.00	2.82	4.29	95.71	552.70	22.57	2.26	63.94	19.45
11:00am	384.30	2.67	4.11	94.91	552.80	24.39	2.16	66.39	16.73
1:00pm	470.50	3.20	4.97	95.48	468.00	25.36	2.06	71.22	13.46
3:00pm	470.50	2.82	5.22	95.14	474.10	27.34	2.00	71.20	14.25
5:00pm	448.00	2.61	4.67	95.91	530.90	23.45	2.20	63.32	15.14
7:00pm	477.40	2.63	5.60	96.18	569.90	23.81	2.13	68.48	15.66
9:00pm	531.20	2.82	4.40	95.16	575.00	21.43	2.34	65.09	16.41
11:00pm	492.10	2.82	4.85	94.89	540.00	25.89	2.16	66.18	15.80
1:00am	459.70	2.63	4.51	95.39	538.60	27.79	2.28	65.56	17.51
3:00am	296.20	2.67	5.47	94.90	536.00	26.68	2.20	68.66	15.23
5:00am	888.70	2.82	4.71	95.22	557.30	26.32	2.28	67.19	14.65

Concentration of Infusate = 115.55 g/l

APPENDIX TABLE 11.
Doudenal Digesta Composition. Animal 1, Block 3
(Experiment 3)

Sample Time	Digesta PEG Cnc. (mg/100ml)	I.R (ml/min)	Digesta DM (%)	Sample [-- Freeze Dried --- DM (%)	Yb Cnc. (mg/g)	Cr Cnc. (mg/g)
7:00am	379.77	2.56	4.96	95.01	610.00	3.50
9:00am	265.85	2.56	4.16	95.00	641.60	3.82
11:00am	478.84	2.56	5.01	94.88	619.80	3.85
1:00pm	488.75	2.56	3.46	95.27	651.80	4.93
3:00pm	518.47	2.65	3.88	95.34	653.60	5.44
5:00pm	478.84	2.70	5.22	95.14	579.00	3.62
7:00pm	531.35	2.56	5.44	95.39	538.40	4.19
9:00pm	419.40	2.56	4.06	95.19	603.10	4.08
11:00pm	617.51	2.70	6.56	95.42	501.20	3.88
1:00am	434.26	2.56	3.83	95.25	546.90	4.88
3:00am	444.14	2.56	3.88	95.24	581.30	5.07
5:00am	459.03	2.70	4.75	95.20	598.90	3.78

Concentration of Infusate = 129.10 g/l

APPENDIX TABLE 12.
Doudenal Digesta Composition. Animal 3, Block 3
(Experiment 3)

Sample Time	Digesta PEG Cnc. (mg/100ml)	I.R (ml/min)	Digesta DM (%)	Sample [-- Freeze Dried --- DM (%)	Yb Cnc. (mg/g)	Cr Cnc. (mg/g)
7:00am	502.10	2.58	3.77	94.67	539.90	4.81
9:00am	406.20	2.42	5.39	95.34	542.10	4.37
11:00am	483.10	2.58	3.75	95.24	506.40	4.25
1:00pm	416.60	2.58	4.15	95.13	496.20	4.85
3:00pm	549.50	2.50	5.42	95.11	445.30	4.50
5:00pm	397.60	2.51	5.04	95.33	385.80	4.74
7:00pm	584.70	2.58	5.59	95.20	569.50	4.14
9:00pm	409.00	2.42	4.39	95.53	539.70	4.50
11:00pm	466.00	2.51	5.52	95.00	515.90	4.61
1:00am	480.20	2.58	5.15	95.00	515.90	4.63
3:00am	470.80	2.42	4.86	95.29	582.30	4.50
5:00am	530.60	2.51	5.11	94.97	551.80	4.07

Concentration of Infusate = 146.04 g/l

APPENDIX TABLE 13.
Doudenal Digesta Composition. Animal 4, Block 3
(Experiment 3)

Sample Time	Digesta PEG Cnc. (mg/100ml)	I.R (ml/min)	Digesta DM (%)	Sample [-- Freeze Dried --- DM (%)	Yb Cnc. (mg/g)	Cr Cnc. (mg/g)
7:00am	371.50	2.55	4.70	95.67	643.30	4.81
9:00am	298.50	2.41	3.93	96.37	589.80	4.37
11:00am	345.60	2.55	4.06	95.00	519.20	4.25
1:00pm	600.00	2.55	3.80	96.32	541.50	4.86
3:00pm	465.90	2.53	4.53	95.09	604.20	5.38
5:00pm	418.00	2.54	4.82	96.16	599.60	4.74
7:00pm	505.70	2.55	5.25	96.10	606.00	4.14
9:00pm	465.90	2.41	4.53	95.75	590.70	4.17
11:00pm	411.50	2.54	4.73	96.04	521.50	4.62
1:00am	388.00	2.55	4.48	96.37	527.50	4.63
3:00am	465.90	2.41	4.53	96.64	576.00	4.67
5:00am	854.30	2.54	5.04	95.62	564.10	4.07

Concentration of Infusate = 132.50 g/l

APPENDIX TABLE 14
Doudenal Digesta Composition. Animal 1, Block 4
(Experiment 3)

Sample Time	Digesta PEG Cnc. (mg/100ml)	I.R (ml/min)	Digesta DM (%)	Sample [-- Freeze Dried --- DM (%)	Yb Cnc. (mg/g)	Cr Cnc. (mg/g)
7:00am	512.60	2.56	5.77	95.92	741.53	5.48
9:00am	584.80	2.60	5.11	96.29	724.97	5.97
11:00am	555.10	2.56	3.82	95.81	644.97	5.04
1:00pm	555.10	2.59	5.24	95.85	682.97	4.33
3:00pm	654.00	2.70	5.12	95.71	697.67	8.43
5:00pm	649.00	2.54	5.19	96.22	742.70	5.84
7:00pm	565.00	2.59	5.24	95.93	589.40	4.01
9:00pm	520.00	2.70	4.53	95.90	776.53	5.91
11:00pm	555.10	2.54	4.47	96.33	646.57	5.70
1:00am	540.30	2.56	4.08	96.55	548.57	5.77
3:00am	537.40	2.60	5.28	95.99	649.13	7.84

Concentration of Infusate = 124.05 g/l

APPENDIX TABLE 15.
Doudenal Digesta Composition. Animal 2, Block 4
(Experiment 3)

Sample Time	Digesta PEG Cnc. (mg/100ml)	I.R (ml/min)	Digesta DM (%)	Sample [-- Freeze Dried --- DM (%)	Yb Cnc. (mg/g)	Cr Cnc. (mg/g)
7:00am	672.80	2.50	6.36	94.78	667.80	7.37
9:00am	619.00	2.49	5.35	95.19	651.93	6.68
11:00am	881.00	2.50	5.09	94.92	763.47	6.56
1:00pm	576.40	2.49	5.50	94.96	626.73	7.99
3:00pm	657.60	2.58	4.89	95.22	657.07	8.71
5:00pm	738.80	2.52	5.84	94.97	637.70	7.17
7:00pm	688.10	2.49	5.14	95.04	707.93	7.92
9:00pm	698.10	2.58	6.02	95.18	617.63	8.23
11:00pm	624.10	2.52	4.32	95.46	665.47	6.31
1:00am	611.90	2.50	7.05	94.81	560.23	7.56
3:00am	591.60	2.49	4.89	95.00	618.57	8.35

Concentration of Infusate = 134.87 g/l

APPENDIX TABLE 16
Doudenal Digesta Composition. Animal 3, Block 4
(Experiment 3)

Sample Time	Digesta PEG Cnc. (mg/100ml)	I.R (ml/min)	Digesta DM (%)	Sample [-- Freeze Dried --- DM (%)	Yb Cnc. (mg/g)	Cr Cnc. (mg/g)
7:00am	423.90	2.45	4.91	95.45	728.47	5.74
9:00am	473.80	2.45	4.96	95.74	694.87	5.89
11:00am	614.50	2.45	4.22	95.75	727.53	5.49
1:00pm	417.13	2.47	5.62	95.05	469.47	6.99
3:00pm	621.60	2.52	4.62	95.34	535.97	6.68
5:00pm	711.00	2.48	4.03	95.81	731.03	4.92
7:00pm	358.00	2.47	4.64	94.87	640.27	5.99
9:00pm	461.50	2.52	4.60	95.01	490.93	6.95
11:00pm	663.90	2.48	3.97	95.92	752.97	4.92
1:00am	680.90	2.45	4.31	94.69	681.33	6.46
3:00am	454.00	2.45	4.00	94.86	642.60	6.44

Concentration of Infusate = 78.27 g/l

APPENDIX TABLE 17.
Doudenal Digesta Composition. Animal 4, Block 4
(Experiment 3)

Sample Time	Digesta PEG Cnc. (mg/100ml)	I.R (ml/min)	Digesta DM (%)	Sample [-- Freeze Dried --- DM (%)	Yb Cnc. (mg/g)	Cr Cnc. (mg/g)]
7:00am	709.60	2.69	5.42	95.00	848.25	6.39
9:00am	603.40	2.72	5.13	95.00	752.27	6.51
11:00am	593.30	2.69	4.70	95.00	929.13	5.42
1:00pm	805.70	2.69	4.74	95.00	831.13	8.00
3:00pm	659.70	2.76	5.33	95.00	754.83	6.43
5:00pm	679.30	2.87	5.11	95.00	809.20	8.04
7:00pm	775.30	2.69	6.10	95.00	759.03	8.16
9:00pm	765.20	2.76	5.01	95.00	785.87	5.93
11:00pm	581.10	2.87	4.93	95.00	759.73	7.34
1:00am	578.10	2.69	5.38	95.00	773.50	7.34
3:00am	522.50	2.72	3.82	95.00	705.13	7.93

Concentration of Infusate = 146.70 g/l

APPENDIX FIGURE 1.
PEG Analysis (Carbowax 4000)
[Malwar and Powell, 1967]

Reagents Needed:

1. Standard solutions 300-1100 mg PEG/100 ml
2. 10 % (w/v) anhydrous $BaCl_2$ solution
3. 0.3 N $Ba(OH)_2$ solution
4. 5 % $ZnSO_4 \cdot 7H_2O$ solution [a]
5. Gum arabic solution (conc. 2 - 12 mg/l)
6. 30 % TCA + 5 % $BaCl_2$ solution

Element	Symbol	Molecular Wt.
Barium	Ba	137.4
Chlorine	Cl	35.5
Zinc	Zn	65.4
Sulfur	S	32.1

Procedure (Step wise)

1. To a 50 ml Erlenmeyer add: Swirl after each addition
   a. 1 ml - sample, standard or blank solution
   b. 10 ml H_2O
   c. 1 ml 10 % $BaCl_2$ solution
   d. 2 ml 0.3 N $Ba(OH)_2$ solution
   e. 2 ml 5 % $ZnSO_4$ solution
2. Cap with parafilm and shake vigorously.
3. Let stand for 10 min. then filter through double thick Wattman # 42.
4. Transfer 1 ml of filtrate to 16 X 150 mm test tube.
5. Add 3 ml of gum arabic solution and aggitate gently.
6. Add 4 ml 30 % TCA - 5 % $BaCl_2$ solution, cap with parafilm and immediately invert 5 times.
7. 60 - 90 min. later read O.D. on a Beckman DU spectrophotometer at 650 mu and slit width of 0.04 mm.

[a] gum arabic concentration will affect O.D. readings, therefore, it is necessary to determine the optimum concentration of gum arabic which will give the maximum O.D. readings under the conditions of the experiment and the PEG concentration range found in samples.

APPENDIX FIGURE 2.
Preparation of Cr:EDTA
[Binnerts et.al.,1968 (adaptation)]

Reagents needed:

1. $CrCl_3 - 6H_2O$
2. EDTA (free acid form)
3. NaOH pellets
4. $CaCl_2$

Procedure (step wise):

Utilizing a 6 l Erlenmeyer flask on a stirring/hot plate.

1. To 4 l of H_2O add 400 g EDTA.

2. Heat to boiling.

3. To hot solution add 284 g $CrCl_3 - 6H_2O$.

4. Very carefully add 100 g NaOH to hot solution
   - the addition of NaOH may result in an extreamly vigorous boil thus exercise extream caution !!

5. Bring solution to a boil and maintain for 1 hour.
   - or untill volume has returned to 4 l.

6. Carefully add to solution 25 g $CaCl_2$.

7. Cool to near room temperature.

8. If pH of solution is less than 5 add additional NaOH to bring pH up. If white precipitate appears add HCl to solution untill dark purple color returns.

APPENDIX FIGURE 3.
Yb and/or Cr Analysis

Reagents Needed

1. KCl solution (1000 mg K/l)
   - if analyzing for Cr only distilled water can be used
2. Concentrated nitric acid (HNO_3)
3. Concentrated perchloric acid ($HClO_4$)
4. Standard solutions (1 - 5 ug Yb and/or Cr/ml)

Procedure

1. Number and record weight of 250 ml Phillips beaker

2. Weigh into a beaker enough sample to contain 300 - 1000 mg Yb and/or Cr. Record weight.

3. Add concentrated nitric and perchloric acids as follows:
   - Low fat samples:
     - if sample wt. 1 g — 9 ml HNO_3 and 3 ml $HClO_4$
     - if sample wt. 1.0 - 1.5 g — 15 ml " " 5 " "
     - if sample wt. 1.5 - 2.0 g — 21 ml " " 7 " "
     - sample weights above 2 g can be digested but it is better to keep the levels of Yb and Cr high enough to keep the ash content of the digesiton solution low this allows the flame to run cleaner and minimizes clogging of the nitrous oxide head (following)
   - High fat samples require the use of more $HClO_4$.

4 Heat beakers on hot plate (high) untill red smoke appears then turn heat down slightly.
   - if samples foam excessively turn heat down more.

5. Digest untill white vapor appears (perchlorate is being driven off). Observe closely if sample is allowed to dry the perchlorate may explode! If charring occures blackening fo portions of solution) take off burner and allow sample to cool then add a small amount of HNO_3. Place back on burner and digest untill white vapor appears.

6. After appearance of white vapor allow digestion to continue for a few minutes (do not allow sample to dry)

7. Remove from burner and cool to room temperature.

8. Add approximately 200 ml of KCl solution
   -an easy way is to place beaker on a scale and add by weight (assume solution weighs 1 g/ml)
   -dilution factor = (beaker wt. + H_2O) - empty beaker wt.

9. Using a nitrous oxide flame on an atomic emmision spectrophotometer read at wave length of 425.4 nm for Cr and 398.8 nm for Yb. (slit width of 0.5 mm).

APPENDIX FIGURE 4.
Rare Earth Binding Procedure
(adapted from Oklahoma procedure)

This procedure has been used for Yb, Er and La.

1. Plaae feed in plastic garbage can.
   - use can large enough to accomodate feed and water plus allow for swelling of feed if using grains.
2. Dissolve choride form of rare earth in distilled water.
3. Pour rare earth solution over feed in garbage can.
4. Completely immerse the feed with tap water and let soak.
5. After soaking over night, cover can with window type screening, invert and allow water to drain.
   - takes about 1 hour.
6. Completely immerse feed in water again and soak for at least 2 hours.
7. Invert can again and allow to drain.
8. Repeat steps 6 and 7.
9. Remove feed from can and allow to dry.
   - it is not recomended to dry in very hot oven as the digestion charateristics of the feed may be affected
   - we generally spread the feed on the ground (on a plastic tarp) in a warm room or in the sun and allow it to dry.
10. Marked feed is then divided into enough equal portions to provide sufficient marker for each days feeding.

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