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Evaluation of Markers for Determining Site and Extent of Digestion and Digesta Flow Patterns in Steers Fed Corn Silage Diets

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ABSTRACT

Evaluation of Markers for Determining Site and Extent of Digestion and Digesta Flow Patterns in Steers Fed Corn Silage Diets

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The effect of marker choice (lignin, ytterbium, lanthanum, chromium EDTA) upon estimates of site and extent of digestion of corn silage based diets was investigated. An abomasal infusion of polyethylene glycol (PEG) was utilized to evaluate digesta flow patterns and marker recovery at the duodenum. Superimposed upon these investigations was an evaluation of the effect of several supplemental protein sources (urea, soybean, corn gluten meal, wet distillers grains) upon digestion of major dietary constituents in steers fed corn silage based diets. Studies utilized Latin square, crossover designs with Holstein steers having duodenal (T) cannulae, or in addition, abomasal infusion cannulae. Estimates of apparent rumen digestibility of dietary constituents was significantly affected by marker selected. Lignin levels were elevated in duodenal samples, perhaps due to elevated acid detergent fiber levels. Estimates of total tract digestibility of dietary constituents do not vary significantly due to marker choice.

Fecal lignin recovery tended to be elevated (average, 110% of intake) and produced slightly higher digestibility estimates than other markers. Fecal chromium levels, when corrected for an estimated 5% absorption, provided estimates of digestibility similar to other markers. Ytterbium satisfies the requirements of an ideal marker in more respects than any other marker evaluated. Digesta flow patterns, estimated by PEG infusion, indicated the presence of a phasic pattern of duodenal flow of digesta. The pattern appeared to vary with supplemental protein source. Corn silage diets supplemented with urea, soybean and corn gluten meal exhibited nearly equal ruminal and total tract digestion of nitrogen, organic matter and acid detergent fiber. Corn silage diets supplemented with wet distillers grains exhibited reduced ruminal and total tract nitrogen availability as compared to diets supplemented with urea ans soybean meal.

DEDICATION

This thesis is dedicated in loving memory of my father, Alan Weber. My father's commitment to excellence in all aspects of life set a precedent I am proud to follow. I will always remember..."like sparks from flint and steel".

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INTRODUCTION

The ruminant occupies a unique niche in animal agriculture. Ruminants harness the cellulolytic and protein synthesis capabilities of the rumen microbes. They are therefore capable of utilizing diets composed of fibrous materials and low biological value proteins. As a result of the symbiotic relationship between rumen microbes and host, the ruminant animal can produce meat, milk, fiber and serve as beasts of burden without competing with humans for high quality dietary components. No other group of livestock occupy a position of such importance in animal agriculture.

Realistically, it is currently not possible to maximize the production of meat, milk and fiber without the incorporation of some high quality dietary components in ruminant diets. However, a thorough understanding of factors influencing the efficient utilization of dietary components by ruminants will allow maximum productivity with a minimum of competition for human food resources. Efforts to evaluate the efficiency of production in ruminants typically involves an evaluation of feed input versus product output; i.e. feed efficiency. It has long been understood that certain combinations of dietary components result in optimal animal performance whereas other combinations result in poorer performance than expected. The phenomena of negative

associative effects, where performance on a particular diet or digestibility of a diet are not as expected, does exist. Intuitively, situations must exist where combinations of dietary ingredients act synergistically and result in positive associative effects. The exact nature of negative or positive associative effects, with respect to digestibility, are most commonly attributed to changes in site and extent of digestion of dietary constituents.

Systems to estimate digestibility and nutrient flow through the digestive tract must be utilized to evaluate the interactions of dietary components upon the site and extent of digestion of the total diet.

Markers which are indigestible, nonabsorbable, do not influence digestibility of dietary components and can be analytically quantitated, are used to evaluate the site and extent of digestion as well as nutrient flow through the digestive tract.

The purpose of this research was to evaluate the use of various markers to estimate the site and extent of digestion of corn silage diets supplemented with several common protein sources. The specific goal was to more explicitly define and develop appropriate marker systems for the determination of site and extent of digestion in ruminants.

LITURATURE REVIEW

Markers in Ruminant Nutrition

There are literally hundreds of substances which can be used in ruminant nutrition as markers for flow and digestibility of dietary constituents. Kotb and Luckey (1972) provided an outstanding review of markers used in nutrition research. Table 1 lists a partial summary of several markers used in ruminant nutrition and the principal researchers involved with the markers.

In recent years, rare earth lanthanide series elements have been very popular for nutrition research. Kyker (1961) discussed the chemistry of rare earth elements. The chemistry of the rare earth elements dictates their acceptibility as markers for specific types of nutritional research.

The intent of this literature review is to discuss and emphasize the principles of marker studies. It is not the author's intent to evaluate the efficacy of every available element or compound for marker studies. An understanding of the principles pertaining to marker use is essential. The principles represent the foundation for decision making regarding the choice of markers used in nutrition research.

Marker Systems

Markers can be used to evaluate three general areas of interest to ruminant nutritionists. The three areas of marker application require one to make three sets of assumptions regarding specific criteria which are critical for marker derived data to be valid and reliable.

Three areas of marker application include; 1) Digestibility and nutrient intake estimates based on marker ratio techniques (Bergeim, 1926; Stanley and Cheng, 1957; Kleiber, 1961), 2) Estimation of the rate of passage of specific dietary components, particles or phases (Blaxter et al., 1956; Grovum and Williams, 1973b; Ellis et al., 1979), 3) Estimation of the contribution of microbial components to digesta flow (Siddens et al., 1982). These three areas provide researchers with detailed information. However, as stated earlier, each technique requires the establishment and adherence to a specific group of assumptions and analytical techniques. The use of markers of microbial flow were reviewed by Siddens et al. (1982). The use of microbial markers is not discussed in the literature review, although the general principles of digesta markers do apply to the use of microbial markers.

Marker Ratio Techniques

The selection of a suitable marker for use in digestibility studies requires an evaluation of the proposed marker in relation to several important criteria. Table 1 is a brief list of the more commonly used markers.

Specific criteria for marker ratio determination of digestibility have been discussed by Alvarez (1950), Engelhardt (1974) and Kotb and Luckey (1972). The important criteria for marker ratio digestibility studies requires that markers be:

1) Nonabsorbable
2) Inert, Indigestible
3) Flowing with the total digesta: steady state
4) Nontoxic to gut tissues or microbes
5) Noninterfering with respect to digestibility of dietary components
6) Analytically quantifiable by specific and sensitive means
7) Noninterfering with analysis of other dietary components

Few, if any, of the available substances can fulfill all of these criteria in all respects.

An evaluation of the importance of each assumption and the resultant effect on data when an assumption is violated is necessary to completely understand the role of markers in ruminant nutrition.

Table 1 Digestibility and Rate of Passage Markers

Marker	Phase	Absorption	Reference
PEG 4000	liquid	slight	Winne and Gorig, 1982 Clemens, 1982 Neudoerffer et al.,1982 Ulyatt, 1964 Kay, 1969
CrEDTA	liquid	10% 2% 5%	Corbet, 1981 Goodall and Kay, 1973 Faichney, 1975
Cr-51 EDTA	liquid	22%	Downes,McDonald, 1964 Uden et al., 1980
CoEDTA	liquid	3-28%	Uden et al., 1980
Phenol red	liquid	slight	Schedl et al., 1966 Miller,Schedl, 1970 Kunihara,Teruhiko, 1981
Chromic oxide	particles	?	Drennan et al., 1970 Wilkinson,Prescott, 1970 Prigge et al., 1981 Purser,Mori, 1966
Mg ferrite	particles	?	Neumark et al., 1975 Neumark et al., 1981
Lignin	solids	variable	Thonney et al., 1979 Balch, 1957 Galyean et al., 1979 Fahey and Jung, 1983
IADF (Indigestible acid detergent fiber)	solids	?	Waller et al., 1980 Penning,Johnson, 1983
AIA (Acid insoluble ash)	solids	?	Frape et al., 1982 Van Keulen,Young, 1977 Thonney et al., 1979 Furuichi,Takahashi, 1981
Chromium mordanted fiber	solids		Uden et al., 1980
Ru-103 Phenanthroline	particles		Tan et al., 1971 Warner, 1981
Cerium	particles/solids		Huston and Ellis, 1968 Ellis and Huston, 1967
Dysprosium	particles/solids		Ellis, 1968 Young et al., 1976
Lanthanum	particles/solids		Crooker et al., 1982 Stern et al., 1983
Samarium	particles/solids		Crooker et al., 1982
Ytterbium	particles/solid		Teeter et al., 1979 Prigge et al., 1981 Sklan et al., 1975

Nonabsorption of Markers

Utilization of the marker ratio technique for calculating digestibility of any dietary component requires that the marker utilized be nonabsorbable. This is a very important criteria. Absorption of marker from the digestive tract results in an increased estimate of nutrient flow and a concurrent underestimation of component digestibility.

Equation 1 illustrates the role of marker concentration of digesta samples (digesta= feces, ileal, duodenal or abomasal) and feed samples in calculation of nutrient flow through the digestive tract and consequently digestion of nutrients.

(1)

$$\left(\frac{(\text{Nutrient Conc.}^{A} / \text{Marker Conc.}^{B}) \text{ Ratio in Digesta Sample}^{C}}{(\text{Nutrient Conc.}^{A} / \text{Marker Conc.}^{B}) \text{ Ratio in Feed Sample}^{D}} \right)^{G}$$

1. $(\quad)^{G} \times 100 = \%$ Nutrient flow of Intake
2. $1 - (\quad)^{G} \times 100 = \%$ Nutrient Digestibility
3. $(\quad)^{G} \times$ Nutrient Intake = Nutrient Flow
4. $\dfrac{(\text{Nutrient Intake} - \text{Nutrient Flow})}{\text{Nutrient Intake}} \times 100 = \text{Digestibility}$

A) Nutrient concentration expressed as grams/kg of dry matter or other suitable measure

B) Marker concentration can be expressed as a percentage of dry matter or g, mg/kg of dry matter or other suitable measure

C) Digesta can be of abomasal, duodenal, ileal or fecal material

D) Feed could be replaced in this ratio by rumen, abomasal, duodenal or ileal data and nutrient flow and digestibility calculated from this point of origin to any site further down the digestive tract, i.e. duodenal to fecal, etc.

G) A ratio of ratios

It is clear a decline in the concentration of a marker in the digesta sample, in Equation 1, will result in elevated flow and concurrent depression in digestibility estimates for all components of interest.

Absorption of markers from the digestive tract is well documented. Absorption of low molecular weight components of PEG 4000 has been reported (Winne and Gorig, 1982). Chromium absorption from CrEDTA complexes has been observed by Corbett (1981), Goodall and Kay (1973) and Faichney (1975). Cobalt absorption from CoEDTA complexes has been reported by Uden et al. (1980).

Many researchers have adjusted marker concentration in digesta samples to account for absorption. Although this can theoretically be done, book values for marker absorption cannot be adapted to apply to all situations. Therefore, correction of marker concentration can only be done, with any significant degree of accuracy, by analysis of urinary excretion of marker and tissue retention. This would significantly increase the complexity of studies. It is obvious that markers which are not absorbable are to be used whenever possible.

Inert, Indigestible Markers

It should be noted that a marker need not be absorbed to create problems of over estimation of dietary nutrient flow. Alterations of marker concentration due to digestive processes or analytical problems with detection of markers in digesta can create errors in the estimation of nutrient flow and digestion.

The problem with alteration of marker concentration due to digestive processes may be of special significance when a marker such as lignin is used. Apparent digestibility, absorption and/or failure of gravimetric methodology to accurately detect lignin residues has been observed by Thonney et al. (1979) and Muntifering et al. (1981).

Marker and Digesta Flow

As indicated, an important assumption is that markers move with the total digesta. As a result, compositing of digesta from numerous samples will provide a representative sample of total digesta with true concentration of markers and nutrients. Unfortunately, numerous problems arise when digesta composites do not represent the true digesta which has moved through the digestive tract during the sampling period. Many researchers use a sampling sequence similar to the one described by Faichney (1980). With this technique,

twelve samples of digesta (abomasal, duodenal, ileal or fecal) are taken over a three day period, with 4 to 6 hours between sampling. These samples are then composited on an equal wet weight basis. This assumes that each sample represents an equal contribution, with respect to volume and content, to total daily digesta flow. This is the point at which many digesta passage and digestibility studies confront severe data evaluation problems. The existance of steady state digesta flow may not be valid under many conditions. It does appear that, with respect to fecal and perhaps ileal sampling, the assumption of steady state flow and therefore equal weight compositing may be adequate.

It has been well documented that repeated sampling and compositing of fecal samples is equal to total collection schemes for many species of livestock and nonfarm animals: Beef steers; Young et al., (1976), Thonney et al., (1979), Beef cows; Prigge et al., (1981), Sheep; VanKeulen and Young, (1977), Horses; Sutton et al., (1977), Rabbits; Furuichi and Takahashi, (1981).

Numerous studies have shown that abomasal or duodenal samples will not produce the same degree of accuracy when compared with total collection schemes. Corbett and Pickering (1983) have shown, in grazing sheep, marker and nutrient concentrations in abomasal samples varied with time of sampling . They observed a variation of plus or minus 30% from values calculated from a 24 hour mean concentration of marker. These data illustrate the presence of nonsteady flows in animals eating several meals each day while grazing.

Uden et al., (1980) has illustrated the different rates of passage of the liquid phase marker CoEDTA and a solid phase marker, chromium mordanted fiber. These data illustrate the existance of differential flow rates. Therefore, material moving with the liquid phase leaves the rumen at a more rapid rate than those materials moving with the solid phase.

As a result of these flow differences, the estimation of digestibility or flow can be grossly affected by marker choice. It would appear that compositing of duodenal or abomasal samples on an equal weight basis will not correct for the rapid movement of liquid phase markers and slower movement of solid phase markers. These observations coupled with the difficulty to collect representative samples of digesta passing abomasal or duodenal cannula led Faichney (1980a) to propose the two marker and double marker systems. Faichney (1980) discussed the work of Hogan and Weston (1967) who observed differences between samples collected from a simple cannula and digesta flowing past the cannula. This failure to acquire a representative sample is a problem, however, careful sampling may minimize the differences between samples and true digesta.

Nontoxic Markers

There is not a significant amount of information regarding the toxicity of markers to gut tissues, microflora or the whole animal. Toxicity of rare earth elements to

bacteria has been evaluated by Johnson and Kyker (1966). Toxic effects of ruthenium phenanthroline on rumen microbes is reported by Evans et al. (1977) and Beever et al. (1978).

Analytical Quantification of Markers

Evaluation of marker concentrations in digesta samples is a very important consideration. Table 2 illustrates the detection limits of several elements used in marker studies as summarized by Ellis et al. (1980).

Due to the nonspecific nature of gravimetric procedures for estimation of markers such as lignin, indigestible acid detergent fiber (Penning and Johnson, 1983) and acid-insoluble ash (Van Keulen and Young, 1977) analysis of each animal's feed, digesta and fecal samples must be conducted within an animal set, under the same laboratory conditions. With gravimetric methodology, laboratory conditions must be standardized. This includes standardization of reagents, lab temperature, reaction times and sample dry matter corrections. Evidence of the need for strict control of gravimetric analysis is discussed by Mueller (1956) with respect to lignin analysis and by Van Keulen and Young (1977) with respect to acid-insoluble ash analysis.

Table 2 Minimal Analytical Levels for Markers

Element	Procedure		
	Activation Analysis	Atomic Absorption	
	ug/sample	ug/ml[a]	ug/g[b]
Cobalt	.5	1	10
Chromium	1.5	2	20
Dysprosium	-	4	40
Lanthanum	.06	-	-
Ytterbium	.07	2	20

a) ug/ml aspirated or extracted
b) assumes 2g sample ashed, extracted by 20ml, analyzed in nitrous oxide supported flame

Marker Effect upon Digestibility of Dietary Constituents

When a marker is used to calculate the rate of passage of a particular feed component through the digestive tract, its effect on digestion must be considered. Theoretically, feed particles small enough to pass through the omasal orifice prior to digestion will not be affected by reduction in rate of digestion due to marker inhibition. Teeter et al. (1979) report no effect of marker upon dacron bag degradability of feedstuffs. However, Teeter et al. (1981) report that ytterbium did reduce digestibility (disappearance from dacron bags). Due to the role of digestion upon rate of passage of fibrous fed particles, reduction in digestibility

due to markers is more critical than with most protein sources. Uden et al. (1980) report increasing chromium content in mordanting fiber decreased digestibility of the material.

Marker Association and Movement

In order to calculate the rate of passage of specific fed components the marker must be closely associated with the feed components. Hartnell and Satter (1979) discuss the binding of several rare earth elements and marker movement to other feed components. The nature of rare earth binding characteristics has been discussed by Kyker (1961), Ellis and Huston (1968) and Ellis et al. (1979). In general, the marker should not move independently of the feed which it is to mark. Teeter et al. (1979) indicate that the method of marking feeds will affect the proportion of marker bound tenaciously to feedstuffs. There appear to be numerous sites on feed particles which bind rare earth elements. These sites have differing binding affinities. Ellis et al. (1983) discussed the presence of acid resistant binding sites on feedstuffs for ytterbium. These sites have very high affinities for ytterbium. When utilizing a marker for the calculation of rate of passage of a particular feed component, studies must be conducted or evaluation of existing data must be done to insure that the marker is actually moving with the particular feed component.

Estimation of Feed Component Rate of Passage

It is well known that the retention time of a feed component in the rumen is a key factor in determining the extent of digestion which can occur. Retention time is a function of liquid turnover for soluble and small feed components. Digestion rate is a key factor in determining the retention time of feed particles too large to leave the rumen through the omasal orifice.

Marker technology has been applied to the quantification of rate of passage of specific feed comonents. Calculation of the rate of passage of specific markers has been discussed in detail by Grovum and Phillips (1973), Grovum and Williams (1973b) and Grovum and Williams (1977).

Briefly, the rate of passage of a marked feed component or marked digesta phase can be calculated from pulse dose techniques. Assuming rules of kinetics apply to the passage of digesta through the digestive tract, mathematical analysis of flow as described by Shippley and Clark (1972) can be accomplished. A dose of marker placed in the rumen will move from the rumen at a rate proportional to the amount remaining. Samples of duodenal digesta can be acquired or feed samples can be taken sequentially over a period of several days to calculate the rate of decline of marker concentration. Multiple pool analysis using curve peeling techniques has been discussed by Grovum and Williams (1973a).

However for most marker studies a single pool model is suitable to reach conclusions regarding the flow of a marked feed particle or phase. The rate constant (k) is the fraction of marker or percent excreted per unit of time based on analysis of the semilog plot of the log of marker concentration in sequential samples versus time. Equation 2 can be used to calculate numerous attributes of digesta flow.

(2)

$$Y = Y_0 \cdot e^{-kt}$$

Y = concentration of marker in a sample at time t
Y_0 = concentration of marker in a sample at $t=0$

Equation 2 can be used to solve for rate constant (k) if only the time between two sampling periods and marker concentrations are known. Turnover time (T) for a marker can be calculated from Equation 3; this is also the mean retention time value.

(3)

$$T = 1/k$$

The half life of the marker in the digestive tract is calculated from Equation 4.

(4)

$$T_{1/2} = \frac{.693}{k}$$

Researchers have utilized marker technology to calculate the specific rate of passage of particular feed components. Uden et al. (1980) bound chromium to fiber components and followed their passage through the digestive tract. It is possible to couple rate of passage of marked feed components with dacron bag, in-situ, degradability studies described by Orskov et al. (1980) and Mehrez and Orskov (1976). With estimates of rate of feed component passage and rate of degradation within the rumen, estimates of feed components escaping rumen digestion can be acquired.

Several assumptions must be evaluated before data derived from marker rate of passage and dacron bag degradability studies are utilized. These assumptions include the following:

1) Markers must be closely associated with the particular component of interest.
2) Markers must not interfere with digestion of feed components, if digestion is required for passage through the omasal orifice.
3) Dacron bag degradability estimates must represent actual degradation of free floating components.

In-situ Dacron Bag Digestibility Studies

A key factor involved with coupling rate of passage data with digestion rate is the effect the dacron bag has on digestibility rates. Factors affecting the accuracy of dacron bag data have been evaluated by Mehrez and Orskov (1977). In general, a key problem exists in reference to coupling dacron bag data to passage data. One must assume

for purposes of calculation that disappearance of nitrogen and dry matter from nylon bags is synonymous with digestion. In reality, small particles may leave the dacron bag and are not necessarily digested and may pass to the lower tract intact.

An important consideration, which has not been discussed in detail in the literature, is the effect of diet upon digestibility of dietary ingredients. It appears protein source digestibility within the rumen may be a function of the other dietary components being fed. This raises questions with regard to digestibility estimates derived from dacron bag studies which may have been conducted in cattle fed diets which could alter protein degradability within the dacron bags. Research indicating the effect of diet upon dacron bag degradability of protein sources has been conducted by Loerch et al. (1983). In-situ degradability estimates may need to be studied with particular feed components evaluated in animals fed the feed components. Further research needs to be conducted to establish the nature of these problems.

Integration of Rate of Passage and Degradability Calculations

Orskov and McDonald (1979) and Orskov (1982) discuss the calculation of the amount of material remaining in dacron bags after a unit of time. Equation 5 illustrates the calculation for a feed component which has little or no rapid

release of water soluble or rapidly digestible material.

$$P = 100(1 - e^{-ct}) \quad (5)$$

Where P is the amount degraded after time "t" and "c" is the degradation rate for the component of interest.

Many feed components exhibit a complex digestion pattern. In essence, there are water soluble and rapidly degradable portions, slowly degraded portions and components which may never be degraded during their normal residence time in the rumen. This more complex type of digestion was discussed by Orskov and McDonald (1979). Equation 6 is used to describe digestion of a more complex nature.

$$P = a + b\,(1 - e^{-ct}) \quad (6)$$

In this equation, P is the amount degraded at time "t". In the equation, "a", "b" and "c" are constants where "a" represents the percent of feed rapidly degraded and "b" the remaining material which is degraded at a rate described by "c". In the equation, the sum of "a" and "b" cannot exceed 100% but the value of "a" + "b" is normally the percent digested when time nears infinity.

As discussed earlier, the rate of passage of labeled feeds can be coupled to estimates of rumen degradability and estimates of rumen outflow calculated. This technique is subject to the assumptions evaluated previously. As described by Ganev et al. (1979) and Orskov (1982), Equation 7 can be used to calculate the amount of a feed component degraded in the rumen after a unit of time.

$$P = a + \frac{(b \cdot c)}{(c + k)} (1 - e^{-(c+k)t}) \quad (7)$$

In this equation, as in 5 and 6, P is the percent of the feed component degraded after a unit of time "t". The unit "a" is the percent of feed components degraded rapidly, "b" the percentage of feed components degraded at rate "c" after time "t" and "k" is the fractional outflow rate of the feed component calculated from the pulse dose decay curve of marked feed. Essentially, after any significant length of time, the equation is most easily represented by Equation 8.

$$P = a + \frac{(b \cdot c)}{(c + k)} \quad (8)$$

The rate constant "k" can be calculated from pulse dose, sampling systems of marked feed components as discussed earlier. Broderick (1978) also discused the application of feed component movement through the digestive tract coupled with the rate of degradation within the rumen, to estimate a percent of feed component escaping rumen degradation. The following equation for estimated feed component escape from the rumen is illustrated in Equation 9.

$$\% \text{ escape} = [Kr/(Kr + Kd)] \times 100 \quad (9)$$

In the equation "Kr" is the rumen flow rate constant for the feed component and "Kd" is the rate constant for degradation in units per hour.

This calculation assumes a constant degradation rate constant for the feed components. Evaluation of the amount of a feed component leaving the rumen must be done with care.

One should consider the importance of the major assumptions listed earlier.

Double Marker Method

Faichney (1980a,b) described the calculation procedure for the double marker method as follows for samples separated into filtrate and filtrand by straining through Terylene cloth and utilizing a constant rumen infusion of both solid and liquid phase markers.

Equation 10 illustrates Faichney's reconstitution factor calculation. Marker concentrations are expressed as a fraction of marker infused each day per kg of digesta or filtrate, dry matter (DM) concentrations are expressed as a fraction of the original sample and nitrogen (N) concentrations were expressed as g/kg of digesta or filtrate. Concentration of markers, such as CrEDTA were corrected for absorption based on data of Faichney (1975).

The reconstitution factor (R) is the number of units of filtrate which must be added to or removed from one unit of digesta to obtain true digesta. Assuming the liquid and solid phase markers are in equilibrium within the rumen, by virtue of constant infusion, the reconstitution factor would be equal to 1 if the digesta samples (abomasal, duodenal, ileal or fecal) are representative of true digesta.

(10)

$$R = \frac{(\text{Conc. Digesta Solid Marker} - \text{Conc. Digesta Liquid Marker})}{(\text{Conc. Filtrate Liquid Mkr.} - \text{Conc. Filtrate Solid Mkr.})}$$

(11)

$$\text{True Digesta Marker Concentration} = \frac{\text{Conc. Digesta Liquid Mkr.} + (R \times \text{Filtrate Liquid Mkr. Conc.})}{1 + R}$$

(12)

$$\text{True Digesta Flow} = 1/\text{True Digesta Marker Conc.}$$

The flow of any dietary constituent is calculated by replacing marker concentrations in Equation 11 by the concentration of the constituent of interest. This value is then multiplied by the flow calculated with Equation 12.

The double marker method, in summary, corrects for difference in marker ratio (digesta solid phase marker to digesta liquid phase marker ratio should be equal to the infused ratios or ratios in the feed). This correction is necessary if a nonrepresentative sample of digesta is acquired due to simple cannula characteristics. The technique assumes that steady state conditions exist within the rumen as a result of feeding multiple meals through the day. In the discussion of the double marker method, Faichney does not evaluate the role of discrete intervals of water consumption which could easily disrupt steady state.

Two Marker Method

Faichney (1980) discussed a two marker method for the calculation of digesta flow. This method differs from the double marker method in that it is designed for studies where animals are fed only once or twice daily. In essence, steady state is not exhibited by Faichney's definition, however, both solid and liquid phase markers are constantly infused into the rumen. This method requires marker concentrations be adjusted for absorption anterior to the sampling site. Digesta samples may be composited on an equal weight basis to represent feeding cycles. The composited samples are filtered through Terylene cloth. The samples must not be centrifuged as can be done with the double marker method. The sample, after filtration, is divided into a liquid phase filtrate and a solid phase filtrand. It is proposed, by virtue of the differential movement of the filtrate and filtrand, that dietary constituents move with one phase or the other. Estimates of each phase rate of flow coupled with the content of dietary constituents within each phase could predict digesta flow accurately. The two marker method (Faichney, 1980a) includes the following calculations.

Digesta Sample Filtrate Fraction (FF)

FF= (1-Digesta DM) / (1-Filtrate DM)

Effective Infusion Fraction for Liquid Marker

Liquid Mkr.=(Filtrate Liquid Mkr.)/(Digesta Liquid Mkr.) x FF

Corrected Liquid Marker Concentration

Filtrate Flow = FF/Digesta Liquid Marker

Effective Infusion Fraction for Solid Marker

I=· 1- (Filtrate Solid Marker x Filtrate Flow)

$$\text{Filtrand Solid Marker} = \frac{[\text{Digesta Solid Marker} - (\text{FF} \times \text{Filtrate Solid Marker})]}{(1 - \text{FF}) \times \text{I}}$$

Filtrand Flow= 1/Filtrand Solid Marker

Digesta Flow= Filtrate Flow + Filtrand Flow

Calculation of Nutrient Flow

$$\text{Nitrogen Flow} = (\text{Filtrate N} \times \text{Filtrate Flow}) + \frac{[\text{Digesta N} - (\text{FF} \times \text{Filtrate N})]}{1 - \text{FF}} \times \text{Filtrate Flow}$$

The two marker method assumes that dietary constituents associated with the solid and liquid markers move with the marker fractions. Ellis and Huston (1968) and Faichney (1980b) have shown that digesta flow represents a complex mixture of different flow rates for different constituents. Liquid markers move the most rapidly followed by particulate

markers such as chromic oxide and ruthenium phenanthroline and the slowest movement is for lignin or other fiber associated markers. Mudgal et al.(1982) reported the retention time of lignin in the rumen was 2.1 to 2.3 times longer than ruthenium phenanthroline. Therefore, as indicated by the two marker method, materials moving with ruthenium phenanthroline would move more rapidly than those associated with lignin.

Effects of Cannulation Upon Digestion of Diets in Ruminants

It is a common practice to use cannulated animals in digestibility studies with ruminants. Several types of cannula are available for use with ruminants. An innovative re-entrant cannula design has been discussed by Ivan and Johnson (1981). A modified T-type cannula design has been developed and tested by Komarek (1981a and 1981b).

Investigations of the effects of cannulation on intestinal motility and digesta flow in ruminants have been conducted by Wenham and Wyburn (1980), Sissons and Smith (1982), Hayes et al. (1964) and Poncet et al. (1982). In general, surgical modification of the digestive tract alters flow of digesta. This is especially true of the re-entrant cannula as discussed by Wenham and Wyburn (1980). The re-entrant cannula appears to be the most troublesome of all cannulation procedures. Hayes et al. (1967) report that rumenal and abomasal fistulation did not alter digestibility

coefficients of their diets. Poncet et al. (1982) report the Y re-entrant cannula created marked reductions in the rate of digesta flow through the duodenum. The effect of slowing digesta movement might result in longer digesta retention throughout the digestive tract and alter digestibility estimates.

Digestibility of Corn Silage and Supplemental Protein Sources by Ruminants

The digestibility and utilization of components present in ruminant diets involves a complex interaction of level of intake, concentrate level, particle size, roughage source, nitrogen level and source and many other variables. The rumen environment represents the result of its microbial population interacting with and being selected by dietary ingredients. Within the rumen, the optimal situation would include prolific microbial growth resulting in maximal digestion of dietary roughage with a minimum digestion of high quality dietary proteins.

Data indicate that there is a range of rumen ammonia levels which maintain optimal growth of the microbial population. Pilgrim et al. (1970) indicated that 50 to 75 percent of the nitrogen present in rumen bacteria originated as ammonia. Satter and Slyter (1974) have reported optimal microbial growth occured at a theoretical rumen ammonia level of 5 mg/dl. Hume et al. (1970) observed maximum microbial growth in sheep occuring at ammonia concentrations of 9

mg/dl. In contrast to these data, Mehrez et al. (1977) report optimal microbial activity, not necessarily growth, occured at 23.5 mg/dl of rumen fluid.

Recent interest in bypass proteins has raised concerns that low rumen degradability of dietary proteins could result in levels of rumen ammonia too low for optimal digestion of dietary roughages. Wohlt et al. (1976) has provided data indicating that rumen solubility of dietary proteins is directly correlated to degradability. Wohlt et al. (1976) formulated a series of low and high solubility diets. When these diets were fed to sheep, the low solubility diets produced rumen ammonia levels of from 5 to 6.8 mg/dl. These levels of ammonia are near the minimum required for optimal microbial growth and certainly well below the level indicated by Mehrez et al. (1977) to provide optimal digestion. Data presented by Spears et al. (1980) and Sharma et al. (1972) indicate chemical treatment of protein sources can result in drastically low levels of rumen ammonia. Sharma et al. (1972) also reported calves fed formaldehyde treated rapeseed had reduced dry matter digestion.

There is limited data that selection of a low rumen degradable protein source for corn silage diets will reduce digestibility of the roughage components. Cottrill et al. (1982) report the inclusion of fish meal (a protein source of low rumen degradability) for urea in corn silage diets tended to reduce rumen organic matter digestion. As discussed by Bergen et al. (1974), some of the nitrogenous constituents of corn silage may not be readily available to rumen microbial

digestion and assimilation into microbial protein. These observations indicate there may be a need to provide some soluble proteins in corn silage diets to maintain adequate microbial digestion of dietary constituents. Soluble or readily degradable protein sources may also provide the alpha keto acids; phenylacetic, isobutyric, isovaleric and two methyl butyric acids which have been shown to be required by many cellulolytic bacteria for protein synthesis (Hume et al., 1970; Bryant and Doetsch, 1955 and Cline et al., 1966). These alpha keto acids are carboxylated and aminated to form phenylalanine, valine, leucine and isoleucine respectively (Allison et al., 1966 and Allison and Bryant, 1963).

Factors Affecting the Digestibility of Supplemental Protein Sources

As discussed earlier, the digestibility of supplemental protein sources may affect the extent of digestion of dietary roughage sources. Protein sources which are resistant to microbial proteolytic activity have been labeled bypass protein sources. Many researchers regret the popularity of the phrase "bypass protein" since these protein sources are more appropriately "microbial protease resistant proteins" since they do not actually bypass the rumen.

The importance of bypass protein sources in ruminant diets has been discussed by Chalupa (1975) and Clark (1975). Many researchers have studied animal performance in relation to bypass protein supplementation. Klopfenstein et al.

(1976, 1978) have conducted numerous experiments and reported data evaluating protein sources and animal performance.

Rock et al. (1983) reported steers fed corn gluten meal, as the only supplemental protein source, gained slower than steers fed dehydrated alfalfa. Ruminants fed bypass protein sources, such as corn gluten meal, benefit from the addition of urea as a source of supplemental, readily degradable nitrogen for microbial protein synthesis and growth. This response is referred to as a "complementary" effect by Klopfenstein et al. (1978). This effect may also be described as a positive associative effect. These observations are also supported by Cottrill et al. (1982) where increasing level of fish meal, a "microbial protease resistant protein" replacing urea resulted in a decrease in digestibility of organic matter in the rumen. It is clear that one must formulate a diet to provide nitrogen for maximum microbial growth and sufficient total nitrogen flow to to the lower tract to support optimal animal performance.

Protein Chemistry

The availability of nitrogen from supplemental protein sources for microbial growth is directly related to the chemistry of proteins, including their secondary and tertiary structures. The chemistry of cereal proteins determines the potential for degradation by microbial proteases. Brohult and Sandegren (1954) have discussed the chemical nature of cereal proteins.

Cereal proteins are made up of combinations of four general classes of proteins. These classes include: albumin, globulin, prolamin and glutelin fractions. Table 3 illustrates the composition of several cereal proteins.

Table 3 Protein Content of Cereal Grains[1]

Item	% Crude Protein (dry basis)	Protein Fractions, % of Total Protein			
		Albumin	Globulin	Prolamin	Glutelin
Corn	7-13	+/-	5-6	50-55	30-45
Barley	10-16	+/-	10-20	35-40	35-45
Wheat	10-15	3-5	6-10	40-50	30-40
Oats	8-14	1	80	10-15	5
SBM[2]	30-50	+/-	85-95	-	-

1. Brohult and Sandegren (1954)
2. Bailey et al. (1935)

As discussed by Osborne (1924), the albumins are soluble in water and the globulins are soluble in water and dilute saline solutions. Protein sources high in albumins and globulins, such as soybean meal, are very soluble in the rumen and as discussed by Wohlt (1973), this is highly correlated to rumen to rumen degradability. As a result, soybean meal is very degradable in the rumen. The glutelins are soluble in very dilute acid or alkaline solutions, insoluble in water, saline or solutions of alcohol. Prolamins are only soluble in solutions of alcohol. Proteins high in prolamin and glutelin fractions, such as corn gluten meal, are relatively insoluble in rumen fluid and resistant to microbial protease attack. Solubility, however, is not

the only factor affecting protein degradability in the rumen.

Mahadevan et al. (1980) reports the protein, bovine serum albumin, is highly soluble in the rumen. Studies show this protein is very resistant to microbial protease activity. Mahadevan states the resistance may be due to the proteins 16 disulfide linkages which may inhibit microbial protease activity. Casein is very soluble in rumen fluid, lacks disulfide linkages and is readily degraded in the rumen.

Many researchers have attempted to modify the rumen degradability of high quality dietary protein sources. Methods have included: formaldehyde (Spears et al., 1980), aldehyde treatments (Peter et al., 1971), tannic acid treatment (Driedger and Hatfield, 1972) and heat treatments (Nishimutu et al., 1973).

Formaldehyde has been used extensively to alter rumen degradation of dietary proteins. Formaldehyde treatment results in the formation of methyl groups on terminal alpha amino groups of lysine, followed by condensation of these groups with primary amide groups of asparagine, glutamine and guanidyl groups of arginine. These condensations form intermolecular and intramolecular methylene bridges. The treated proteins are stable and resistant to microbial protease activity. The methylene bridges may prevent degradation by altering tertiary structure similar to the action of disulfide bonds. The methylene bridges are broken in the acid environment of the abomasum. Excessive formaldehyde treatment may render the proteins indigestible throughout the total tract. Formaldehyde treatment of high

quality dietary proteins has increased nitrogen retention under several feeding regimes (Faichney, 1971; Tamminga, 1979).

There are factors other than the direct chemistry of proteins which affect solubility and degradability of cereal proteins in the rumen. However, these other factors are the result of the rumen environment interacting with the chemistry of the cereal proteins. Smith et al. (1959) and Isaacs and Owens (1972) evaluated the solubility of protein sources at varying pH and salt concentrations. In general, soybean meal is least soluble at a pH range of 4 to 5. Solubility of soybean meal plateaus at a pH of 8. Corn proteins are more soluble at the lower pH ranges. Smith et al. (1959) reports an increase in salt concentration of their solvent solution, in vitro, from 0 to .1M depress solubility of soybean meal from 90% at 0 to 50% soluble at .1M. Salt concentrations above .25M increased soybean meal solubility.

Loerch et al. (1983) have illustrated the effects of in-vivo pH on in-situ dacron bag degradability of supplemetnal protein sources. Feeding a diet to the fistulated steers, in which the in-situ study was conducted, of 80% corn, as compared to 20%, produced a lower rumen pH. The rate of soybean meal nitrogen disappearance was 2.9% per hour in the 80% corn diet and 7.8% per hour in the 20% corn diet. This was not observed for blood meal, meat and bone meal or corn gluten meal. These studies also indicate the supplemental protein source fed the fistulated steers also affected nitrogen disappearance from the dacron bags.

Protein Bypass Estimates

It is obvious that estimates of rumen degradability protein sources are a function of several factors. The primary factors include: microbial protease activity, protein chemistry, secondary and tertiary structure, rumen pH, ionic strength of rumen fluid and perhaps some unkonwn dietary interactions. Specific rates of protein outflow from the rumen are as important, under many circumstances, as protein chemistry. Calculation of protein outflow rates an estimates of total degradability have been discussed by Orskov and McDonald (1970) and Orskov and McDonald (1977). Table 4 provides a summary of current estimates of degradability of supplemental protein sources. The range of estimates for protein bypass are quite large. It is interesting to note the difference in bypass value of soybean and corn gluten meal with only a small increase in level of intake (Zinn et al. (1981). Estimates of protein bypass are only significant and unique for a given level of intake relative to rumen/total tract volume and nature of the total diet, especially with respect to the level of concentrates in the diet due to their affect on rumen pH.

Table 4 Protein Bypass Estimates

Feedstuff	Bypass %	Reference
Soybean meal	61	Hume, 1974
Soybean meal	20	Kropp et al., 1976
Soybean meal	19-24	Orskov and McDonald, 1970
Soybean meal	35	Stern and Satter, 1982
Soybean meal	15 (1)	Zinn et al., 1981
Soybean meal	18 (2)	Zinn et al., 1981
Corn gluten meal	57	Stern et al., 1983
Corn gluten meal	46 (1)	Zinn et al., 1981
Corn gluten meal	61 (2)	Zinn et al., 1981
Dried distillers grains	32	Stern and Satter, 1982
Dried distillers grains with solubles	55	Santos and Satter, 1980

1) Steers, intake 1.2 x maintenance
2) Steers, intake 1.6 x maintenance

Level of Intake: Effects on Site and Extent of Digestion

Several authors have discussed the role of level of intake and its effects on site and extent of digestion of dietary constituents (Zinn et al., 1983; Zinn et al., 1981; Miller, 1973; Tamminga et al., 1979 and Mudgal et al., 1982). Evans, 1981a and 1981b, has reviewed the relationships between dietary parameters and liquid, solids turnover rates. In brief; liquid turnover rates increase as feed intake increases, liquid turnover decreases as digestible energy content of the diet increases, liquid turnover decreases as forage intake decreases, solid turnover increases as feed intake increases, solid turnover declines as digestible energy content of the diet increases (for sheep, not related in cattle), solids turnover increases as forage percentage of

the diet increases. In general, increasing the level of intake increases the turnover of both liquids and solids from the rumen. This reduces rumen retention time, reduces digestion of dietary constituents and produces a shift in the site of digestion. Bypass of dietary protein as a result of increasing turnover is beneficial; bypassing fiber to the lower tract may not be beneficial.

Independent of level of intake, compounds such as monensin reduce liquid turnover as described by Lemenager et al. (1978). The result is a longer ruminal retention time. Despite longer rumen retention in animals fed monensin, there is less rumen degradation of dietary constituents due to a depression in microbial growth. Muntifering et al. (1981) report a shift in nitrogen and starch digestion to the lower tract in steers fed monensin.

Effects of Shifting the Site and Extent of Digestion

As discussed earlier, increasing the rate of passage of feeds from the rumen increases dietary protein flow to the lower tract. This is a preferred situation since it is more efficient to have high quality dietary proteins absorbed by the animal intact and provide nonprotein nitrogen to rumen microbes. A shift in starch digestion to the lower tract is an efficiency promoting event. Glucose absorption from starch in the small intestine is energetically more efficient than conversion to volatile fatty acids and their absorption

from the rumen. Black (1971) has discussed the theoretical basis for improved efficiency of energy and protein utilization when there is increased rumen bypass of the diet.

Fiber components bypassing digestion in the rumen pass through the small intestine without significant digestion. Fiber components may be digested in the cecum and proximal colon and partially compensate for reduced ruminal digestion of fiber due to increased rate of passage from the rumen. Data indicate a significant digestion of dietary fiber can occur post ruminally. Putnam and Davis (1965) indicate that alfalfa and wood cellulose fiber had a digestion coefficient of 29 percent when placed directly into the abomasum as compared to 43 and 63 percent respectively when fed per os. Dixon and Nolan (1982) report significant fermentation and absorption of dietary constituents bypassing the small intestine does occur in the cecum and proximal colon and to a much less extent in more distal portions of the large intestine.

SUMMARY

It is apparent that factors affecting the amount of passage of dietary proteins depends on a large number of variables. These variables include; protein chemistry, rumen environment, level of intake and addition of antibiotics such as monensin to the diet.

Maximization of dietary protein bypass must not be accomplished at the expense of providing for microbial

growth. Microbial growth can be improved when readily available nitrogen sources are included in diets containing "microbial protease resistant proteins". There is also potential improvements in ruminal fiber digestion when microbes are properly supplemented with nitrogen and sources of iso-acids.

The cecum and proximal large intestine do provide significant contributions to total tract digestion of dietary fiber and may play a major role when bypass of dietary constituents occurs to any great extent.

Extreme care must be taken when selecting a system of markers to evaluate site and extent of digestion. This thesis evaluates several aspects of marker function which affect estimates of site and extent of digestion. The diets studied contain corn silage and several common supplemental protein sources. The effects of protein source and marker selection upon the site and extent of digestion of corn silage based diets are studied and evaluated in detail.

It is evident that the level of intake in these studies is quite low. This will most likely bias the digestibility estimated toward the the upper limits of digestibility of corn silage based diets. This is most likely the case due to longer residence times of dietary constituents allowing for maximum microbial attack and consequently optimal rumen digestion of dietary constituents. Care must be taken to evaluate the level of intake relative to the total capacity of the rumen and total tract not merely relative to percent of body weight. The rumen and total digestive tract reaches

its maximum capacity much sooner than body weight reaches a maximum.

Evaluation of digestibility estimates for use in models of animal performance must take into account level of intake, source of dietary constituents and markers utilized to provide the parameter estimates. Hopefully this thesis will provide some insight into these considerations.

PRELIMINARY EVALUATION: MARKER BINDING BEHAVIOR

Introduction

At the onset of this research program, little data was available regarding the binding behavior of the rare earth elements Yb and La. This evaluation procedure was conducted to establish potential rare earth elements binding affinity with corn silage, soybean and corn gluten meals and wet distillers grains. This was required to estimate the amount of marked feed required to conduct these research programs.

Uden (1978) discussed the relationships between rare earth molecular weight and ionic radius to binding with feedstuffs. Rare earth elements of high molecular weight and small ionic radius bind more tenaciously to feed components than lower molecular weight high ionic radius rare earths. For this reason, ytterbium (Yb) appears to be a better marker than lanthanum (La), assuming binding affinity is an important criteria. It has been discussed by Allen et al. (1982) that rare earth elements may be released from their binding sites by an acid environment.

Both corn silage and wet distillers grains have pH values of 4.2 or less and it is of interested as to study their affinity to bind Yb and La.

Materials and Methods

Corn silage, soybean meal and wet distillers grains were soaked in either ytterbium or lanthanum chloride solutions containing 50 mg of rare earth per gram of feedstuff dry matter. Feeds (50 grams of dry matter) were soaked for 24 hours under refrigeration in 1,000 ml beakers. After the 24 hour soaking period, the solution was drained off and a sample of each feedstuff was taken for evaluation of initial marker concentration. The feeds were soaked for 6 hours with distilled water, liquid was drained off, material was rinsed once and feed samples were taken for analysis of marker concentrations. This cycle of soaking for 6 hours in distilled water and one rinse was conducted for three cycles.

Concentrations of rare earths on feed samples were evaluated by neutron activation analysis as discussed in experiment one materials and methods.

Results and Discussion

Corn silage marked with Yb lost 29.5% of the associated Yb after the first soaking-washing cycle. When La was utilized as a marker, 64.8% of the initial marker concentration was removed by the first soaking-rinsing cycle.

Soybean meal marked with Yb lost 22.5% of the initial Yb content after the first washing-rinsing cycle and 8.4% of the

La.

Wet distillers grains lost 64% of the Yb and 67.3% of the La upon the first soaking-rinsing cycle.

Corn silage, soybean meal and wet distillers grains contained 10.5, 9.9; 22.0, 7.4; 11.5, 7.5 mg of Yb and La per gram of dry matter respectively after the 3 cycles of soaking and washing.

The 24 hour soaking and 3 cycle soaking-rinsing scheme was applied to 45 kg batches of feed. Utilizing soaking solutions contributing twice the potential rare earth concentration present after 3 soaking-rinsing cycles, the bound marker levels were slightly higher than those found in the laboratory. Corn silage contained 15.1 to 24.2 mg Yb/gram of dry matter. Soybean meal contained 14.5 to 40.4 mg La/gram, wet distillers grains contained 14.8 mg La/gram and corn gluten meal contained 17.5 mg La/gram.

These levels were higher than values found when small amounts of feeds were processed. Likely this represents an inability to rinse the feeds as effectively under large scale settings.

The higher levels of rare earths bound to feedstuffs provided the basis for determining the dose size to achieve 2 to 3 grams of marker intake per day during periods of adaptation (5 days) and digesta sampling (3 days) for experiments one and two.

PRELIMINARY EVALUATION: FECAL EXCRETION OF MARKERS

Introduction

An important consideration involved in any passage study is the period of time required to reach stable marker concentrations in fecal samples. This is critical to achieve valid total tract digestibility estimates, which require steady state flow of markers from the total tract. This requirement must be balanced against time constraints and the cost of markers required for the marker adaptation period.

Materials and Methods

In this study, two duodenally cannuulated steers from experiment one were fed 323 grams of Yb marked corn silage (31% DM, 15.1 mg Yb/gram) and 250 mls of CrEDTA (9.2 mg Cr/ml) mixed with 6.8 kg of diet dry matter. Fecal samples were taken from each steer at 10, 20, 50, 80, 100 and 105 hours after the initial dose. Steers were fed the same level of markers for each subsequent day of the adaptation period. Fecal samples were freeze dried and analyzed for marker concentrations as discussed in experiment one materials and methods.

Results and Discussion

Figure 1 illustrates the pattern of marker concentration in feces averaged across the two steers. The concentration of Yb in the feces reached a peak at the 100 hour sample while Cr level reached a peak at 50 hours with some slight variation after this point.

Teeter et al. (1981) indicated 'peak fecal Yb concentrations, associated with long hay, occured 31.8 hours after dosing. Welch and Smith (1978) found polypropylene ribbon excretion peaked by 48 hours in steers and 72 hours in sheep. Uden (1978) reported peak excretion of several markers occured 40 and 50 post dosing in large and small heifers respectively.

These data indicate that peak marker concentrations occur at 48 to 72 hours after dosing. In our research, peak concentrations of Yb occured at 100 hours after dosing and Cr peak was reached essentially 50 hours post dosing.

It was concluded from this data and literature sources that a minimum 72 hour marker feeding period would be required for liquid markers and a 96 hour period for solid markers to reach suitably stable levels. We chose to feed marker for a minimum of 5 days (120 hours) prior to initiation of duodenal or fecal sampling.

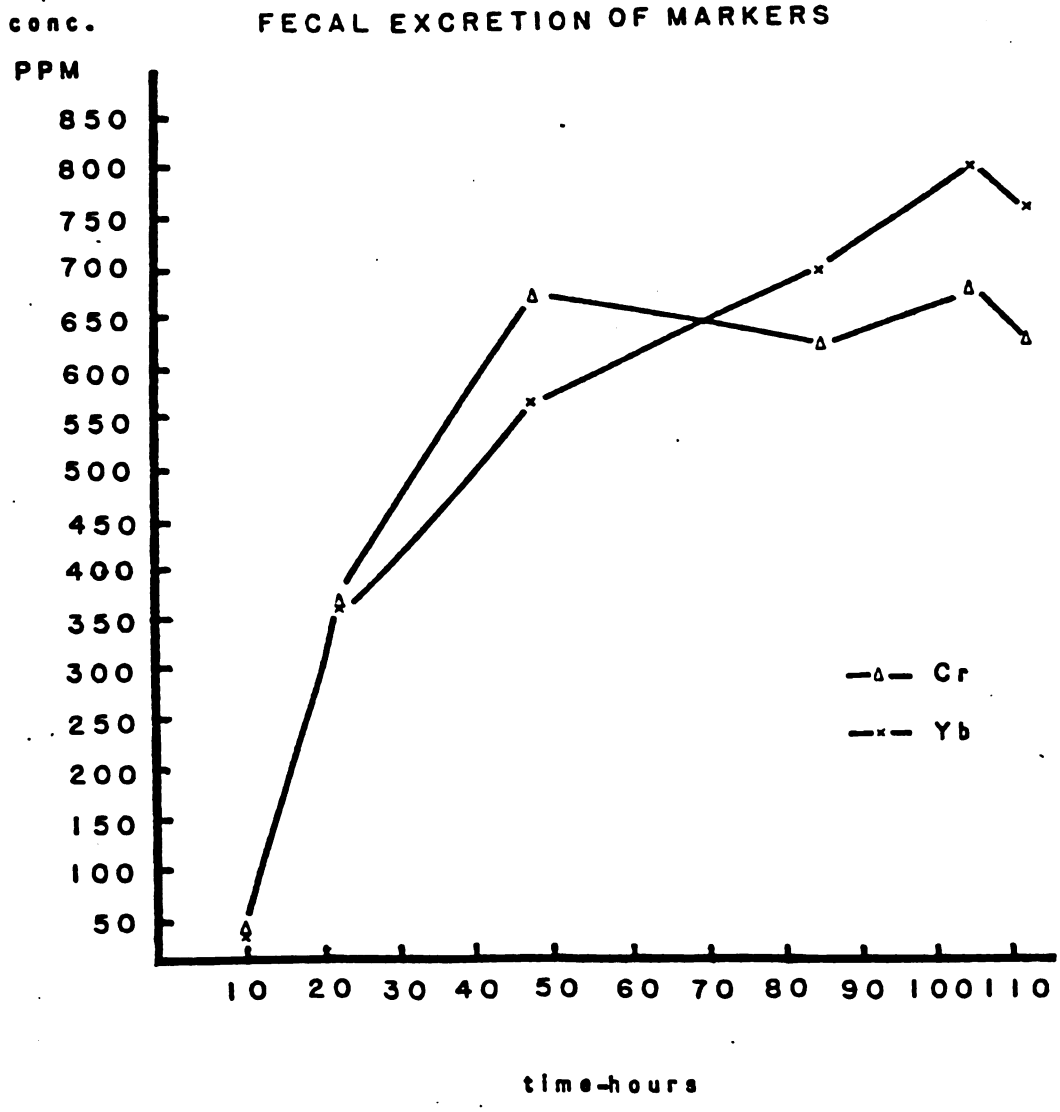

Figure 1 Fecal Excretion of Digesta Markers

MATERIALS AND METHODS

Experiment One

Experimental Design

This study was designed as a 4 x 4 Latin square. It utilized four, duodenally cannulated, 600 kg, Holstein steers. The experiment consisted of four periods within which each of the steers received one of four corn silage based diets. Figure 2a shows the arrangement of animal, periods and dietary allocation. Within each diet, three digesta markers were evaluated to study the consequences of marker choice upon estimates of site and extent of digestion.

Statistical evaluation was conducted, as with standard Latin square crossover experiments, utilizing blocking for animal and period effects. Markers were evaluated, as to their effects upon digestibility, within diets with four observations for each marker. With this arrangement, animal and periods are confounded and cannot be separated from the error term. Means were compared utilizing the all pair-wise comparisons of the Tukey's test (Gill, 1973; Gill, 1978) as opposed to orthogonal comparisons which would not provide logical comparisons but would be more sensitive to differences between treatment means.

	Period			
Animal #	1	2	3	4
788	B	A	C	D
789	C	D	A	B
790	D	C	B	A
791	A	B	D	C

Diets	Composition
A	Corn Silage-Soybean Meal
B	Corn Silage-Urea
C	Corn Silage-Wet Distillers Grains
D	Corn Silage-Wet Distillers Grains with Urea

Figure 2a Experiment One Design

Cannula Design and Insertion

A T-type cannula was surgically placed in the duodenum approximately 10 cm from the phylorus, anterior to the bile duct. The cannulae initially were made from a viscous plastisol (Norton's Plastic and Synthetics Div., Akron, Ohio and M & R Plastics and Coatings, Maryland Heights, Missouri). The mold utilized to make the cannulae was a copy of one acquired from Dr. L.D. Satter, University of Wisconsin. These cannulae were not durable. Portions of the cannulae in contact with digesta became brittle and resulted in cannula losses due to cracking off of the T portion. Cannulae were replaced with cannulae made from 15.9 mm ID X 22.22 OD Silastic tubing (Silastic Medical Grade Tubing, catalog #601-685, Dow Corning Corp. Medical Products, Midland, Michigan). The 20 cm barrel section was placed through a hole in the center of the 15 cm T portion of the cannula. The T section was prepared by longitudinally cutting, in half, a 15 cm piece of 15.99 mm ID x 22.22 OD tubing, A 22.22 diameter hole was cut in the center of the T section for barrel insertion. The inner and outer surface of the junction of barrel and T section were joined by placing a liberal bead of Silastic medical adhesive, silicone type, at the junction (adhesive #891, Dow Corning Corp.). The cannula was allowed to cure for 24 hours at which time excess adhesive can be removed and rough edges smoothed with a razor blade. This type of cannula can be sterilized by autoclave or ethylene

oxide techniques prior to surgical insertion.

Diet Formulation

In this study, four corn silage based diets were fed. Diets were mixed daily using fresh corn silage from a 1000 metric ton capacity upright silo. Silage averaged just under 8% crude protein and had a large amount of corn grain, equivalent to approximately 7.0 bushels per ton of 33% dry matter silage. Four diets were designed to be isonitrogenous. A mineral or mineral-urea supplement was designed for each diet. Table 5 lists the average composition of each diet on a dry matter basis. Table 6 lists the supplement composition. The protein sources were soybean meal, urea, wet distillers grains and wet distillers grains plus a mineral- urea supplement. Table 7 lists the source of nitrogen for each diet. Diets supplemented with wet distillers grains plus urea have 50% of the supplemental nitrogen from the wet grains and urea respectively.

The diets were formulated to contain 13% crude protein with calcium and phosphorus levels set for those required by 225 kg steers. Table 8 lists the average analysis of the diets.

The wet distillers grains were generated from ethanol production at the MSU pilot scale ethanol plant located at the Beef Cattle Research Center. The wet distillers grains were collected from whole stillage immediately after distillation of the mash. The grains were separated from

whole stillage on a 24 x 24 mesh, inclined, vibrating screen. The wet grains averaged 22% dry matter, 24% crude protein and contained less than 5% starch. After collection, the wet grains were mixed in a horizontal mixer to insure homogeniety and packaged individually into 10 kg packages. The grains were frozen for storage. Wet distillers grains were thawed at room temperature overnight prior to feeding.

Table 5 Diet Formulation: Experiment One

Component	TREATMENT			
	CS-Soy	CS-Urea	CS-WDG	CS-WDG,Urea
	---------% of Total Diet Dry Matter---------			
Corn Silage[1]	85.6	93.4	66.7	79.7
Soybean Meal[2]	12.5	-	-	-
WDG[3]	-	-	31.8	16.5
Urea[4]	-	1.8	-	.9
Supplement	1.9	6.8	1.5	3.8

1) Corn silage ensiled, well eared. 33% dry matter, 7.9% CP.
2) Soybean meal, 92% dry matter, 50% CP.
3) Wet distillers grains, 22% dry matter, 24% crude protein, collected from whole stillage by 24x24 mesh vibrating, inclined screen, frozen, stored.
4) Urea was contained in the supplement.

Table 6 Supplement Composition: Experiment One

Component	TREATMENT			
	CS-Soy	CS-Urea	CS-WDG	CS-WDG,Urea
	% of Total Dry Matter			
Ground Corn	16.7	44.7	16.7	37.7
Limestone	25.6	7.4	29.3	11.7
Calcium Sulfate	25.6	7.3	29.3	11.6
Monosodium Phosphate	19.0	8.8	8.1	9.1
TM Salt[1]	13.1	3.8	16.6	6.5
Urea	--	28.0	--	23.4

[1] Trace mineral salt contained a minimum of: 0.35% Zn, 0.20% Fe, 0.03% Cu, 0.005% Co, 0.007% I, 96% NaCl.

Table 7 Source of Nitrogen in Corn Silage Based Diets[1]

Component[2]	Diet			
	CS-Soy	CS-U	CS-WDG	CS-WDG-U
Corn Silage N	61.5	61.5	61.5	61.5
Urea N	--	38.5	--	19.2
Natural Protein N	38.5	--	38.5	19.3
ADIN % of Total N[3]	3.6[a]	4.4[a]	10.2[b]	8.0[c]

[1] Corn silage based diet, 13% crude protein

[2] Expressed as a % of total nitrogen in the diet

[3] ADIN-Nitrogen bound to acid detergent fiber, means with different superscripts differ ($P<.05$)

Table 8 Diet Analysis: Experiment One

		Treatment			
Component[1]	Unit	CS-Soy	CS-Urea	CS-WDG	CS-WDG-Urea
Dry Matter	%	36.06	34.34	26.65	29.88
Crude Protein	%	14.04	13.13	13.68	13.35
Organic Matter	%	93.94	94.38	94.79	94.73
ADF	%	20.48	22.47	21.32	22.49
Lignin	%	2.36	2.56	2.81	2.92
Ca[2]	%	.48	.48	.48	.48
P[2]	%	.34	.34	.34	.34
K[2]	%	1.08	.90	.66	.79
Salt	%	.25	.25	.25	.25

[1] Average values, based upon 4 composited samples from the four periods.

[2] Values calculated based upon similar feed ingredients.

Markers

In experiment one, three markers (lignin, ytterbium, CrEDTA) were used to evaluate the site and extent of digestion of major feed constituents; nitrogen, organic matter and acid detergent fiber (ADF).

Lignin represents an internal marker and was analyzed in feed, duodenal and fecal samples using the 72% sulfuric acid, gravimetric procedure to determine acid detergent fiber lignin (Van Soest, 1963 and Van Soest and Wine, 1967).

Ytterbium (Yb) was utilized as a marker for the solid phase of digesta. It was bound to corn silage by a soaking procedure. Teeter et al., (1979) discussed the binding of ytterbium to feeds by soaking or spraying methods. Teeter et al., (1981) reported the presence of binding sites on feeds which had varying degrees of affinity for ytterbium. Preliminary studies on corn silage used in our research have shown tenacious binding of ytterbium at a level of 10 mg Yb/kg of corn silage dry matter. This is the level of Yb remaining after soaking corn silage for 24 hours in a solution providing the potential of 50 mg Yb/kg of corn silage, followed by 3 cycles of washing and soaking in distilled water for 6 hours.

In an effort to efficiently utilize Yb, marker solutions were prepared to provide the potential Yb concentration on the marked corn silage of 20 mg Yb/kg of corn silage dry matter. It was assumed 3 cycles of washing and soaking for 6

hours with water would remove the loosely bound Yb. Ytterbium chloride (Research Chemicals, P.O. Box 14588, Phoenix, Arizona) contains a variable number of water molecules of hydration with 6 being the average value and averages 44.64% Yb.

Corn silage was soaked in the ytterbium chloride solution for 24 hours, in large plastic containers, under refrigeration. The corn silage was soaked with enough solution to suspend the material and allow for easy mixing. Following 24 hours of soaking, the plastic containers were covered with 2 layers of cheesecloth and a layer of screen, which was wired in place. The containers were inverted, allowing the soaking solution to drain off. The containers were refilled with water, agitated and drained. The containers were refilled with water and soaked for 6 hours followed by draining. This washing, soaking procedure was repeated 3 times.

When the final rinse solution was removed, the corn silage was spread out in a thin layer on plastic and allowed to air dry to 30% dry matter. At this time, the marked corn silage was mixed in a horizontal mixer to insure homogeneity, packaged in plastic bags with 323 grams per bag, subsampled for marker concentration and dry matter analyzed. Corn silage marked with Yb for this study averaged 31% dry matter and 15.1 mg Yb/g of silage dry matter.

The chromium complex of ethylenediamine tetracetic acid was prepared by adapting the procedure of Downes and McDonald (1964) for use of the free acid form of ethylenediamine

tetracetic acid (EDTA) rather than the disodium salt. The chromium complex (CrEDTA) can be prepared in the following manner:

1. Dissolve 400 grams of free acid ethylenediamine tetracetic acid dissolved in 4 liters of distilled water. Add 100grams of NaOH to facilitate solubilization of the EDTA. Stir constantly on a hot plate, heat to a gentle boil.
2. Dissolve 284 grams of chromium chloride in 2 liters of distilled water.
3. Combine solutions 1 and 2. Heat to a gentle boil while stirring. Hold at a gentle boil (100 degrees C), for 1 hour.
4. Add 80 mls of 1 N calcium chloride to the EDTA solution, stir well. The added Ca binds to unoccupied Cr binding sites on the EDTA molecule.
5. Adjust the pH of the CrEDTA solution with NaOH. Final pH should be 5.0 to 5.5.

CrEDTA prepared in this manner will contain 9 to 10 mg of Cr per ml of solution.

Housing, Adaptation, Feeding

This research project was initiated October 1, 1981 and was completed April 7, 1982. The four duodenally cannulated Holstein steers were confined during the adaptation and collection phases of the project in 1.8 x 2.4 m pens in an environmentally controlled facility. They had free access to water and there was no bedding as the pens have slatted floors.

Four diets were fed to each of the four Holstein steers during four periods. Steers were adapted to each diet for 21 days prior to any duodenal or fecal sampling. Figure 3 illustrates a typical adaptation and sampling schedule.

As illustrated by Figure 3, after the 21 days of adaptation, the steers were fed marker for 5 days followed by 3 days of marker feeding with duodenal and fecal sampling.

The slatted floor facility creates severe feet and leg problems, especially with large steers as utilized in this research project. The steers were allowed to rest in bedded pens, outdoors for 14 days between collection periods. During the rest periods, the steers received the corn silage, soybean meal diet.

Typical Adaptation and Sampling Schedule

Animal	Day 1 to 21, adaptation to new diet
Diet + Markers	Day 22-25
Diet + Markers	Day 26- Feed sample
Diet + markers	Day 27- Feed sample, doudenal and fecal samples: 12 am, 4 am, 12 pm, 6 pm
Diet + markers	Day 28- Feed sample, duodenal and fecal samples: 6 am, 10 am, 2 pm, 8 pm
Diet + markers	Day 29- Duodenal and fecal samples: 2 am, 8 am, 4 pm, 10 pm
No markers	Day 30- Duodenal samples: 8 am, 12 pm, 3 pm
No markers	Day 31- Duodenal samples: 8 am, 12 pm, 3 pm
No markers	Day 32- Duodenal samples: 8 am, 12 pm, 3 pm
No markers	Day 33- Duodenal samples: 8 am, 12 pm, 3 pm
	Rest period, outdoors: 14 days

Figure 3 Adaptation and Sampling Protocol

Diets were mixed fresh daily. Corn silages, protein source and the appropriate mineral supplement were weighed on an electronic balance to the nearest tenth of a kg. The diets were mixed in a horizontal mixer. During periods of marker feeding, marked corn silage replaced fresh corn silage on an equal weight basis and was mixed in the horizontal mixer. At the time of mixing, 250 mls of CrEDTA was added slowly during the mixing process. Each diet was allowed to mix for several minutes. As the diet was run out into its feed container, a .25 kg subsample was taken to make up a diet composite sample. This type of mixing and sampling procedure was conducted for each diet in each period. Three .5 kg feed samples from each diet within a period were taken for future analysis and frozen until analyzed.

The steers were fed once each day at 7:30 am. Each steer received 6.8 kg of diet dry matter each day during the experimental periods. This level of intake represented a feeding regime of 1.1 x maintenance calculated based on a requirement of 100 kcal ME/kg$^{.75}$ body weight. It was assumed each steer initially weighed 500 kg and the diets contained 1.56 mcal ME/kg of diet dry matter.

Sampling, Compositing, Processing

Feeds

As illustrated in Figure 3, three 250 gram feed samples were taken from the mixed diets, one sample the day before duodenal and fecal samples were taken and one sample each of the first two days of the collection periods. It was assumed the samples were representative of the complete mixed diet. The three feed samples from each diet, within a period, were composited on an equal wet weight basis. This resulted in a total of 16 composite feed samples for the entire experimental period. A subsample of each composite was oven dried at 105 degrees C for 24 hours to estimate original sample dry dry matter. The remaining feed samples were freeze dried for a period of 3 days in a Virtis model 25,SRC freeze dryer (Virtis Co., Gardiner NY). The freeze dried samples were ground in a Wiley mill with a 1 mm screen and stored in sealed plastic containers. Figure 4 shows the feed processing scheme.

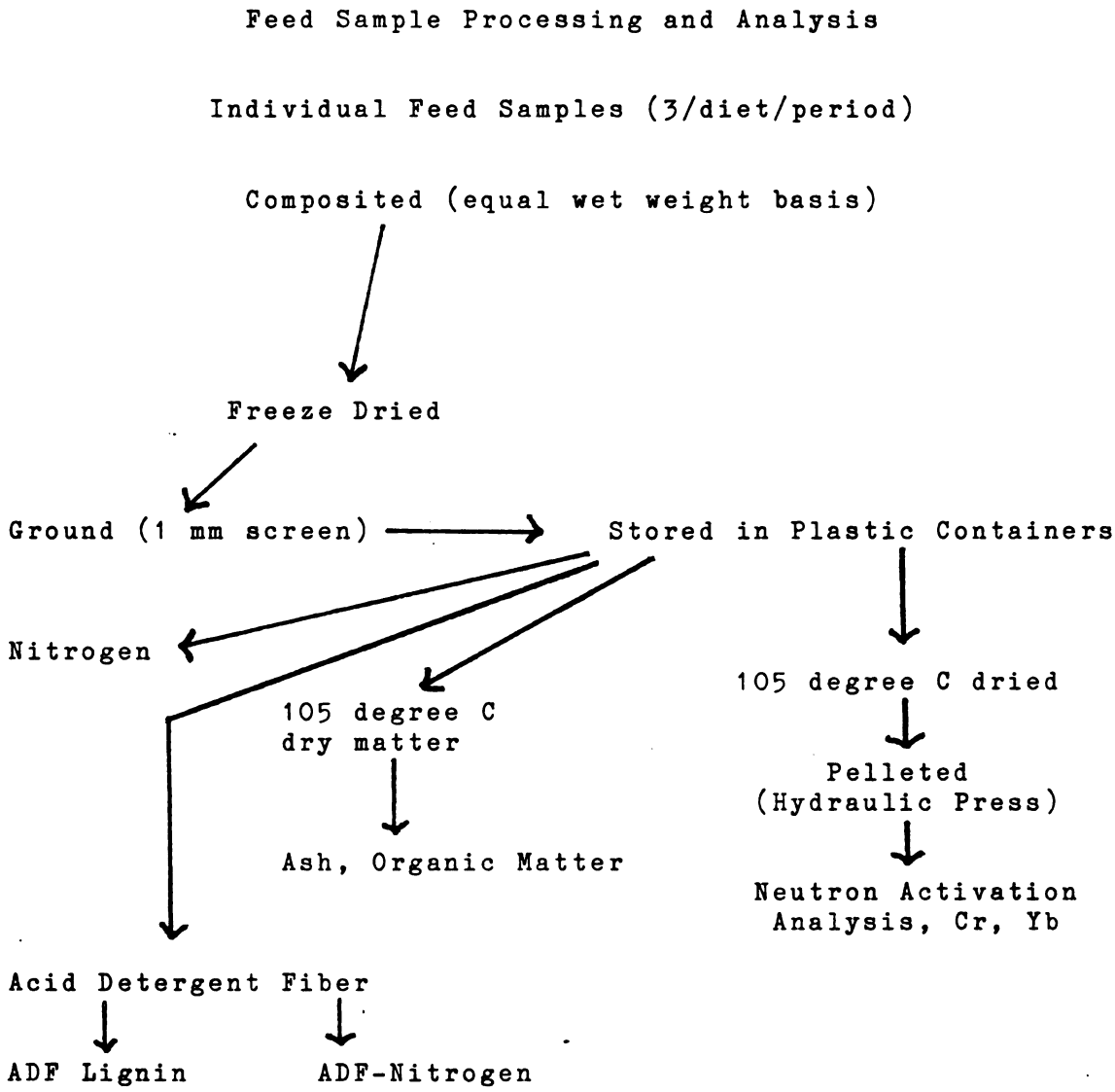

Figure 4 Feed Processing System

Duodenal

Figure 3 illustrates a typical collection protocol used in this experiment. Twelve duodenal samples were taken from each steer, each period of the experiment. The duodenal samples were collected in individually labled, 400 ml capacity, square plastic containers with lids. An attempt was made to collect at least 300 mls of duodenal digesta from each steer at each sampling time. Care was taken to discard the first 100 mls of duodenal digesta leaving the cannula after the plug is removed. This material is often of very high solids content and should not be used. After the initial surge of duodenal contents leaves the cannula, often there is a period of several minutes where no additional flow is occuring. It has been observed by the author that replacing the plug and moving to the next steer, purging his cannula and replacing the plug and then going back to sample the first steer, is a system which works well. Surges of 100 mls of duodenal contents are not uncommon, however, under no circumstances should collections be made by leaving the digesta dribble from the cannula. It is much more efficient and intuitively more correct to replace the plug and collect surges of digesta which can only help maintain a correct collection of duodenal solids and liquids. Samples of duodenal digesta were frozen immediately after collection.

An alternative to immediate freezing is homogenization in a Waring blender, subsampling and analysis of digesta dry matter in each sample, then freezing the remainder. At the time of homogenization, a subsample can be separated, (50-60 ml) and frozen in a separate container for future analysis of duodenal ammonia nitrogen.

In experiment one, the twelve duodenal samples from each steer were homogenized separately in a Waring blender. At this time, 100 g of each homogenized sample are combined in a large beaker and placed on a stirring plate. The 1200 gram duodenal composite is placed in four plastic containers, being careful to have equal representation of digesta in each container. Three of the four containers are freeze dried (Virtis freeze dryer) as discussed earlier regarding the feed samples. The fourth container is stored in a freezer as a backup in case a sample is lost or other problems are encountered.

After freeze drying, the three containers of dry duodenal digesta (94-98% DM) are ground in a Wiley mill with a 1 mm screen. At this time, the contents can be placed in one container and stored for future analysis. Storing feed and duodenal samples prior to analysis should be done under freezing or at least refrigeration. Samples of duodenal digesta of 94 to 98% dry matter content will begin to support a type of fungal growth in a few months after storage if not kept refrigerated. Figure 5 summarizes the steps involved with processing of duodenal samples.

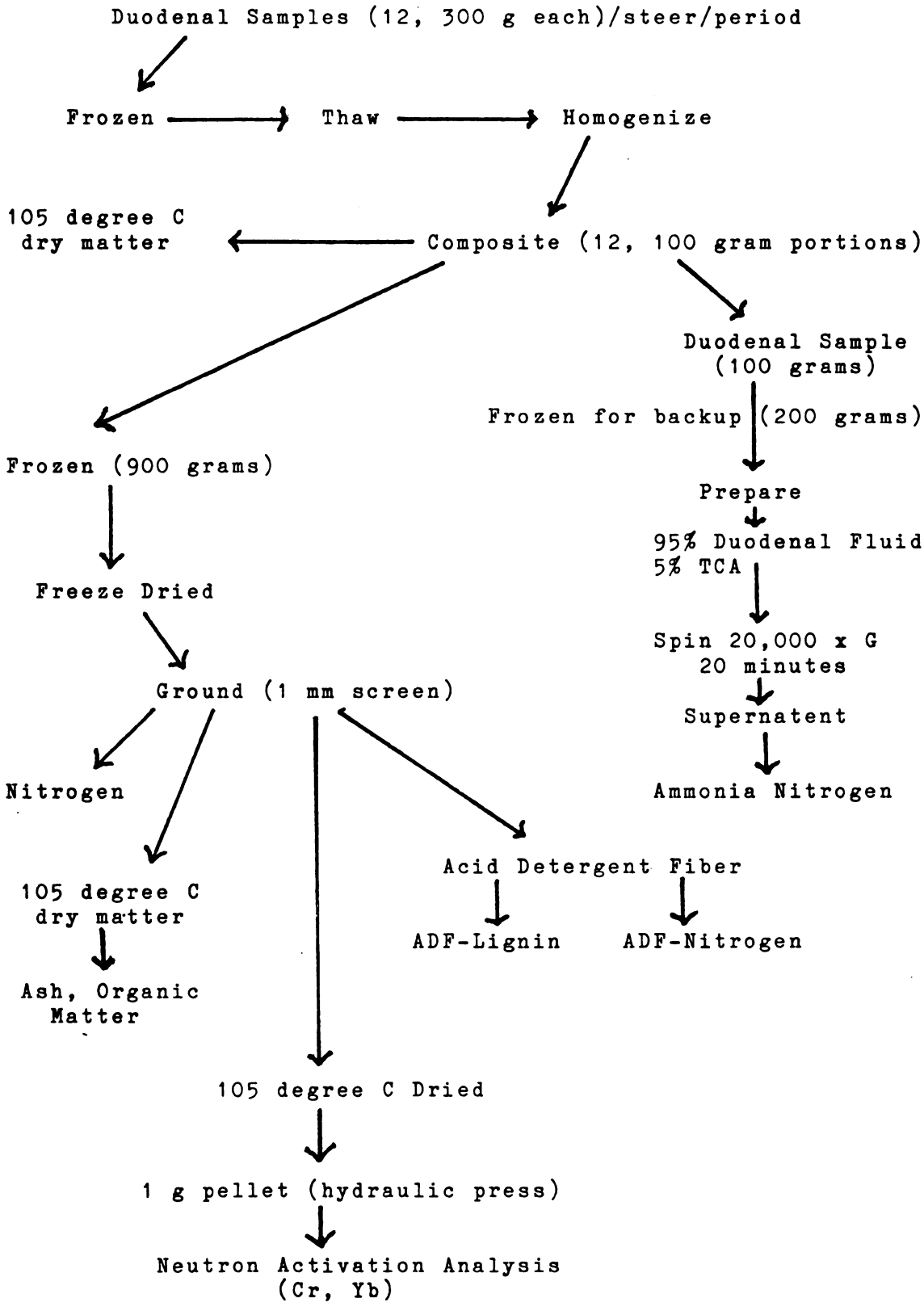

Figure 5 Duodenal Sample Processing

Fecal

A total of twelve fecal samples were taken from each steer, each period, at the same sampling intervals as the duodenal sample collections as illustrated in Figure 3. The fecal samples were taken by rectal palpation and grab sampling of fecal contents. It is desirable to acquire approximately 200 grams of feces at each sampling interval. The fecal samples were frozen and stored prior to compositing.

Fecal samples were composited on an equal wet weight basis. The frozen samples are thawed and 100 grams from each individual sample is placed in a large container. The 1200 grams of fecal material is mixed well, 300 grams were placed in each of four containers. Three of the containers, (900 grams), containing the fecal composite, were freeze dried.

Freeze dried fecal material was ground in a Wiley mill through a 1 mm screen, placed in 2 plastic containers and stored either frozen or in a refrigerator for future analysis. Figure 6 illustrates procedures for fecal processing.

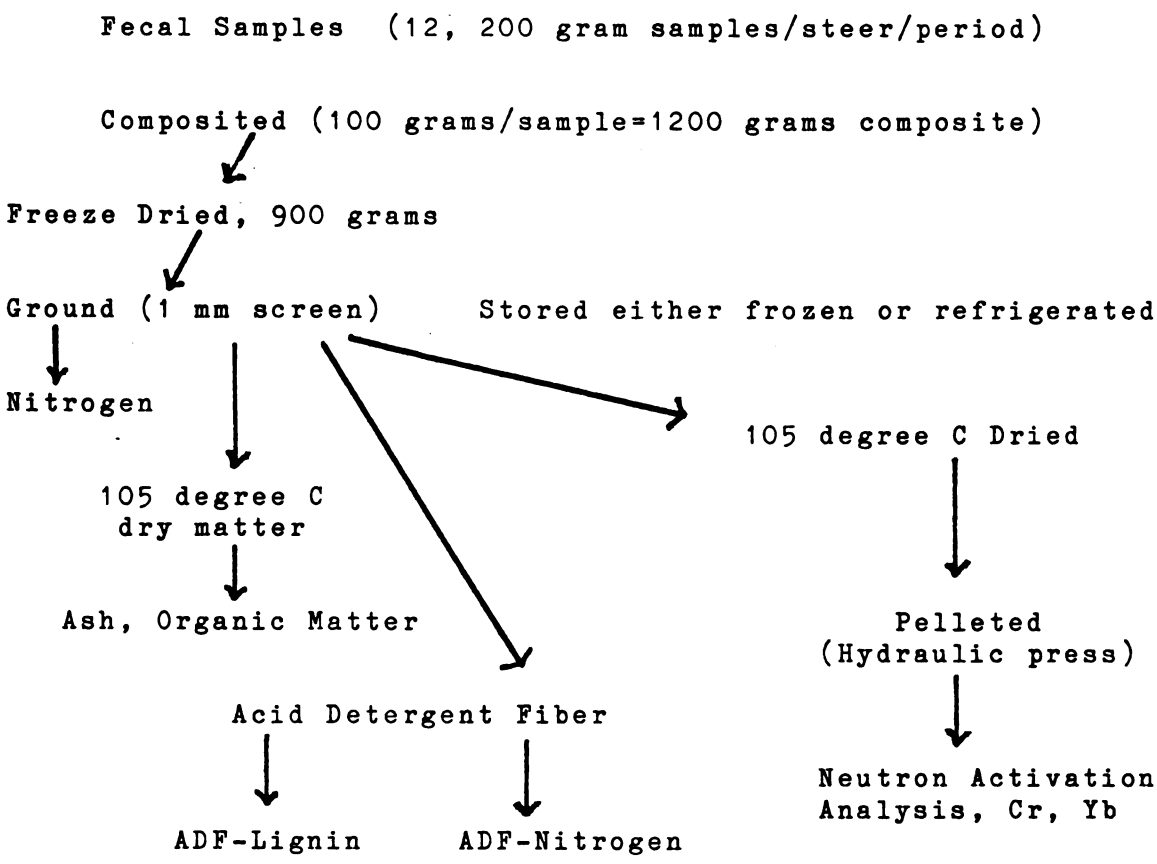

Figure 6 Fecal Sample Processing and Analysis

Analytical Techniques

Nitrogen

The total nitrogen present in feeds, duodenal and fecal samples was determined by sulfuric acid digestion in the presence of a copper sulfate based catalyst (Pope Kjeldahl mixtures, P.O. Box 903, Dallas, Texas 75221). The analysis of free nitrogen was conducted on a Technicon Auto Kjeldahl System. Nitrogen determinations were conducted on samples having dry matter of 92-98 % and adjusted to a 100% dry matter basis.

Ammonia Nitrogen

There are several methods available to determine ammonia nitrogen content of rumen and duodenal fluid samples. The most common methods include; steam distillation of ammonia from samples made alkaline, (Dixon and Nolan, 1982), ammonia electrodes such as model 95-10 Orion (Orion Research Corp., Cambridge, MA), Nesslers reagent system (Sigma Technical Bulletin No. 14), hypochlorite-alkaline phenol method of Berthelot (Ngo et al., 1982) and Technicon Auto Kjeldahl system. Review of these systems shows a number of limitations exist for each analytical technique.

Due to the large number of duodenal ammonia determinations required for experiments one and two, the automated Kjeldahl system was chosen. The Technicon auto analyzer operations manual lists modifications required to analyze ammonia nitrogen in liquid samples. The modifications were designed to increase samples size when analyzing samples with very low ammonia levels such as blood plasma. Duodenal ammonia nitrogen concentrations range from a low of 15 to above 80 mg/100 mls. These levels were within the detection range for analysis without sample flow modifications.

The analysis of ammonia nitrogen in duodenal samples require the following sample processing:

1. Pipet carefully 9 mls of duodenal fluid. Let thawed sample solids settle before sampling.
2. Place 9 mls of duodenal fluid and 1 ml of 50% trichloroacetic acid (TCA) in 12 ml centrifuge tube.
3. Centrifuge at 20,000 x g for 20 minutes.
4. Place supernatant in a clean test tube, seal and refrigerate until analyzed.
5. Analyze in duplicate on Technicon Auto analyzer. 100 and 200 ppm nitrogen standards (from ammomiun sulfate). Utilize ammonia free duodenal fluid prepared as discussed in this section, as the base line, rather than distilled water.

Several attributes of duodenal fluid affect analysis of ammonia nitrogen. In general, duodenal sample pH was sufficiently acid (pH less than 4) to provide a long storage life (frozen) and adequate hydrogen ion concentration to prevent ammonia loss. Precipitations of proteins by TCA and centrifugation will not remove free amino acids from the

dilution of duodenal fluid by TCA and other additions, of 2 to 3 ppm of ammonia nitrogen equivalent. The 100 and 200 ppm duodenal samples produced readings equivalent to 1-2 ppm ammonia nitrogen above the 100 and 200 ppm standards. The 1 to 2% difference between duodenal based standards and normal standards is considered not significant enough to warrant using ammonia free duodenal samples spiked with ammonia-nitrogen as standards. However, it would appear advantageous to utilize the ammonia free duodenal fluid (0 ppm ammonia-nitrogen) to produce the baseline readings for the Technicon autoanalyzer system.

Acid Detergent Fiber, Lignin, Nitrogen

The analysis of acid detergent fiber was conducted in quadruplicate when both lignin and acid detergent fiber nitrogen data were required. Acid detergent fiber analysis was conducted by the technique discussed by Goering and Van Soest (1970).

Analysis of lignin utilized the 72% sulfuric acid method as described by Van Soest (1963) and Van Soest and Wine (1967). In order to reduce error in use of lignin as a marker, each animal's feed, doudenal and fecal composite samples were analyzed as a set to reduce variation due to changes in environmental temperature and chemical reagents.

Acid detergent fiber nitrogen, also known as acid detergent insoluble nitrogen, was analyzed by micro Kjeldahl, digestion in concentrated sulfuric acid and with Pope

solutions. It is known certain amino acids react with automated Kjeldahl reagents and are measured as ammonia complexes by the analyzer. Final sample dilution on the analyzer is 3,333:1 and as a result, amino acids can only contribute a small amount of "ammonia like" activity with the Kjeldahl reagents used in the Technicon system.

In an attempt to evaluate the potential contribution of non-ammonia nitrogen components to the total quantification of ammonia nitrogen, the following analysis was conducted.

A 180 ml sample of duodenal fluid, pooled from several samples, was centrifuged at 20,000 x g for 20 minutes with 20 ml of 50% TCA. The sample supernatant pH was elevated to 11 with sodium hydroxide and it was heated to 100 degrees C. The solution was held at 100 degrees C for 1 hour. This was conducted to drive off all of the ammonia nitrogen. The original sample pH (3 to 4) and volume was restored by addition of sulfuric acid and distilled water.

Aliquates of ammonia free duodenal fluid were prepared to contain 0, 100 and 200 ppm of ammonia nitrogen by the addition of ammonium sulfate. These samples were run on the Technicon autoanalyzer and compared to distilled water as a baseline and 100 or 200 ppm ammonia nitrogen standards. The 100 and 200 ppm standards were prepared in the standard Technicon manner; ammonium sulfate as the nitrogen source, sulfuric acid, distilled water, Pope Kjeldahl salts and nitrogen free filter paper.

The results indicate the 0 ppm ammonia-nitrogen free duodenal fluid produced a chart reading, corrected for

Kjeldahl catalyst. The samples used were the acid detergent fiber residue remaining after the normal acid detergent fiber analysis.

All data were corrected to a 100% dry matter basis, based on 105 degrees C oven dry matter determinations.

Dry Matter, Ash, Organic Matter

All feed, duodenal and fecal samples were freeze dried. The freeze dried samples were 92 to 98% dry matter. All feed samples and composite duodenal and fecal samples were analyzed for dry matter, ash and organic matter. Dry matter analysis of each sample is conducted in duplicate by weighing 1 to 2 grams of sample into ashing crucibles. The samples were dried at 105 degrees C for 24 hours. The samples were allowed to cool, are re-weighed and then ashed at 500 degrees C for a minimum of 2 hours at the ashing temperature. This technique of incorporating several analysis steps into one was very efficient. This dry matter value was then used to correct all other analysis data to a 100% dry matter basis.

Activation Analysis: Ytterbium and Chromium

Activation analysis represents one of the most sensitive analytical techniques for evaluation of elements, both quantitatively and qualitatively. With nuclear reactors, the large neutron flux can produce radioisotopes as the result of neutron bombardment. When the neutron flux and period of

bombardment are known, the isotopes are produced in proportion to the masses of elements present. Activation of a known mass of an element and an unknown, if exposed to the same flux and time period, will allow for quantification of the mass of the element in the unknown.

Activation analysis is a non-destructive method of analysis which works well for detection and quantification of elements, especially the rare earths and chromium. In experiment one, analysis of ytterbium and chromium, in asamples of feed, duodenal and fecal material, were conducted in duplicate.

A total of 96 samples (48 samples duplicated) and 6 standards were analyzed as a set. Feed, duodenal and fecal samples from each animal were analyzed together, within an activation analysis run. This prevented variation due to standard differences or other factors.

Four grams of feed, duodenal and fecal samples for each animal and period were placed in small glass bottles. These samples were oven dried at 105 degrees C for 24 hours and sealed with screw-on caps while hot.

Standards were prepared by drying corn silage collected during the experiment, grinding in a Wiley mill through a 1 mm screen and oven dried at 105 degrees C for 24 hours. A 100 gram sample of this 100% dry amtter corn silage was placed in a 500 ml beaker. A solution containing 2 ml of a certified 10 mg/ml ytterbium standard (20 mg total Yb), 40 ml of a certified 1 mg/ml chromium standard (40 mg total Cr) and 158 ml of distilled water was prepared. This solution was

mixed with the dry corn silage in the 500 ml beaker. The mixture was oven dried at 105 degrees C for 48 hours, reground in a Wiley mill with a 1 mm screen, redried at 105 degrees C for 24 hours and placed in an oven dried container and sealed. These standards contained 200 ppm of ytterbium and 400 ppm of chromium.

Preliminary evaluation of neutron activation of ytterbium, lanthanum and chromium was conducted at Michigan State University using the nuclear reactor in the Chemical Engineering department. Small, 2 dram, polyethylene vials were used to contain samples for irradiation in the nuclear reactor. After irradiation, the samples were allowed to decay for a week to ten days to reduce the amount of Na-24 in the sample. The samples were then counted with a germanium (lithium) [Ge(Li)] detector. At this time, two problems were discovered. One problem was the 100% dry matter samples were coating the sides and top of the polyethylene vials. The other problem was that if two identical samples had different total sample weights and thus filled the vials to different levels, the amount of element detected per unit of samples mass was different. It was verified by the reactor operator that sample geometry will affect the efficiency of sample counting on Ge(Li) detectors.

In order to prevent sample geometry from affecting element analysis, the sample geometry must be standardized. A pellet formed by a hydraulic press, as used for bomb calorimetry work, seemed to provide a solution to the geometry problem and associated static charge related coating

of vial walls with sample materials.

All feed, duodenal, fecal and standard samples were formed into pellets with a hydraulic press. The pellet ranged in mass from .8 to 1.4 grams but due to specific gravity differences, size of the pellets did not vary to any significant degree. The pellets were 100 mm in diameter and varied from 50 to 75 mm in height. The pellets were placed in 4 dram polyethylene vials (Olympic Plastics Co. INc., 5800 W. Jefferson Blvd., Los Angeles, CA 90016) and were held in place with 2 cotton balls.

The standards ranged in mass from 1.0040 to 1.4317 grams and contained 200 ug/g Yb and 400 ug/g Cr. Six pellets of exactly the same mass (plus or minus .007 grams) were used for each activation analysis run. A group of 40 to 50 standard pellets were prepared at one time from which, 6 of equal mass were chosen. The remaining pellets were placed in numbered vials, with their mass recorded and were used in subsequent analysis runs.

Sets of 48 duplicated samples and 6 standards were sent to the University of Wisconsin Research Reactor for activation analysis (Reactor Lab, 130 Mechanical Eng. Bldg., Univ. of Wisc., Madison WI 53706).

The four dram polyethylene vials were heat-sealed and irradiated in the nuclear reactor for 3 hours at a thermal flux of approximately 5×10^{11} neutrons cm^{-2} sec^{-1}.

In order to reduce the presence of interfering, short lived isotopes such as Na-24, the samples and standards were allowed to decay for 10 to 14 days. The samples and

standards were counted for 900 sec. of live time. The elements, Cr-51 and Yb-169 were counted at 319.8 Kev and 198.1 Kev respectively. The counting of the gamma radiation emmitted from the samples and standards was accomplished by a Ge(Li) detector coupled to a Tracor Northern TN-11 pulse height analyzer. The effects of interfering energy peaks were removed and corrections for different decay times were accomplished by a computer program developed by the Nuclear Engineering department at the University of Wisconsin.

Calculations: Flow and Digestibility

Calculations of flow and digestibility of dietary constituents can be accomplished without an accurate estimate of feed intake. This can be accomplished when a suitable marker is homogeniously distributed throughout the diet.

It was observed that animals, housed in the slotted floor metabolism room, would lose feed from their mouth while masticating. For some reason, several steers preferred to masticate feed with their heads outside of the feedbunk. Feed loss into the manure pit below the steers was evident. An estimate of feed loss was not possible. As a result, actual ingested feed intake is unknown, although feed provided is measured.

The calculation of flow and digestibility of dietary constituents was calculated, as a percent of intake, by assuming the ratio of feed constituents and markers present was not altered by feed waste. The ratio of feed nutrient

concentrations and feed marker concentrations can be compared to their ratio at other sampling points to determine nutrient flow and digestibility. Equations 13, 14, 15 and 16 illustrate the calculation site of nutrient flow and digestibility at various points along the digestive tract.

Equation 13 was used to calculate the percentage of nutrient intake which has flowed to the duodenum (duo), grams of nutrient flow per day and percent apparent ruminal digestion.

(13)

$$\frac{\text{(Nutrient Conc. Duo. DM/Marker Conc. Duo. DM)}}{\text{(Nutrient Conc. Feed DM/Marker Conc. Feed DM)}}$$

x 100= % Nutrient Flow of Nutrient Intake x

= Nutrient Intake (g/day)= Nutrient Flow to Duodenum (g/day)

(100 - % Flow of Nutrient)= % Apparent Ruminal Digestion

Total tract nutrient flow and digestion was calculated in a similar manner as illustrated by equation 14.

(14)

$$\frac{\text{(Nutrient Conc. Fecal DM/Marker Conc. Fecal DM)}}{\text{(Nutrient Conc. Feed DM/Marker Conc. Feed DM)}}$$

x 100= % Nutrient Flow of Nutrient Intake to Feces x

= Nutrient Intake (g/day)= Nutrient Flow to Feces (g/day)

(100 - % Flow of Nutrients to Feces)=

% Apparent Total Tract Digestion

(15)

% Apparent Total Tract Dig. - % Apparent Ruminal Dig.=

% Apparent Lower Tract Digestion

The percent apparent digestion of nutrients entering the lower tract was estimated by equation 16.

(16)

$$\frac{\text{\% Apparent Lower Tract Digestion of Nutrient}}{\text{\% Apparent Flow of Nutrient from Rumen}} \times 100 = \text{\% Apparent nutrient digestion of nutrient entering duodenum and lower tract.}$$

MATERIALS AND METHODS

Experiment Two

Experiment Design

The design of this study was a replicated 2 x 2 latin square, crossover experiment. It utilized four duodenally and abomasally cannulated, 350 kg, Holstein steers. The experiment consisted of two periods within which each of the steers received one of two corn silage based diets. Figure 2b shows the arrangement of animals, periods and dietary allocation. Within each diet, four digesta markers were evaluated and an infusion of PEG into the abomasum was conducted to establish the patterns of digesta passage.

Statistical evaluation was conducted as with standard latin square, crossover experiments utilizing anlaysis of square and treatment effects. Markers were evaluated, as to their effect upon digestibility, within diets, with four observations for each marker. With this arrangement, animals, periods and squares were confounded and could not be separated in the analysis of variance. Means were compared utilizing the all pair-wise comparisons of the Tukey's test (Gill, 1973; Gill, 1978).

Square 1

Animal	Period 1	2
813	A	B
820	B	A

Square 2

Animal	Period 1	2
819	A	B
550	B	A

Diets	Composition
A	Corn Silage-Soybean Meal
B	Corn Silage-Corn Gluten Meal

Figure 2b Experiment Two Design

Cannula Design and Insertion

A T-type cannula prepared and inserted in the same manner as in experiment one was utilized for sampling of duodenal contents. In addition, each steer had a surgically inserted abomasal infusion cannula. This small cannula was placed in the non-glandular region of the abomasum, near the junction with the omasum.

The cannula was prepared with a 25 cm barrel portion of 9.53 mm ID x 12.7 mm OD Silastic brand tubing (Catalog #601-525, Silastic medical grade tubing, Dow Croning Corp. Medical Products, Midland MI). The T portion was prepared with an 8

cm, 1/3 section of 15.99 mm ID x 22.22 mm OD Silastic medical grade tubing (catalog #601-685). The barrel portion was placed through a 12.7 mm hole in the center of the T portion. Five cm of the barrel projected from one side of the cannula, 20 cm on the other. The two portions were bonded together by a liberal bead, on both sides of the T portion, around the junction of barrel and T portions (Silastic brand Medical Adhesive, catalog #891). The infusion cannula was held in the abomasum with a purse string suture of nonabsorbable material.

Both the duodenal and abomasal cannulae were inserted at the same time through a single incision site. The steers require at least a one month recovery period before the experiment was conducted.

Diet Formulation

In this study, two corn silage diets were fed. One was supplemented with soybean meal (SOY), the other with corn gluten meal (CGM). Diets were mixed daily using fresh corn silage ensiled in an upright silo. The silage averaged nearly 8% crude protein and was not as high in grain content as the corn silage utilized in experiment one. The silage was estimated to contain 6 bushels of corn equivalent per ton of silage at 33% dry matter.

Table 9 lists the composition of each diet on a dry matter basis. The diets were formulated to contain 13% crude protein with calcium and phosphorus levels set for those

required by 225 kg steers. Table 10 lists the average analysis of the diets. Table 11 lists the supplement composition.

Table 9 Diet Formulation: Experiment Two

Component	Treatment	
	CS-Soy	CS-CGM
	% of Dry Matter	
Corn Silage[1]	85.6	89.7
Soybean Meal[2]	12.5	--
Corn Gluten Meal[3]	--	9.0
Supplement	1.9	1.3

[1] Corn silage 6 bu/ton at 33% DM, 7.9% crude protein

[2] Soybean meal 92% dry matter, 50% crude protein

[3] Corn gluten meal, 90% dry matter, 66% crude protein

Table 10 Supplement Formulation: Experiment Two

Component	Treatment	
	CS-Soy	CS-CGM
	% of Dry Matter	
Ground Corn	16.7	15.46
Monosodium phosphate	19.0	34.28
Calcium sulfate	25.6	16.75
Limestone	25.6	16.75
Salt-TM[1]	13.1	16.75

1 Trace mineral salt contained a minimum level of: 0.35% Zn, 0.20% Fe, 0.03% Cu,0.005% Co, 0.007% I and 96% NaCl

Table 11 Diet Analysis, Experiment Two

Component	Unit	Treatment	
		CS-Soy	CS-CGM
Dry Matter		33.34	31.85
Crude Protein		13.07	12.84
Organic Matter		93.72	94.30
ADF		22.16	24.56
Lignin		2.52	2.59
Ca[2]		.48	.47
P[2]		.34	.35
K[2]		1.08	.85
Salt		.25	.25

1) Average values based upon 4 composite samples, 2 periods
2) Values calculated based upon similar feed ingredients

Markers

In experiment two, four markers, (lignin, ytterbium, lanthanum, CrEDTA) were utilized to evaluate their effects upon estimates of site and extent of digestion of dietary constituents.

Lignin served as an internal, naturally occuring marker. The lignin evaluated was 72% sulfuric acid lignin as described in experiment one.

Ytterbium was bound to corn silage by the soaking and washing procedure as discussed in experiment one materials and methods. The corn silage was individually packaged. Each package contained 1362 grams of marked corn silage at 18.1% dry matter. The marked silage was frozen and stored until required. Prior to mixing into the diets, the corn silage was thawed overnight.

As illustrated by Figure 3, markers were placed in each animals' diet for a total of 8 days. A package, containing 1362 grams of marked corn silage, replaced 1362 grams of normal corn silage at the time of diet preparation. The marked corn silage was 18.1% dry matter and contained an average of 24.17 mg Yb/gram of silage dry matter. After removing a subsample of the mixed diets for diet analysis, each steer received an average of 5.76 grams of Yb per day.

Lanthanum was bound to both soybean meal and corn gluten meal by the soaking procedure utilized to mark corn silage.

A soaking solution was prepared to contain an estimated 40 mg La/gram of feed to be marked. Lanthanum chloride (7.40% La, Research Chemicals P.O. Box 14588, Phoenix, Arizona 85063) was utilized as a source of Lanthanum which is highly soluble.

Soybean and corn gluten meal were oven dried at 105 degrees C for 48 hours. The marked feeds were then ground in a burr mill because after drying, the material had formed hard clumps of feed. The marked feeds were individually packaged. Packages contained 150 grams of soybean meal at 81.4% dry matter and 40.37 mg La/gram of dry matter. Corn gluten meal was packaged in 170 gram units at 83.8% dry matter and 17.47 mg La/gram of dry matter.

During periods of marker feeding the 150 grams of marked soybean meal or 170 grams of corn gluten meal replaced the same amount of soybean meal or corn gluten meal at the time of diet mixing. Steers receiving the soybean meal and corn gluten meal diets consumed an average of 4.83 grams and 2.24 grams of lanthanum respectively during periods of marked feed addition.

During periods of marker feeding, 250 mls of CrEDTA were added to each diet at the time of mixing. The CrEDTA complex was prepared as discussed in experiment one materials and methods. Steers daily Cr intake averaged 2.25 grams per day during periods of marker feeding. The CrEDTA complex averaged 9.2 mg Cr/ml.

A polyethylene glycol solution (PEG 4000) was infused into the abomasum. The solution contained 135 mg PEG/ml and was infused at a rate of 2.7 to 2.9 mls/minute. Infusion was

conducted by using a Harvard type peristalic pump. The infusion was initiated 12 hours prior to duodenal sampling. Measurement of infusion rate was conducted by evaluation of weight change of flasks containing the infusate. Flasks were weighed prior to and immediately after each duodenal sampling period. This provided 12 measures of infusion rate for calculation of duodenal flow at each sampling time.

The steers were connected to 7.6 meters of 9.5 mm Tygon tubing. The tubing ran through a series of pulleys and was weighted to keep the tubing tight and yet allowing some freedom of movement of the steers. Steers had to be secured with a halter to prevent their damaging the tubing.

Housing, Adaptation, Feeding

This research project was initiated January 7, 1982 and completed April 5, 1983. The four Holstein steers were confined during the adaptation and collection phases in an environmentally controlled metabolism room. Figure 3 illustrates the typical adaptation and sampling schedule. The only differences are the steers were infused with polyethylene glycol, into the abomasum, beginning 12 hours prior to duodenal sampling and terminated after the last duodenal samples were taken.

Diets were mixed fresh daily. During periods of marked feed addition, feeds bound with Yb and La replaced equal amounts of respective unmarked feed ingredients. While the diets were mixing in a horizontal mixer, 250 ml of CrEDTA

were added slowly.

Steers were fed once daily at 90% of ad lib intake of the steer consuming the lowest average amount of dry matter per day. Steers averaged 350 kg and their average dry matter intake was 7.2 kg for both diets and periods.

Three .5 kg feed samples were taken from each steer's mixed diets over 3 days as indicated in Figure 3. The samples were frozen for future compositing and analysis.

Sampling, Compositing, Processing

Feeds

The three .5 kg feed samples, obtained as illustrated in Figure 3, were analyzed in the same manner as described in the materials and methods in experiment one, Figure 4. Due to analytical difficulties experienced with determination of acid detergent fiber nitrogen, this analysis was not conducted in experiment two. Lanthanum in feed samples was analyzed as Yb and Cr were in experiment one , Figure 4.

Duodenal

Figure 3 illustrates a typical collection protocol, as used in experiment one and two. The duodenal samples were processed as described in experiment one, Figure 5, but were not composited. Duodenal samples were analyzed i ndividually for all components and markers. Acid detergent insoluble

nitrogen was not analyzed in this experiment. Lanthanum was analyzed by activation analysis as indicated for Yb and Cr in Figure 5.

Fecal

Fecal samples were taken following the schedule illustrated in Figure 3. Figure 6 summarizes the compositing and processing sequence conducted for fecal samples obtained in experiment two. Acid detergent fiber insoluble nitrogen was not analyzed in fecal samples. Lanthanum was anlayzed by activation analysis as described in Figure 6 for Yb and Cr.

Analytical Techniques

Nitrogen

The total nitrogen in feeds, duodenal and fecal samples was analyzed as samples in experiment one analytical techniques.

Ammonia Nitrogen

Ammonia nitrogen in duodenal samples was analyzed as described in experiment one analytical techniques. The modifications include that each of the 96 duodenal samples were analyzed individually for ammonia nitrogen.

Dry Matter, Ash, Organic Matter

Feed, duodenal and fecal samples were analyzed for dry matter, ash and organic matter as discussed in experiment one, analytical techniques.

The modifications include that after homogenization of duodenal samples, individual duodenal digesta dry matter was analyzed in duplicate for each of the 96 samples. This was conducted by placing 20 to 30 mls of homogenized duodenal digesta into small aluminum dry matter pans and dried at 105 degrees C for 24 hours.

Analysis of ash, organic matter and freeze dried sample dry matter was conducted on each duodenal samples in duplicate.

Acid Detergent Fiber, Lignin

The analysis of acid detergent fiber and lignin were conducted in duplicate on composite samples of feeds and feces as discussed in experiment one, analytical techniques. All 96 duodenal samples were analyzed in duplicate for acid detergent fiber and lignin.

Activation Analysis

Activation analysis on samples collected in experiment two was conducted as discussed in experiment one, analytical techniques. All 96 duodenal samples and feed, fecal

composites were analyzed in duplicate.

Lanthanum 140 radioisotope was counted at 487 Kev. Standards contained 300 ppm La and were prepared as discussed in experiment one.

Polyethylene Glycol Analysis

Figure 7 illustrates the processing sequence, conducted on each of the 12 duodenal samples from each steer, for PEG analysis. The technique represents only slight modificaiton to an improved, turbidimetric, PEG analysis developed by Malawar and Powell (1967). Standards for this analysis should be prepared with centrifuged duodenal fluid from cattle fed diets similar to those used in the experiment.

The presence of interfering materials, especially tannins, can be corrected for in this manner. The standards utilized in this study contained 0, 300, 500 and 700 mg/100 mls of PEG and were processed in the same sequence and at the same time as the duodenal samples. Analysis of a 1:25 dilution of the individual infusion solutions was also conducted within the same set as the respective duodenal samples.

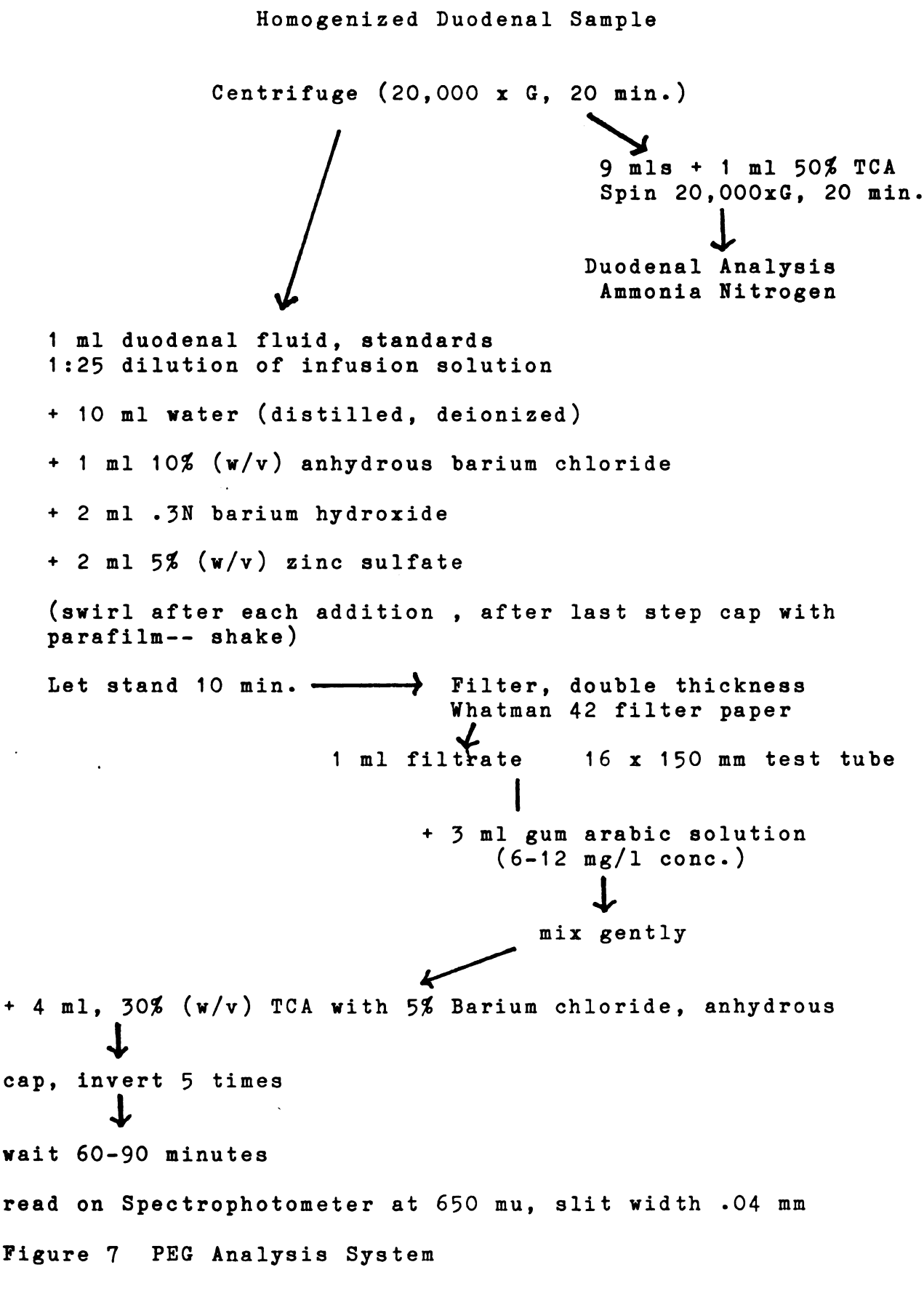

Figure 7 PEG Analysis System

Calculations: Flow and Digestibility

In this experiment, digestibility of dietary constituents was estimated by marker ratio techniques based on lignin, Yb, Cr and La concentrations in feeds and fecal composites and duodenal concentrations based on averaging the levels in the 12 duodenal samples on the equal whole digesta basis. Marker concentrations were also estimated in duodenal samples based upon weighted values resultant from abomasal PEG infusion and PEG concentration in duodenal samples. These marker concentrations were compared with those based on averages derived from equal whole digesta compositing.

Digesta flow at each sampling time was calculated as illustrated in equation 17. Percent of dietary constituents flowing to the duodenum at each sampling time was calculated as illustrated in equation 18.

The following calculations were conducted for each duodenal sample within a steer/diet set of data.

(17)

Digesta Flow per Hour

$$\frac{\text{Infusate Conc. (mg PEG/ml) x Infusion rate (ml/min)}}{\text{PEG conc./ml whole digesta (mg/ml)}}$$

= ml digesta flow/min

x 60 min/hour

= Digesta flow/hour

x % DM

= Digesta dry matter flow/hour at each sampling point

(18)

Percent of Total Flow at each Sampling Time

$$\frac{\text{Digesta flow/hr}}{\sum_{i=1}^{12} (\text{Dry matter flow/hr})_i} \times 100$$

= % of total daily digesta flow at sampling time

$$\frac{\text{(Digesta flow/hr x nutrient or marker conc. in digesta DM)}}{\text{(Digesta flow/hr x nutrient or marker conc. in digesta DM)}} \times 100$$

= % of total nutrient or marker flow at sampling time

Duodenal samples were mathematically composited based on an equal whole digesta weight basis and based on percent of total daily flow occuring at the sampling time based on PEG infusion.

Ruminal digestion was calculated based on an equal amount dry matter compositing system and the marker ratio techniques as well as PEG determined nutrient flow versus total intake of each nutrient.

PEG contributed to apparent organic matter content of duodenal and fecal samples. The amount of PEG infused per day was used to correct the duodenal and fecal samples to actual organic matter contents prior to determination of organic matter digestion.

Based upon the percent of total daily flow occuring at each of the 12 duodenal sampling times, the data can be examined by Fourier analysis. The Fourier transform

can help describe the circadian variation in flow. This analysis technique was not utilized to evaluate the infusion system studied in this research, how ever we intend to evaluate the techniques at a later date.

The Fourier analysis system was used by Corbett and Pickering (1983). A similar evaluation of rumination patterns was conducted by Murphy et al. (1983) using cosinar analysis and similar circadian rhythms was described but not mathematically predicted by Gordon and McAllister (1970).

RESULTS AND DISCUSSION

Experiment One

The Effect of Supplemented Protein Source Upon Site and Extent of Digestion of Corn Silage Based Diets by Steers.

Rumen Disappearance and Flow

There were no statistically significant effects of protein source upon apparent rumen disappearance of nitrogen (N), organic matter (OM), acid detergent fiber (ADF) or non-ammonia nitrogen flow (NAN flow) to the lower tract (duodenum). However, specific trends exist which are evident regardless of the marker (lignin, Yb or Cr) selected to predict digestibility and flow (see tables 13,14,15).

The absolute numerical values of digestiblity vary based on the marker selected. The qualitative differences between dietary constituent digestibility and flow are likely actual occurances, not representations of artifacts of protein source or marker choice.

The NAN flow as a percent of nitrogen intake was the least for the soybean meal (SOY) supplemented diet and highest for the wet distillers grains with urea combination (WDG-Urea). The SOY diet NAN flow as a percent of N intake was 44.9, 65.7 and 73.2 while WDG-Urea averaged 54.3, 70.3 and

82.4 for lignin, Yb and Cr markers respectively. Crickenberger et al. (1979) reported NAN flow of 50.9% of N intake on a corn silage-soy diet at 13% crude protein, based on lignin as a marker. The WDG-Urea diet NAN flow exceeded the SOY supplemented diets by 20.8, 7 and 12.4% based on lignin, Yb and Cr as markers respectively.

Diets supplemented with WDG-Urea exhibited less apparent rumen disappearance of organic matter than SOY supplemented diets. The organic matter disappearance was 22, 32.3 and 57.7 % less for WDG-Urea diets compared to SOY diets, based on lignin, Yb and Cr(EDTA) markers respectively. Merchen et al. (1979) reported a brewers dried grains and urea combination, in a corn cob based diet, had 5.5% less apparent rumen organic matter disappearance when compared to a similar diet supplemented with urea alone. They also report 9.8% less microbial nitrogen incorporated per kg of apparent organic matter digestion for diets supplemented with brewers dried grains with urea versus urea alone.

The quantity of apparent organic matter fermented or digested in the rumen is directly related to microbial growth and the amount of dietary nitrogen incorporated per kg of apparent rumen organic matter fermented. Estimates of grams of microbial nitrogen incorporated per kg of apparent fermentable organic matter range from 14.6 for low nitrogen diets to 21.3 for high nitrogen diets (Hume et al., 1970, based upon grams of microbial protein divided by 6.25).

Merchen et al. (1979) reported 13.3, 14.5 and 12.0 g microbial N/kg OM fermented in diets supplemented with urea,

soybean meal and urea, dried brewers grains and urea respectively. The brewers grains and urea diet provided the lowest total microbial nitrogen contribution to the lower tract. Hembry et al. (1975) report 9.6, 13.0, 6.2 and 11.9 grams of microbial N/kg fermented OM for diets supplemented with soybean meal, casein, zein and urea respectively. They also report the addition of isovaleric and valeric acids to diets containing urea improved microbial growth.

Diets supplemented with corn based proteins (wet distillers grains, dried distillers grains, and zein) or other proteins resistant to degradation, may not support optimal microbial growth. The addition of urea to diets supplemented with corn based proteins may not improve the efficiency of microbial growth as expected.

It has been shown that the alpha keto acids; phenylacetic, isobutyric, isovaleric and two methylbutyric are required by many cellulolytic bacteria for protein synthesis (Hume et al., 1970; Bryant and Doetsch, 1955 and Cline et al., 1966). Perhaps, due to the resistant nature of corn based proteins to digestion within the rumen, there is a deficiency of both nitrogen and specific alpha keto acids for microbial growth. The potential benefits of alpha keto acids and urea to these diets has not been evaluated.

Apparent rumen disappearance of ADF in WDG-Urea supplemented diets was the lowest compared to urea supplemented diets, except when Yb was used as a marker. In this instance, the soybean meal supplemented diets had a slightly higher ADF disappearance than the urea supplemneted

diet. The WDG-Urea diets had 22, 32.3 and 57.7% less apparent ADF disappearance from the rumen compared to the urea diets with lignin, Yb and Cr as markers, respectively.

The trend for low rumen digestion of nitrogen, organic matter and acid detergent fiber in diets containing wet distillers grains with urea is interesting. Corn based proteins, such as wet distillers grains, have low rumen degradability as illustrated by Zinn et al. (1978) and Stern et al. (1983). As described by Pilgrim et al. (1970), 50 to 75% of the microbial nitrogen originates as ammonia. Hembry et al. (1975) has shown in sheep that diets supplemented with the corn protein zein supported the lowest microbial growth.

The low microbial growth on diets supplemented with corn based proteins may be related to low rumen ammonia levels generated in these diets. Rumen ammonia levels of 5 mg/100 ml (Satter and Slyter, 1974) and 9 mg/100 ml (Hume et al., 1970) have been reported to support optimal microbial growth. Mehrez et al. (1977) report that optimal digestion within the rumen requires rumen ammonia levels of 23.5 mg/100 ml. Klopfenstein et al. (1976) has reported peak rumen ammonia levels of 2 mg/100 ml in the rumen of sheep fed dry distillers grains and by 6 hours, values of 0. In comparison, sheep fed urea supplemented diets had peak rumen ammonia levels of 35 mg/100 ml and .6 at 6 hours. Clearly, rumen ammonia levels in sheep fed distillers grains are below those shown optimal for either microbial growth or digestion of dietary constituents.

Urea is often added to diets containing corn based proteins or other proteins resistant to rumen degradation. Klopfenstein et al. (1978) reports a "complementary" effect of urea in these diets, where animal performance is significantly improved relative to the diets containing the resistant proteins alone. Aderibigbe and Church (1983) report increased in vitro dry matter digestion when urea was added to diets containing feather meals. Church et al. (1982) report increased utilization of feather meal when diets are supplemented with urea.

Despite the beneficial effects of urea upon ruminal digestion of dietary constituents, (Church et al. 1982) in diets supplemented with some resistant proteins, this has not been observed in diets containing dried brewers grains (Merchen et al., 1979) or in our studies.

Apparent rumen disappearance of ADF was 8.4, 2.3 and 16.9% less in WDG-Urea diets compared to WDG diets with lignin, Yb and Cr markers respectively.

Based upon the "complementary" effects of urea addiiton to these diets, as reported by Klopfenstein et al. (1978), one must contemplate the source of these performance advantages. The advantages may be unrelated to urea improving ruminal microbial growth. It appears the urea resulted in an increased amount of ruminal flow of undegraded dietary constituents. As reported by Utley et al. (1970), increased urinary output as a result of urea supplementation may increase water intake. This may increase the flow of

dietary constituents to the lower tract.

Black (1971) has indicated that utilization of nutrients post-ruminally eliminates the energy losses which are associated with fermentation and conversion of dietary protein to microbial protein. Perhaps the addition of urea to diets containing corn based proteins such as distillers grains, wet distillers grains, corn gluten meal and some brewers grains, result in a shift in the site of digestion. Based on Black's theory, this may be the source of the "complementary" effects of urea described by Klopfenstein et al. (1978). The role of urea producing a shift in the site of digestion has not been elucidated. As discussed earlier, a deficiency of isoacids as well as nitrogen in diets supplemented with corn based proteins, may be responsible for low rumen microbial growth and high bypass of dietary constituents.

Lower Tract Digestion

The percent of total digestion occuring in the lower tract (duodenum to rectum) was evaluated (Tables 13,14,15). In addition, the percent digestion of dietary constituents occuring in the lower tract was evaluated.

The apparent digestion of nitrogen occuring at the duodenum, with Cr(EDTA) as a marker, was significantly less for WDG supplemented diets compared to urea diets; 57.41 versus 67.47% ($P<0.10$). Although not significantly different, the WDG diets had less lower tract N digestion of that which arrived, for lignin and Yb markers as well.

It is interesting the quantity of ADF arriving at the duodenum which is digested in the lower tract. Estimates range from a low of 10.77% of the ADF arriving in the WDG diet with lignin as a marker to a high of 44.26% in the WDG-Urea diet with Cr(EDTA) as a marker. Putnam and Davis (1965) report alfalfa and wood cellulose fiber had a digestion coefficient of 29% when placed directly into the abomasum compared to 43 and 63 percent respectively when fed per os. Dixon and Nolan (1982) report significant fermentation and absorption of dietary constituents bypassing the small intestine. The fermentation and absorption occurs in the cecum and proximal colon and to a much less extent in the more distal portion of the large intestine. Clearly, the lower tract plays a major role in digestion and absorption of dietary constituents, including ADF. This illustrates the potential exists to have optimal animal performance despite limited ruminal fermentation. The role of the lower tract is often overlooked, especially the role of the cecum or proximal colon in ADF digestion. This area deserves more study, especially with respect to the contribution when diets such as WDG-Urea are utilized.

Total Tract Digestion

The total tract digestion of dietary constituents is listed in Tables 13, 14 and 15. Diets containing urea had significantly greater total tract N digestion compared to WDG supplemented diets ($P<0.05$), (75.2 vs. 65.3%) for lignin and

(72.1 vs. 62.9%) for Cr as markers. The same trend was exhibited when Yb was utilized as a marker (71.3 vs. 63.9); however, the means did not differ (P>0.10).

Table 16 illustrates an estimate of the digestion of protein from corn silage, soybean meal and wet distillers grains. The calculation represent the pooled means of total tract nitrogen digestibility as predicted by lignin, Yb and Cr as markers. The calculation assumes a 100% digestion of urea and no associative effect of protein source on corn silage digestibility. It appears the corn silage nitrogen is 55.9% digestible in the total tract. Soybean meal and wet distillers grains nitrogen digestibility were 95.9% and two estimates of 76.9 and 69.0% for wet distillers grains.

Diets supplemented with WDG alone exhibited higher total tract OM digestion than diets supplemented with WDG-Urea (77.3 vs. 68.7%) with lignin as a marker (P<0.05) and (72.5 vs. 68.0%) with Cr as a marker (P<0.10). The same trend for OM digestion, total tract, existed when Yb was used as a marker but the means did not differ significantly (P>0.10) (73.8 vs. 71.1%) for SOY vs. WDG-Urea and (73.8 vs. 68.5%) for SOY vs. WDG.

Total tract ADF digestion was significantly greater for diets supplemented with SOY versus WDG (60.2 vs. 53.4%) for lignin as a marker (P<0.05). When Yb was utilized as a marker, differences were not significant (P>0.10) but similar trends existed (54.0 vs. 51.4% for soy vs. WDG). When Cr was utilized as a marker, the differences also were not significant, however, the urea diets had numerically greater

ADF digestion than the WDG supplemented diet (56.0 vs. 51.4%).

Despite a trend for a large amount of rumen bypass of dietary constituents in the WDG-Urea diet, the data illustrate the potential contribution, to total tract digestion, supplied by the lower tract. However, in general, diets containing WDG and WDG-Urea had less total tract digestion of dietary constituents as compared to diets containing soybean meal or urea.

These results are similar to data presented by Waller et al. (1980). Diets supplemented with distillers grains and urea for lambs, tended to have lower total tract OM digestion than diets supplemented with urea. Klopfenstein et al. (1976) show higher total tract digestion of N and DM in diets containing soybean meal and urea than for diets containing either corn distillers dried grains or grains with solubles.

ADIN Flow-Total Tract

Tables 11 and 12 list the percent of total dietary nitrogeh component of acid detergent insoluble nitrogen (ADIN) (Van Soest, 1965) and total intake per day of ADIN respectively.

ADIN is often a significant portion of the total nitrogen present in forages. Goering et al. (1974) report ADIN in dehydrated alfalfa ranging from 6.6 to 20.4% of the total nitrogen. Yu (1976) has reported the role of heat and moisture in the formation of ADIN in alfalfa stored in several

ways. Sutton and Vetter (1971) have shown the decreased nitrogen retention in lambs fed haylage with high ADIN. In our research, corn silage appeared to average 4.4% and wet distillers grains averaged 15% ADIN as a percent of the total N.

Tables 13,14 and 15 illustrate the ADIN total tract flow. It is evident that ADIN present in WDG diets was digestible. ADIN in soybean meal and urea diets flowed from the tract at between 79.1 and 100.5% of intake depending upon protein source or marker selected. This indicated only marginal digestibility, if any, for ADIN in the SOY and urea supplemented diets.

ADIN flow was significantly less ($P<0.05$) in WDG diets as compared to the soy diet, (79.1 vs. 42.6%), 90.1 vs. 43.2%) and (100.5 vs. 46.0%) for lignin, Yb and Cr markers respectively. It is reasonable to assume that ADIN produced during the ensiling and storage of forages is virtually indigestible. However, ADIN present in wet distillers grains did appear to be quite digestible in the total tract.

Rumen flow of ADIN was not measured. ADF residue in duodenal samples could not be effectively removed from the Gooch crucibles without damaging the fritted glass filter portion. The material adhered very tanaciously and could not be remove without washing and subsequent ashing of the crucibles.

Lignin Flow-Total Tract

Total tract flow of lignin was evaluated utilizing Yb and Cr as markers. Tables 14 and 15 illustrate the lignin flow as a percent of intake. Although there were no significant differences between diets for lignin flow, there does appear to be a trend toward lower lignin recovery in WDG supplemented diets than in the soy and urea diets.

Muntifering et al. (1981) has reported the relationship between dietary source of lignin, lignin recovery and gravimetric analysis methodology. It is reported that utilizing the acid lignin method, (72% sulfuric acid treatment of ADF residue, Goering and Van Soest, 1970), lignin recovery often exceeds 100% when lignin is forage related (fescue in Muntifering's research). When lignin in the diet is related to seed coat and related materials, lignin digestion is extensive (seed related i.e. corn cobs, cottonseed hulls in Muntifering's research).

In our research, corn silage diets supplemented with wet distillers grains and wet distillers grains with urea, contained 33 and 16% of these feedstuffs on a dry matter basis (see Table 5). Despite the high levels of wet grains, dietary lignin levels were equal to or higher than in diets supplemented with soy and urea (refer to Tables 11 and 12). Therefore, wet distillers grains were contributing a significant amount of lignin to the diet.

Table 12 Experiment One: Intake of Dietary Constituents

Component		Diet				
		CS-Soy	CS-Urea	CS-WDG	CS-WDG,Urea	SEM
Intake/Day[1]						
Dry Matter	kg	6.80	7.00	6.60	6.80	
Nitrogen	g	152.80	147.00	144.50	145.20	
ADIN	g	5.50[a]	6.58[a]	14.70[b]	11.60[c]	.35
Organic Matter	kg	6.40	6.60	6.20	6.40	
ADF	kg	1.40	1.50	1.40	1.50	
Lignin	g	160.50	179.20	185.50	198.60	
Ytterbium	g	2.61	2.72	2.55	2.56	
Chromium	g	3.76	4.10	3.37	3.86	

[1] Means within the same row with different superscripts differ ($P<0.05$).

Table 13 Experiment One: Lignin as a Marker

Component	Diet				
	CS-Soy	CS-Urea	CS-WDG	CS-WDG,Urea	SEM
Rumen (Apparent,% intake)					
Nitrogen Loss	52.02	47.62	48.10	42.44	5.16
NAN Flow	44.94	49.13	51.35	54.32	4.90
OM Digestion	50.56	51.78	47.60	43.83	3.13
ADF Digestion	49.16	50.74	47.84	43.83	3.13
Lower Tract (Apparent Digestion)					
N % of Intake	22.45	27.60	17.25	24.92	5.32
N % of Arrived	45.88	50.42	33.20	36.98	5.36
OM % of Intake	26.71	24.58	21.71	29.38	3.90
OM % of Arrived	52.17	49.88	44.70	46.50	5.57
ADF % of Intake	11.04	9.07	5.62	10.66	2.26
ADF % of Arrived	19.94	15.60	9.60	15.49	3.52
Total Tract (Apparent Digestion)[1]					
Nitrogen % of Intake	74.48[a]	75.22[a]	65.35[b]	67.36[b]	1.46
OM % of Intake	77.28[a]	76.36[a]	69.32[ab]	68.66[b]	1.98
ADF % of Intake	60.20[a]	59.81[ab]	53.46[c]	54.52[abc]	1.39
ADIN Flow % Intake	79.06[a]	66.76[ac]	42.63[b]	54.30[bc]	5.00

[1] Means within the same row with different superscripts differ ($P<0.05$)

Table 14 Experiment One: Ytterbium as a Marker

Component	Diet				
	CS-Soy	CS-Urea	CS-WDG	CS-WDG,Urea	SEM
Rumen (Apparent,% intake)					
Nitrogen Loss	30.07	26.63	27.62	25.36	7.07
NAN Flow	65.70	68.72	68.42	70.30	7.03
OM Digestion	30.52	32.28	27.22	20.67	6.83
ADF Digestion	27.85	33.77	28.74	28.09	2.66
Lower Tract (Apparent Digestion)					
N % of Intake	40.44	44.65	36.28	41.44	5.32
N % of Arrived	57.83	60.86	50.12	55.51	3.20
OM % of Intake	43.26	39.63	41.29	50.40	3.82
OM % of Arrived	62.28	58.52	56.73	63.53	2.06
ADF % of Intake	26.18	19.19	22.64	24.63	6.14
ADF % of Arrived	36.28	28.97	31.77	34.24	7.58
Total Tract (Apparent Digestion)[1]					
Nitrogen % of Intake	70.50	71.28	63.90	66.80	3.24
OM % of Intake	73.79	71.92	68.51	71.08	2.88
ADF % of Intake	54.00	52.96	51.38	52.72	5.75
ADIN Flow % Intake	90.12[a]	78.46[ab]	43.16[b]	55.53[ac]	8.87
Lignin Flow % of Intake	116.18	115.53	106.44	98.42	14.93

[1] Means within the same row with different superscripts differ ($P<0.05$)

Table 15 Experiment One: Chromium as a Marker

Component	Diet				
	CS-Soy	CS-Urea	CS-WDG	CS-WDG,Urea	SEM
Rumen (Apparent,% intake)					
Nitrogen Loss	20.52	14.26	14.21	12.61	5.62
NAN Flow	73.25	79.66	81.10	82.37	5.65
OM Digestion	19.52	19.89	15.12	8.26	4.80
ADF Digestion	15.44	22.53	15.54	12.92	3.81
Lower Tract (Apparent Digestion)[1]					
N % of Intake	48.47	57.85	48.68	53.09	4.70
N % of Arrived	60.97[xy]	67.47[x]	57.41[y]	60.75[xy]	2.09
OM % of Intake	53.00	52.75	55.44	59.75	4.96
OM % of Arrived	65.85	65.85	65.28	65.12	1.96
ADF % of Intake	36.92	33.50	35.84	38.54	5.35
ADF % of Arrived	43.66	43.23	42.42	44.26	5.51
Total Tract (Apparent Digestion)[1]					
Nitrogen % of Intake	68.98[ab]	72.11[a]	62.89[b]	65.70[ab]	1.78
OM % of Intake	72.52[x]	72.64[x]	70.56[xy]	68.00[y]	1.18
ADF % of Intake	52.36	56.02	51.37	51.46	3.43
ADIN Flow % Intake	100.48[a]	80.48[ac]	46.04[b]	58.59[bc]	6.23
Lignin Flow % of Intake	125.40	116.00	110.40	105.20	8.30

[1] Means within rows with different superscripts (x,y) differ ($P<0.10$) or (a,b,c) differ ($P<0.05$)

Table 16 Experiment One: Digestibility of Nitrogen[1]

Component	Diet			
	CS-Soy	CS-Urea	CS-WDG	CS-WDG,Urea
Total Tract (Apparent Digestion, % of Intake)				
Nitrogen[2]	71.3	72.9	64.0	66.6
Urea[3]	--	100.0	--	100.0
Corn Silage N[4]	55.9	55.9	55.9	55.96
Protein N	95.9	--	76.9	69.0

1) Based upon pooled means of all marker studied (lignin. Yb, Cr)
2) Apparent N digestibility,(feed + endogenous)
3) Assumes a 100 % absorption of urea N
4) Assumes no associative effects of protein source upon corn silage N digestibility

Lignin levels in the wet distillers grains were not assayed, however, the lignin present is most likely of a corn cob or other seed coat related source. As a result, this lignin, as discussed by Muntifering, would tend to be more digestible than lignin from the forage material, corn silage. As a result, lignin recovery in fecal composites tend to be lower in wet distillers grain supplemented diets than in soy and urea supplemented diets. This favored digestibility of dietary constituents in the soy and urea supplement corn silage diets as compared to diets supplemented with wet distillers grains.

Comparison of Digesta Markers: Rumen and Total Tract Estimates

Tables 17 through 20 illustrate the potential differences in rumen nitrogen flow, OM and ADF rumen digestion estimates as the result of marker choice. It is evident that significant differences in estimates exist as a result of marker choice.

The data indicate that lignin estimates of digesta flow from the rumen are generally less than those derived from Cr data. This occured despite increasing duodenal Cr values by 5% to correct for absorption. These data indicate the level of lignin in duodenal samples were elevated relative to Cr. Faichney (1980a) proposed that this is the result of a failure to collect duodenal samples which are representative of true digesta passing the sampling point. The samples

taken may not contain a representative amount of liquid, containing Cr, and a disproportionate amount of solids containing lignin. The low Cr levels in the duodenal samples, thus favoring elevated estimates of flow and less rumen digestion, may be due to the more rapid turnover rate of Cr carried by the liquid phase. This would tend to result in low Cr levels in duodenal composites.

Faichney (1980b) has indicated Cr(51) complex of EDTA had a mean retention time of 12.6 compared to lignin of 59.2 hours. Ellis and Huston (1968) have shown the liquid marker PEG had a mean retention time of 26.5 versus 32.7 hours for cerium attached to alfalfa hay materials. We fed the CrEDTA once with the feeding of the total diet at 8 am. Due to the rapid flow of Cr from the rumen, Cr levels in duodenal samples taken late in the day will contain much less Cr than those taken shortly after feeding. This most likely is related to the significantly different estimates of flow and digestion for lignin and Cr markers.

The data indicate no significant differences exist for total tract digestibility of N, OM and ADF as the result of marker choice. However, as discussed earlier, Tables 14 and 15 indicate that there may be a difference in lignin recovery due to dietary lignin source. The data indicate that lignin from forages tends to produce small increases in total tract lignin recovery based on the acid lignin procedure. In contrast, lignin from seed related products tends to be more digestible resulting in lower lignin recovery, especially in the WDG and WDG-Urea diets. This results in (non-

Table 17 Experiment One: Corn Silage- Soybean Meal

Site and Component	Marker			
	Lignin	Yb	Cr	SEM
Rumen Nitrogen Flow[1,2]	47.0[a]	69.9[ab]	79.5[b]	6.30
Total Tract Nitrogen Digestion	74.5	70.5	68.9	3.50
Rumen OM Flow	49.4[a]	69.5[ab]	80.4[b]	6.20
Total Tract OM Digestion	77.3	73.8	72.5	6.80
Rumen ADF Digestion	49.2	27.8	15.4	7.80
Total Tract ADF Digestion	60.2	54.0	52.4	5.10

1) Expressed as a % of intake
2) Means within the same row with different superscripts differ (P<0.05)

Table 18 Experiment One: Corn Silage-Urea

Site and Component	Marker			
	Lignin	Yb	Cr	SEM
Rumen Nitrogen Flow[1,2]	52.4[a]	73.4[ab]	85.7[b]	8.20
Total Tract Nitrogen Digestion	75.2	71.3	72.1	2.50
Rumen OM Flow	48.2[a]	67.6[ab]	80.1[b]	8.20
Total Tract OM Digestion	76.4	71.9	72.6	2.30
Rumen ADF Digestion	50.7	33.8	22.5	7.80
Total Tract ADF Digestion	59.8	53.0	56.0	4.10

1) Expressed as a % of intake
2) Means within the same row with different superscripts differ (P<0.05)

Table 19 Experiment One: Corn Silage-Wet Distillers Grains

Site and Component	Marker			
	Lignin	Yb	Cr	SEM
Rumen Nitrogen Flow[1,2]	51.9[a]	72.4[ab]	85.8[b]	5.59
Total Tract Nitrogen Digestion	67.4	66.8	65.7	1.50
Rumen OM Flow	52.4[a]	72.8[ab]	84.9[b]	5.97
Total Tract OM Digestion	69.3	68.5	70.6	3.85
Rumen ADF Digestion	47.7[a]	28.7[ab]	15.5[b]	6.40
Total Tract ADF Digestion	53.5	51.4	51.4	5.70

1) Expressed as a % of intake

2) Means within the same row with different superscripts differ ($P<0.05$)

Table 20 Experiment One: Corn Silage-Wet Distillers Grains-Urea

Site and Component	Marker			
	Lignin	Yb	Cr	SEM
Rumen Nitrogen Flow[1,2]	57.6[a]	74.6[ab]	87.4[b]	5.60
Total Tract Nitrogen Digestion	67.4	66.8	65.7	1.50
Rumen OM Flow	60.7[a]	79.3[ab]	91.7[b]	5.30
Total Tract OM Digestion	68.7	71.1	68.0	2.20
Rumen ADF Digestion	43.8[a]	28.1[ab]	12.9[b]	6.50
Total Tract ADF Digestion	54.5	52.7	51.5	3.30

1) Expressed as a % of intake

2) Means within the same row with different superscripts differ ($P<0.05$)

significant) slightly higher, digestion coefficients for soy and urea diets compared to the WDG and WDG-Urea diets.

Evaluation of Markers: Selection Based on Magnitude of the SEM

The magnitude of the standard error of the mean (SEM) is an important component of the data set. The SEM value provides information regarding the size of the error or residual components in the analysis of variance. This term indicates, based on its magnitude, the efficiency of the experimental design, blocking and indicates the variation inherent in the sample population. The lower the SEM value, the easier it is to show significant differences between means. Therefore, experimental designs which reduce the error or residual term, and consequently the SEM, are preferred to other designs. In digesta passage studies, it is a common practice to utilize Latin square designs. These designs partition variation into treatment effects, the contribution of nuisance variables such as periods of time and animals and error or the residual variation.

Table 21 summarizes the SEM values for estimates of flow and digestibility of major dietary constituents based on lignin, Yb and Cr as markers. In general, SEM values are lowest for lignin, followed by Cr and highest for Yb. The SEM values are similar to values found in the literature for similar designs. Crickenberger et al.(1979), utilizing lignin as a marker, reports an SEM value of 9.2 for nitrogen passage,

% of N intake. Galyean et al.(1979) report a SEM for rumen OM digestion of 3.2 with lignin as a marker. Waller et al. (1980) reported dry matter and nitrogen total tract digestibility, utilizing sheep, estimates resulting in SEM values of 1.09 and 1.17 respectively. These values are somewhat less, but very similar to values for total tract digestion of organic matter and nitrogen based on lignin and Cr. These values are lower than those reported for Yb.

The data would indicate, based on the magnitude of the SEM values, that lignin is an excellent marker for digesiton and flow research. However, as discussed earlier, there are problems related to differential recovery of lignin. These problems with lignin recovery are related to the dietary source of lignin (forage or seed related) and the somewhat qualitative nature of the gravimetric method of analysis.

The CrEDTA complex is an excellent marker based on its low SEM values. However, it is well documented there is substantial absorption of Cr from the digestive tract (Goodall and Kay, 1973). It also tends to overestimate flow of dietary constituents, particularly acid detergent fiber, from the rumen. Consequently, suspicious estimates of lower tract ADF digestion are common when Cr(EDTA) is used as a marker.

Despite high SEM values for Yb estimates of digestion and flow, there are significant reasons it is superior to lignin and Cr as a marker. Yb is not absorbed from the digestive tract as CrEDTA and analysis is much more sensitive than the gravimetric lignin procedure. A low SEM value should

not be the sole criteria utilized for marker selection. The nonabsorption and accurate quantification criteria are of equal importance.

Table 21 Experiment One: Comparison of Marker SEM Estimates

Component	Marker		
	Lignin	Yb	Cr
	SEM		
Rumen (Apparent, % Intake)			
N Loss	5.16	7.07	5.62
NAN Flow	4.90	7.03	5.65
OM Digestion	4.40	6.83	4.80
ADF Digestion	3.13	2.66	3.81
Lower Tract (Apparent Digestion)			
N, of Intake	5.32	5.32	4.70
N, of N Arriving	5.36	3.20	2.09
OM, of Intake	3.90	3.82	4.96
OM, of OM Arriving	5.57	2.06	1.96
ADF, of Intake	2.26	6.14	5.35
ADF, of ADF Arriving	3.52	7.58	5.51
Total Tract (Apparent Digestion)			
Nitrogen	1.46	3.24	1.78
Organic Matter	1.39	2.88	1.18
ADF	1.98	5.75	3.43
ADIN Flow	5.00	8.87	6.23

RESULTS AND DISCUSSION

Experiment Two

The Effect of Soybean and Corn Gluten Meal Supplementation Upon Site and Extent of Digestion of Corn Silage Based Diets by Steers.

Rumen Disappearance and Flow

Based on marker estimates (Tables 22, 23, 24, 25) there were no significant effects of protein source upon apparent rumen disappearance of nitrogen (N), organic matter (OM), acid detergent fiber (ADF) or flow of non-ammonia nitrogen (NAN) to the lower tract.

Calculations of abomasal flow based on PEG infusion is shown in Table 26. Based on the dilution of PEG and subsequent flow calculations, diets supplemented with corn gluten meal exhibited greater total dietary nitrogen flow to the lower tract ($P< 0.10$). Estimates of flow were 102.83 and 126.02 percent of dietary nitrogen intake for soybean and corn gluten meal respectively.

In these diets, both soybean (SOY) and corn gluten meal (CGM) proteins supplied approximately 38.5% of the dietary N. Stern et al. (1983) report CGM bypass of 57%; other available estimates include 38% (Waller, 1978), 46% at 1.2 x maintainance, 61% at 1.6 x maintenance (Zinn et al., 1981).

Estimates of soybean meal bypass include 20% (Krop et al., 1976) and 35% (Stern and Satter, 1982).

Based upon soybean protein bypass of 20%, CGM bypass of 61%, 38.5% of dietary N from these sources, assuming equal corn silage N degradability, the CGM diet would theoretically have 15.8 % higher flow of dietary N than SOY diets. Assuming literature values of bypass being equally valid, using SOY bypass of 35% and CGM of 38%, CGM diets would have 1.5% more flow of dietary N, as a percent of intake.

Estimates of dietary N flow are 1.5, 7 and 23% higher for the CGM diets based on lignin, chromium and PEG infusion as markers respectively. Estimates of dietary N flow based on Ytterbium and lanthanum, respectively, were 1 and 9% less for CGM diets compared to SOY diets.

Bypass of nitrogen from soybean meal and corn gluten meal, although not directly measured, appear to be very similar. This would be in agreement with literature values for bypass of nitrogen. The differences between the two protein sources bypass potential is essentially to small to detect with any degree of statistical significance.

Data reported by Loerch et al. (1983) illustrate how decreased rumen pH will reduce the degradation rate of soybean meal to a much greater extent than corn gluten meal. These observations would lend more evidence to support finding of very little difference between N flow to the lower tract when diets contain soybean meal or corn gluten meal.

Rumen disappearance of OM and ADF showed no trends toward differences between the diets. This observation is

not surprising given the very similar amount of nitrogen available in the rumen, regardless of protein source, based on the flow data.

Lower Tract Digestion

The potential contribution of lower tract digestion to total digestion of dietary constituents is illustrated in tables 22, 23, 24 and 25. There were no significant differences in digestibility of N, OM or ADF as a % of intake or as a % of arrived.

Digestion of ADF arriving at the lower tract averaged 10% when diets and marker estimates were pooled together. These data represent lower estimates of lower tract ADF digestion potential than observed in experiment two. Putman and Davis (1965) and Dixon and Nolan (1982) have illustrated the importance of the lower tract for digestion and absorption of both fermentation endproducts from ADF and OM fermentation and nitrogenous constituents. The lower tract is plays a significant role in the digestive processes of the ruminant.

Total Tract Digestion

The total tract digestion of N is reported in Tables 22-25. Apparent nitrogen digestion was 2.9, 4.7, 6.4 and 3.6% greater for the CGM suplemented diets in relation to the SOY supplemented diets based on lignin, La, Yb and Cr as markers

respectively. Differences were significant based on Yb and Cr markers ($P < 0.05$). Klopfenstein et al. (1976) reported lower total tract N digestion for diets supplemented with corn distillers products as compared to SOY or urea supplemented diets. Data in experiment one illustrated these differences as well, where diets supplemented with wet distillers grains had lower total tract nitrogen digestion than diets containing soybean meal.

Reiners and Watson (1975) have evaluated the variation in nutrient content of the high protein (60%) corn gluten meal products from wet milling of corn. DeMuelenaere et al. (1967) reported virtually 100% of the lysine in CGM was available in their studies with rats. Misra and Potter, (1970) report 92 to 96% total tract digestion of nitrogen in turkeys fed CGM. CGM proteins are very available to monogastric species and as observed in this experiment, very available to the ruminant. The nitrogen availability appears to be equal to or slightly greater than soybean meal by our evaluation.

The total tract digestion of OM did not differ ($P > 0.10$) due to source of supplemental protein. There is a trend for a somewhat greater total tract OM digestion in diets containing CGM. The trend was evident regardless of the marker selected.

Total tract ADF digestion did not differ significantly ($P > 0.10$) with either protein supplement. A trend toward slightly greater total tract ADF digestion in diets supplemented with CGM was evident regardless of digesta

Table 22 Experiment Two: Lignin as a Marker

Component	Diet		
	CS-Soy	CS-CGM	SEM
Rumen (Apparent,% intake)			
Nitrogen Flow	88.58	90.03	4.38
NAN Flow	85.02	82.51	6.53
OM Digestion	56.12	57.63	1.90
ADF Digestion	52.16	52.97	.30
Lower Tract (Apparent Digestion)			
N % of Intake	58.11	61.46	4.52
N % of Arrived	65.60	68.27	2.07
OM % of Intake	14.73	14.33	1.62
OM % of Arrived	35.57	33.82	3.71
ADF % of Intake	-1.77	.18	.65
ADF % of Arrived	-3.70	.38	1.34
Total Tract (Apparent Digestion)			
Nitrogen % of Intake	69.53	71.43	.23
OM % of Intake	70.85	72.30	.18
ADF % of Intake	50.39	54.08	.45

Table 23 Experiment Two: Ytterbium as a Marker

Component	Diet		
	CS-Soy	CS-CGM	SEM
Rumen (Apparent,% intake)			
Nitrogen Flow	111.78	110.90	4.12
NAN Flow	108.23	103.22	6.19
OM Digestion	41.62	46.64	1.96
ADF Digestion	38.15	41.55	1.62
Lignin Flow	127.70	128.40	3.65
Lower Tract (Apparent Digestion)			
N % of Intake	78.86	81.17	4.16
N % of Arrived	70.55	72.82	1.13
OM % of Intake	26.86	23.76	1.76
OM % of Arrived	46.00	44.56	1.23
ADF % of Intake	9.12	9.24	.73
ADF % of Arrived	14.76	15.82	.19
Total Tract (Apparent Digestion)[1]			
Nitrogen % of Intake	67.08[a]	70.26[b]	.14
OM % of Intake	68.48	70.41	.18
ADF % of Intake	47.28	50.80	.89
Lignin Flow	108.38	107.62	.58

[1] Means within the same row with different superscripts differ ($P<0.05$)

Table 24 Experiment Two: Lanthanum as a Marker

Component	Diet		
	CS-Soy	CS-CGM	SEM
Rumen (Apparent,% intake)			
Nitrogen Flow	99.50	90.30	2.07
NAN Flow	95.96	82.66	4.14
OM Digestion	49.12	55.25	2.70
ADF Digestion	45.90	51.83	2.21
Lignin Flow	113.02	106.14	5.30
Lower Tract (Apparent Digestion)			
N % of Intake	67.50	62.69	5.40
N % of Arrived	67.40	69.27	3.76
OM % of Intake	20.11	15.66	4.63
OM % of Arrived	38.26	36.99	8.05
ADF % of Intake	2.84	2.42	4.00
ADF % of Arrived	6.18	5.33	5.73
Total Tract (Apparent Digestion)			
Nitrogen % of Intake	68.00	72.39	3.33
OM % of Intake	69.23	73.44	3.66
ADF % of Intake	48.57	54.25	6.09
Lignin Flow	107.30	100.82	12.42

Table 25 Experiment Two: Chromium as a Marker

Component	Diet		
	CS-Soy	CS-CGM	SEM
Rumen (Apparent,% intake)			
Nitrogen Flow	117.70	124.87	9.40
NAN Flow	114.15	117.13	11.40
OM Digestion	37.96	39.00	4.96
ADF Digestion	34.72	39.00	.89
Lignin Flow	134.27	142.89	1.26
Lower Tract (Apparent Digestion)			
N % of Intake	85.28	94.86	9.30
N % of Arrived	72.45	75.97	1.42
OM % of Intake	30.64	31.49	4.36
OM % of Arrived	49.38	51.60	3.18
ADF % of Intake	12.69	12.08	1.17
ADF % of Arrived	19.45	19.80	.83
Total Tract (Apparent Digestion)[1]			
Nitrogen % of Intake	67.58[a]	69.99[b]	.12
OM % of Intake	68.60	70.48	.60
ADF % of Intake	47.42[a]	51.08[b]	.28
Lignin Flow	107.71	107.03	.42

[1] Means within the same with different superscripts differ (P<0.05)

Table 26 Experiment Two: PEG Infusion

Component	Diet		
	CS-Soy	CS-CGM	SEM
Rumen (Apparent,% intake)[1]			
Nitrogen Flow	102.83[x]	126.02[y]	1.46
NAN Flow	99.30	118.37	3.52
OM Digestion	35.05	30.23	.84
ADF Digestion	40.38	31.94	3.71
Lignin Flow	123.83	149.75	11.45
Yb Flow	94.88	110.73	3.99
La Flow	107.61[a]	145.01[b]	.99
Cr Flow	87.70	99.04	6.34

[1] Means within the same row with different superscripts (x,y) differ (P<0.10) or (a,b) (P<0.05).

marker selected.

Lignin Flow: Total Tract

Lignin flow, as a percent of intake, averaged across markers and diets, was 106.5%. Data reported in experiment one indicates a lignin flow of 111.6% when all markers and dietestimates are averaged. These data are consistent with the observations of Muntifering et al. (1981). Muntifering indicates there may be production of an artifact lignin moiety when the acid lignin method is utilized.

Comparison of Digesta Markers: Rumen and Total Tract Estimates

Tables 27 and 28 depict the consequences of marker choice on rumen flow and total tract digestibility estimates. In general, rumen flow of dietary constituents tends to be the lowest when lignin is utilized as a marker, often significantly lower than estimates based on Yb and Cr(EDTA) markers. This is an identical observation as described in experiment one. In this study, Yb was often intermediate in estimation of flow and digestibility. It often produced estimates which were not significantly different from either lignin of Cr(EDTA) marker estimates.

Lanthanum provided rumen flow estimates very similar to those generated by lignin. In addition, the standard errors based on La as a marker were much larger than for other

Table 27 Experiment Two: Corn Silage-Soybean Meal Diets

Site and Component	Marker					
	Lignin	La	Yb	Cr	PEG	SEM
Rumen Nitrogen Flow	88.58	99.50	111.78	117.70	102.83	6.26
Total Tract N Digestion	69.53	68.00	67.08	67.58		5.16
Rumen OM Disappearance[1]	56.12[ab]	49.12[b]	41.62[abc]	37.96[bc]	35.05[c]	3.06
Total Tract OM Digestion	70.85	68.00	68.48	68.60		2.27
Rumen ADF Digestion[1]	52.16[a]	45.90[ab]	38.15[ab]	34.72[b]	40.38[ab]	3.34
Total Tract ADF Digestion	50.39	48.57	47.28	47.42		3.86

1) Means within the same row with different superscripts differ ($P<0.05$)

Table 28 Experiment Two: Corn Silage-Corn Gluten Meal

Site and Component	Marker					
	Lignin	La	Yb	Cr	PEG	SEM
Rumen Nitrogen Flow[1]	90.03[a]	90.30[a]	110.90[ab]	124.87[b]	126.02[b]	.74
Total Tract N Digestion	71.43	72.39	70.26	69.99		2.11
Rumen OM Disappearance[1]	57.63[a]	55.25[a]	46.64[ab]	39.00[bc]	30.23[c]	.48
Total Tract OM Digestion	72.30	73.44	72.30	70.50		2.57
Rumen ADF Disappearance	52.97	51.83	41.55	39.00	31.94	4.94
Total Tract ADF Digestion	54.08	54.25	50.80	51.08		3.65

1) Means within the same row with different superscripts differ ($P<0.05$)

markers utilized.

As observed in experiment one and substantiated in the literature, there were no significant differences between any total tract digestibility estimates resultant from marker choice. Due to elevated lignin recovery (106.5% of intake), total tract digestibility estimates based on lignin are consistantly slightly higher than for other markers.

Rumen flow data generated by abomasal PEG infusion and subsequent calculation of digesta flow by degree of PEG dilution is shown in Table 26. These data are based on an assumption that the sum of the 12 flow estimates (every 2 hours of a 24 hour day) were accurate estimates of flow. This may be and most likely is not a valid assumption as marker recovery appears to be too variable and overestimated. Multiple linear regression techniques, regressing flow estimates against a time transform including one set of polynomials of the harmonic series (Fourier Transform) may prove beneficial.

Evaluation of Markers: Magnitude of the SEM

As discussed in experiment one, the SEM value can provide information regarding the error or residual component of the analysis of variance. Table 29 summarizes the SEM for all components evaluated and markers utilized. The experimental design in this study was a replicated Latin square crossover. This provided additional opportunities to reduce the residual term as compared to single Latin squares.

As a result, the SEM values tend to be less than those reported for experiment one, Table 21. Yb SEM values were much lower than those observed in experiment one. Estimates for Cr are generally less but in some instances greater than in experiment one. Estimates of the SEM values for La were extremely high in this study and as observed in experiment one, this may be representative of error associated with the analytical techniques for detection of lanthanum.

Evaluation and selection of a suitable digesta marker based solely upon low SEM values can result in compromising precision for accuracy. It is possible to predict digestibility with great precision and yet not have an accurate estimate.

The CrEDTA complex represents an instance where precision may be high but accuracy is suspect. Absorption of Cr occurs from the digestive, however, SEM values are often quite low when it is used as a marker.

Forage derived lignin recovery is consistantly overestimated both ruminally and by total tract evaluation. Although SEM values are often quite satisfactory, consistantly elevated estimates of digestibility occur.

The rare earth elements, La and Yb, are excellent markers in terms of the absence of absorption or elevated SEM values. This produces difficulties. In essence, they may appear to be markers of great accuracy but lacking precision. Yb derived SEM values in experiment two are very acceptable. This observation and the documented predictable recovery of

the marker result in it providing excellent characteristics as a digesta marker. Detection, both by neutron activation and atomic absorption spectroscopy, is very sensitive.

Therefore, Yb represents a marker which is analytically quantifiable, totally nonabsorbable and produces acceptable variation in estimates of digestibility. The marker is nontoxic, reasonably priced and provides excellent total tract estimates of digestibility. It is definately a marker of choice for total tract studies and of great potential for estimating rumen flow and total tract digestibility of dietary constituents.

Table 29 Experiment Two: Comparison of Marker SEM Estiments

Component	Marker			
	Lignin	La	Yb	Cr
	SEM			
Rumen (Apparent, % intake)				
N Flow	4.38	2.07	4.12	9.40
NAN Flow	6.53	4.14	6.16	11.40
OM Digestion	1.90	2.70	1.96	4.96
ADF Digestion	.30	2.21	1.62	.89
Lower Tract (Apparent Digestion)				
N % of Intake	4.52	5.40	4.16	9.30
N % of N Arriving	2.07	3.76	1.13	1.42
OM % of Intake	1.62	4.63	1.76	4.36
OM % of OM Arriving	3.71	8.05	1.23	3.18
ADF % of Intake	.65	4.00	.73	1.17
ADF % of ADF Arriving	.34	5.73	.19	.83
Total Tract (Apparent Digestion)				
Nitrogen % of Intake	.23	3.33	.14	.12
Organic Matter % of Intake	.18	3.66	.22	.60
ADF % of Intake	.45	6.09	.89	.28

Evaluation of Digesta Flow Patterns in Steers Fed Corn Silage Based Diets

Data discussed earlier in this thesis illustrated the presence of large variability in estimates of ruminal digestibility as a consequence of marker choice and/or compositing techniques. The variation in digestibility estimates is shown in Tables 17-20 and 27-28. These data indicate the presence of variable recovery of digesta markers either due to analytical techniques, marker absorption or compositing techniques. Similar observations have been discussed by Sachtleben (1980).

As discussed earlier, elevated marker recovery at the duodenum results in elevated ruminal flow and reduced digestibility estimates. Low recovery of digesta markers in duodenal samples has been reported by many researchers (Laughren and Young, 1979; Sutton et al., 1974). Estimates often range from 50 to more than 100% recovery but are usually less than 100%. Nicholson and Sutton (1969) have reported lower marker recovery during the first 12 hours of a 24 hour total collection scheme. It appears the presence of differential rates of digesta and markers, as eluded to by Nicholson and Sutton (1969) indicates the flow rates may be the source of marker recovery problems.

The flow of digesta to the duodenum is quite complex. Singleton (1961) reported duodenal flow to be both phasic and

with variations in the magnitude and frequency of digesta flow gushes. Harris and Phillipson (1962) have reported diurnal variation in flow volume in sheep with reentrant cannulae. Conceivably, the variation in marker recovery, leading to different estimates of digestibility, as a result of marker choice or other causes, may be related in some manner to the variation in flow observed at the duodenum. Data of van't Klooster et al. (1969) indicate extending the period of total collection from 24 to 72 hours improved marker recovery to 100%.

Potentially, a weighting system based upon scaling sample composites taken over periods of time, based on the relative flow occuring during the sampling interval, might improve marker recovery and minimize the interval required to achieve adequate marker recovery.

Harris and Phillipson (1962) clearly illustrated the presence of variation in digesta flow rate throughout the day. Peak flows tended to occur 3 hours after peak rumination activity. James et al. (1981) have reported an increase in duodenal flow rates related to increased amplitude of rumen contractions. Rumen contractions are vigorous during periods of rumination. It has been shown that there is a direct relationship between rumination patterns and duodenal flow (Balch et al, 1951; Oyaert and Bouckaert, 1961 and Topps et al., 1968).

Gordon and McAllister (1970) have shown the circadian rhythm of rumination patterns. Diurnal patterns of duodenal

digesta flow has been reported by Corbett and Pickering (1983) in grazing sheep. Increasing feeding frequency does not seem to dramatically affect the pattern of rumination as reported by Murphy et al. (1983). Rumination is very important for feed passage from the rumen as discussed by Pearce and Moir (1964).

Thompson (1973) has shown that multiple feeding of a diet to sheep reduced the amplitude of the flow patterns. Thompson fed sheep either 24 equal, hourly meals or one meal. It was reported by Thompson (1973), sheep fed 24 times per day had significantly greater total digesta flow and starch flow to the duodenum than sheep fed once per day. Goetsch and Galyean (1983) report steers fed 8 times per day may have significantly altered rates of digesta passage than when fed 2 times per day. Multiple feeding reqimes may produce a more steady flow of digesta from the rumen, allowing for better marker recovery. However, if digestibility and flow of constituents present in the rumen differs significantly from more normal feeding regimes, then the significance of these studies to more typical feeding systems is suspect.

Thompson and Lamming (1972) have shown that particle size of the diet also affects the magnitude of the flow patterns in sheep. A diet of long straw and a pelleted portion of ground corn, soybean meal and minerals, fed once daily, resulted in only minimal diurnal flow patterns. It is well accepted that feeding long hay or straw to ruminants increased the time spent ruminating. Although rumination patterns were not measured in Thompson and Lamming's

research, this probably plays a role in the flow patterns. The diurnal flow pattern was more evident when the straw was chopped to a maximum length of 2.5 cm and the patterns were most pronounced when the straw was ground and fed as a pellet. The largest amount of dietary starch reaching the duodenum occured when long of chopped straw was fed as opposed to ground and pelleted.

There have been several studies designed to evaluated the presence of digesta flow patterns in ruminants. Appendix Tables 30-38 depict the data utilized to prepare Figures 8-17. These data are besed upon abomasal infusion of PEG and calculation of percent of total daily flow occuring at each sampling time. Phillips and Dyck (1964) infused PEG 4000 into the abomasum of sheep to establish the pattern of digesta flow. They reported peak flow occured at the time of feeding and for 1 to 2 hours after feeding with a phasic pattern. Corbett and Pickering (1983) utilized a ruminal infusion of chromium-51 and ruthenium-103 to evaluate duodenal flow patterns in grazing sheep. These researchers report low flow rates at mid-day and greatest after nightfall, perhaps indicating the relationship of rumination and digesta flow. Corbett and Pickering (1983) established the validity and application of applying Fourier transformaiton of the data to predict actual flow over a period of time. They hope to utilize these regression equations to adjust the flow of OM and N to the lower tract in grazing sheep.

The use of digesta flow patterns to weight contribution of digesta samples taken from a simple cannula may provide improvements in digestibility estimate. This technique may be most valuable when animals are fed once per day or when steady state flow is not relevant to the research goals.

This section of our research involved abomasal infusion of PEG 4000. Duodenal samples were collected as illustrated by Figure 3 and PEG was analyzed by the methods described by Figure 7. Fourier analysis of the flow data was not conducted due to the need for further evaluation of the data as described graphically in Figures 8-17 and listed in Tables 29-37.

Table 26 indicates the recovery of markers and flow of digesta constituents based on PEG infusion, weighted flows of digesta. Figures 8-17 illustrate the flow patterns of the corn silage diets supplemented with soybean meal or corn gluten meal. The flow diagrams are based on means of four observations for each time period and each diet. Digesta samples were taken in early February and mid-April and means were adjusted arbitrarily for the change in daylight in a manner similar to that described by Corbett and Pickering (1983). For instance, samples taken at 8, 10, 12 PM, etc. in February were pooled with those taken at 10 AM, 12 PM and 2 PM, etc. in April respectively to correct for the effects of changes in the onset of nightfall.

As indicated in Table 26, independent of Fourier transform adjustment of the flow data, marker recovery of Yb and Cr ranged from 94.88-110.73 and 87.70-99.04%

respectively. These data are well within the range of marker recovery in animals with reentrant cannula and total digesta collection systems (Laughren and Young, 1979; Sutton et al., 1979). It is documented that Cr (CrEDTA) is 2 to 5% absorbed from the rumen; the overall Cr recovery averaged over 93% based on the PEG infusion, an excellent recovery. Yb recovery averaged 102.8%, once again a good recovery estimate. These data indicate the infusion procedure was accurately estimating the flow of these markers and the digesta phases they mark.

Lignin recovery ranged form 121.83 to 149.75% of intake based on the PEG infusion procedure. Total tract lignin flow (Tables 23-25) indicate a flow of 100 to 108%. The elevated lignin in the duodenal samples is the cause for the high rumen digestibility, low flow estimates, based on the marker in the studies reported in thesis.

Figure 12 illustrates diagramatically that lignin and ADF flow patterns are virtually identical. This is not surprising given that lignin is a component of the ADF material.

The samples of digesta most likely are too high in ADF if the lignin recovery is elevated. As a result, ruminal ADF digestion, based on Yb or Cr as markers, will be low; consequently lower tract contribution to total tract ADF digestion will be too high. A coefficient utilized to correct duodenal samples for 100% lignin recovery could be utilized to correct for ADF recovery as well. However, duodenal OM is comprised of ADF and other components. Thus

mearly correcting OM based upon elevated ADF may not be a valid method.

The presence of additional ADF in duodenal samples is similar to observations of Faichney (1974 and 1980b) that duodenal samples often contain more solid material and thus should be corected to account for low liquid recovery.

It is clear that flow of digesta to the duodenum is quite comples. In addition to the diurnal flow patterns often observed, pulses of abomasal emptying may exist which force out large amounts of fibrous materials during periods of peak flow. This may result in duodenal samples which are high in ADF and lignin, especially during periods of peak flow. Due to the complex nature of duodenal flow, it appears that simple Fourier transformation of the flow data and weighting of samples based on flow, will not provide all the solutions to problems encountered. The flow patterns do illustrate some of the aspects of the physiology of the digestive tract and the source of marker recovery problems.

Figures 8 and 13 illustrate the digesta flow patterns of OM, N and ADF in steers fed corn silage based diets supplemented with soybean meal or corn gluten meal respectively. An arrow on each of the figures indicates twhen the once per day feeding (90% of ad libitum) occured. Steers fed diets supplemented with soybean meal had peak digesta flow at 4 to 8 hours after feeding. Steers fed diets supplemented with corn gluten meal had peak flow 12 hours after feeding and low flow 4 to 8 hours after feeding. These data indicate there are diet effects on the flow patterns to

Figure 9 and 14 indicate Yb and N flow patterns are very similar. The Yb was utilized to mark the flow of corn silage material to the duodenum and it may be illustrating the flow of corn silage nitrogen to the lower tract. In contrast, Figures 11 and 16 illustrate the flow pattern of La. The La was used to mark the protein supplements, soybean and corn gluten meal. The flow pattern is quite variable and La recovery, based on PEG (Table 26), is not optimal.

Figures 10 and 15 provide evidence of the flow of Cr (CrEDTA) in relation to Yb and total digesta flow patterns. The pattern exhibits large peaks in flow occuring during peaks in total digesta flow. The CrEDTA complex is assumed to be a marker for the flow of liquid through the digestive tract. Liquid flow appears to be quite large during peak flow of digesta. Liquid flow represents the contribution of water intake and saliva flow to the rumen. Duodenal digesta from corn silage fed steers averages 5 to 6% dry matter with surprising consistency despite sampling time period.

Water (liquid) movement through the digestive tract plays a major role in digesta movement. Water serves as both a solvent and as a carrier phase for suspended material. The consistant dry matter content of duodenal digesta and high liquid flows (Cr as a marker) during periods of high digesta flow illustrate the physiological importance of water.

Sulzman (1982) reported the existance of circadian rhythms of water consumption in mice and monkeys. There is very limited information available in the literature discussing the nature of water consumption in ruminants. The

variations in digesta flow observed may be related to circadian rhythms in water consumption, not directly related to rumination patterns. Rumination patterns, however, may be controlled or influenced by water intake and thus produce diurnal, circadian rhythms in digesta flow.

The role of water intake in digestive physiology of ruminants is provided by data of Phillips (1961). It was reported water restriction, in cattle, increased apparent nutrient digestibility. Balch et al. (1953) present data indicating cows limited to 60% of their normal water consumption exhibited reduced dry matter intake. These data, presented by Phillips and Balch et al., indicate water is required for transit of digesta from the rumen. Lower dry matter intake and increased nutrient digestibilities are most likely the result of reduced rumen turnover. Increased retention time in the rumen would increase the digestion of many feed constituents but also reduce dry matter intake. It seems clear water intake, both in quantity and pattern, plays an important role in the digestive physiology of the ruminant and has been overlooked by many researchers.

Figures 8-17 utilize the following abbreviations listed in alphabetical order.

ADF: Acid detergen fiber

Cr : Chromium (CrEDTA)

DM : Dry matter

L : Lignin

La : Lanthanum (bound to protein sources)

N : Total nitrogen

OM : Organic matter

TD : Total digesta (liquids and solids)

Yb : Ytterbium (bound to corn silage)

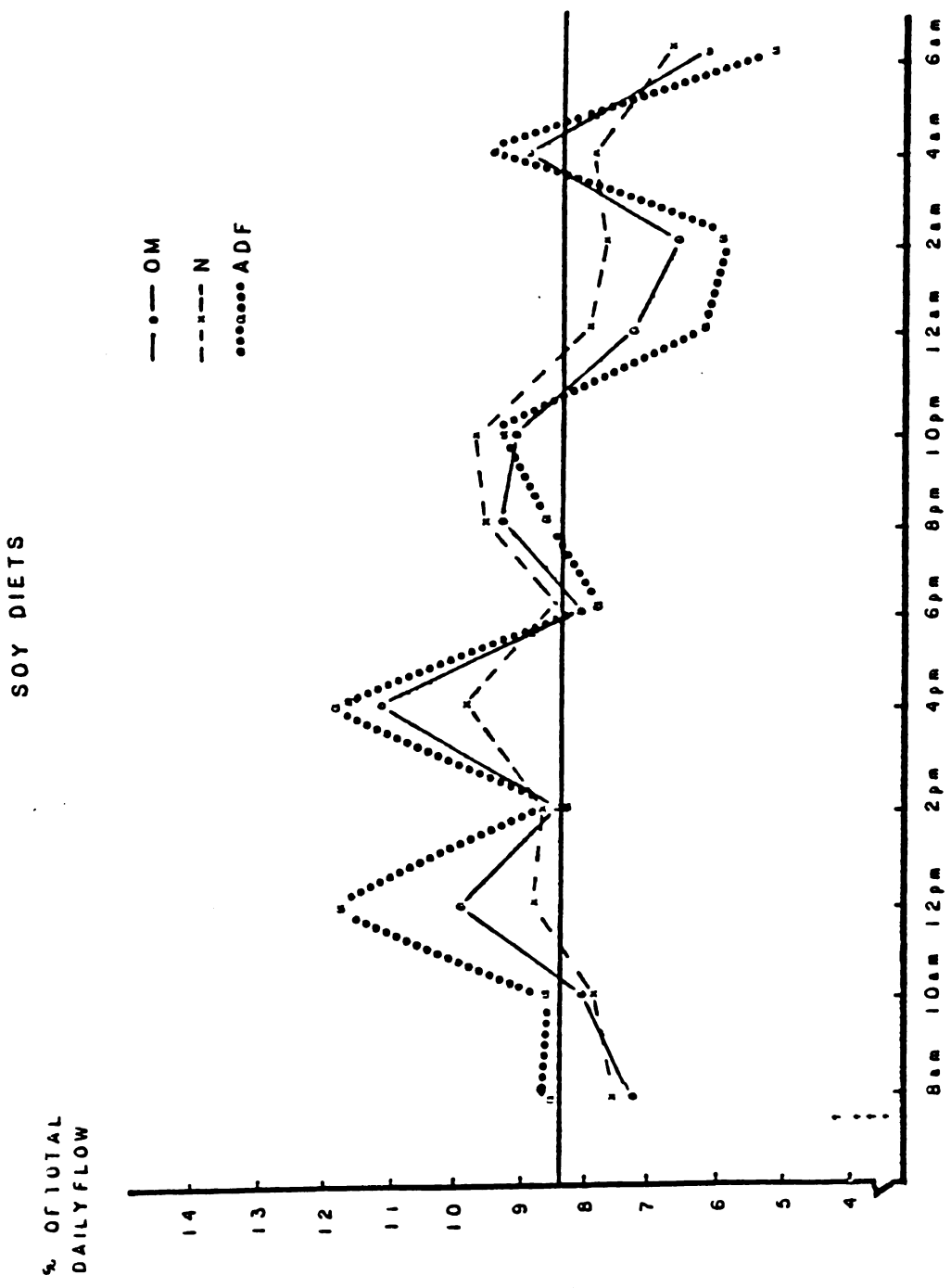

Figure 8 Flow patterns, OM, N, ADF, Soybean Meal Diets

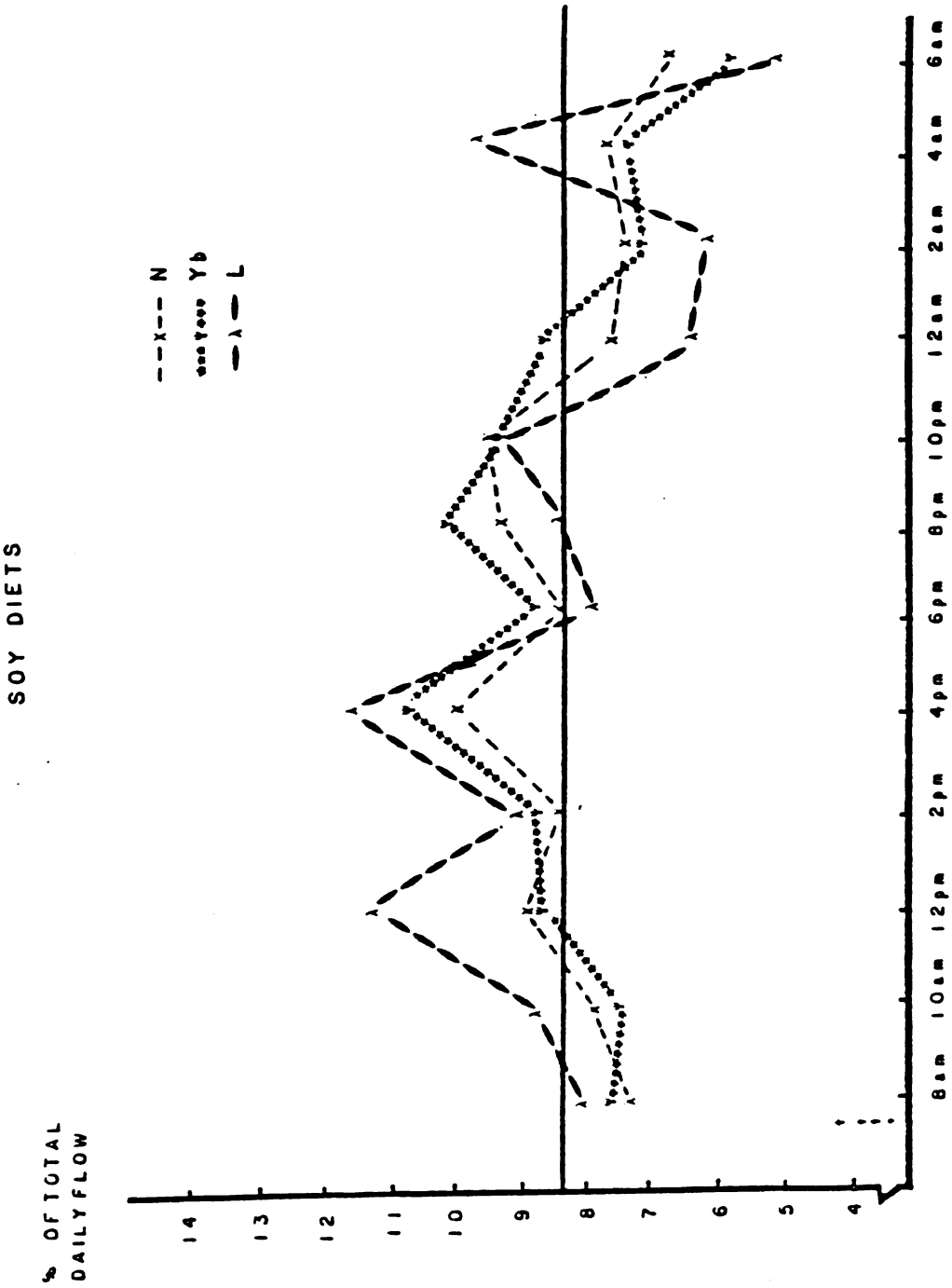

Figure 9 Flow patterns, N, Yb, L, Soybean Meal Diets

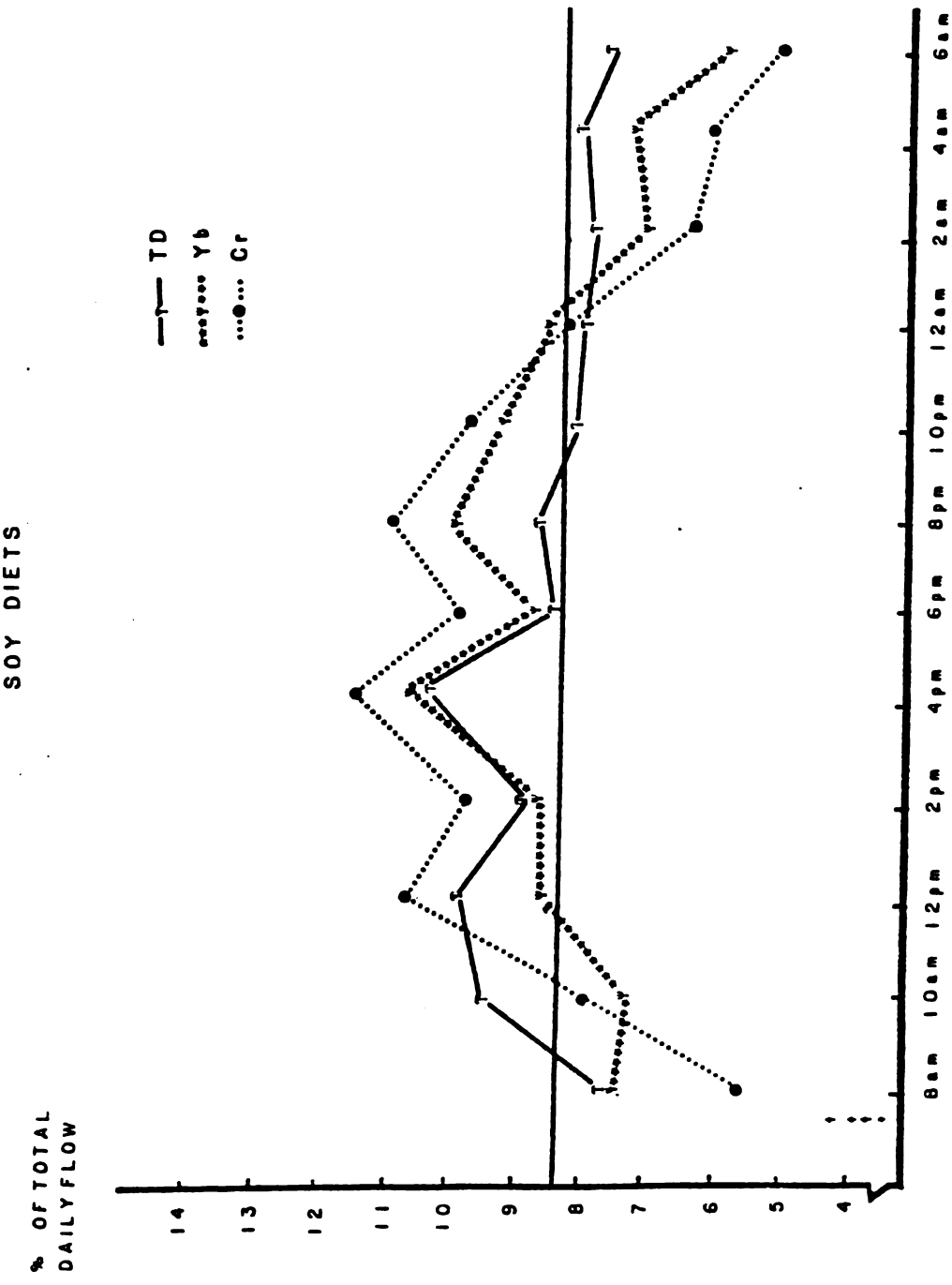

Figure 10 Flow patterns, TD, Yb, Cr, Soybean Meal Diets

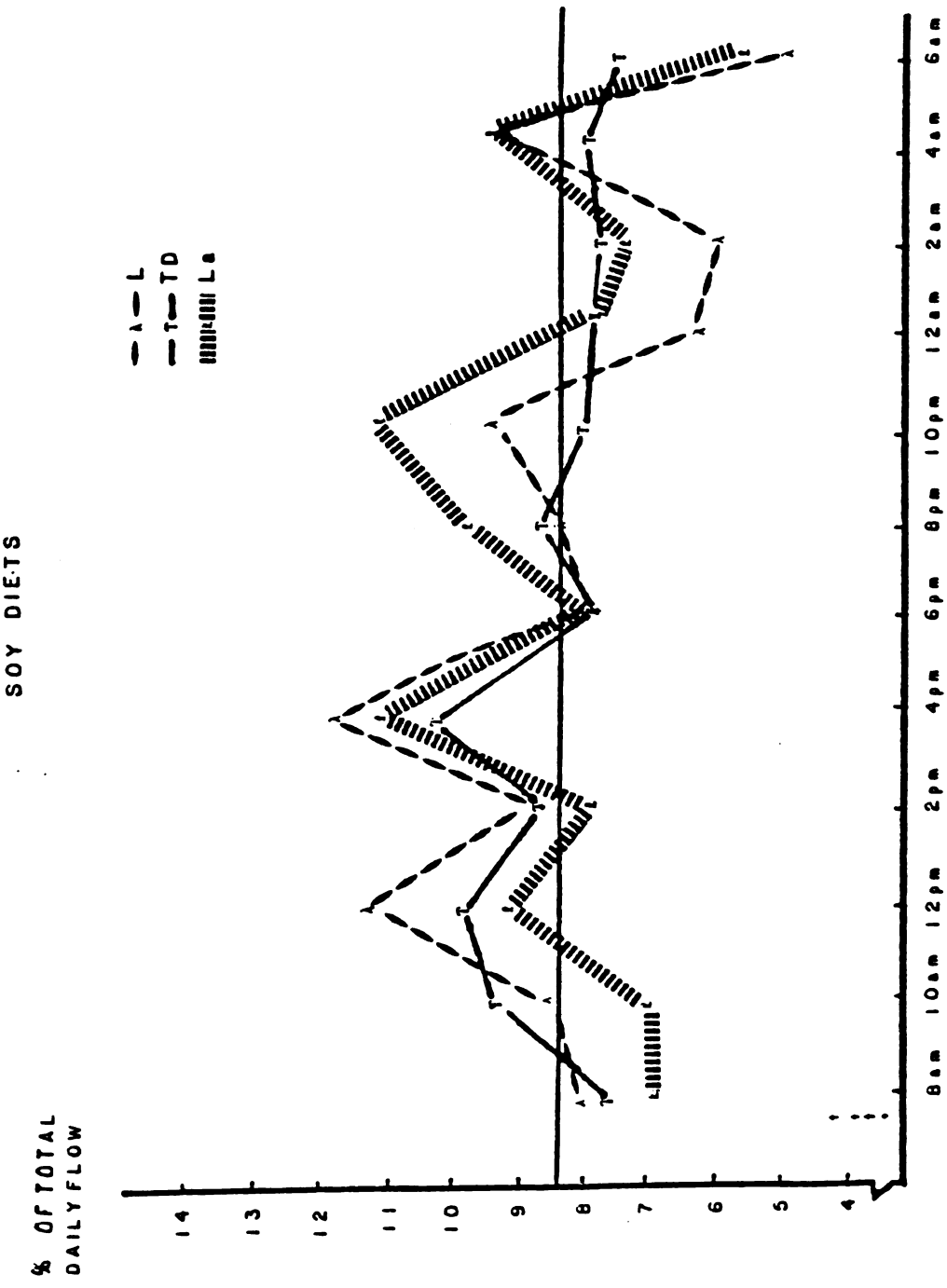

Figure 11 Flow patterns, L, TD, La, Soybean Meal Diets

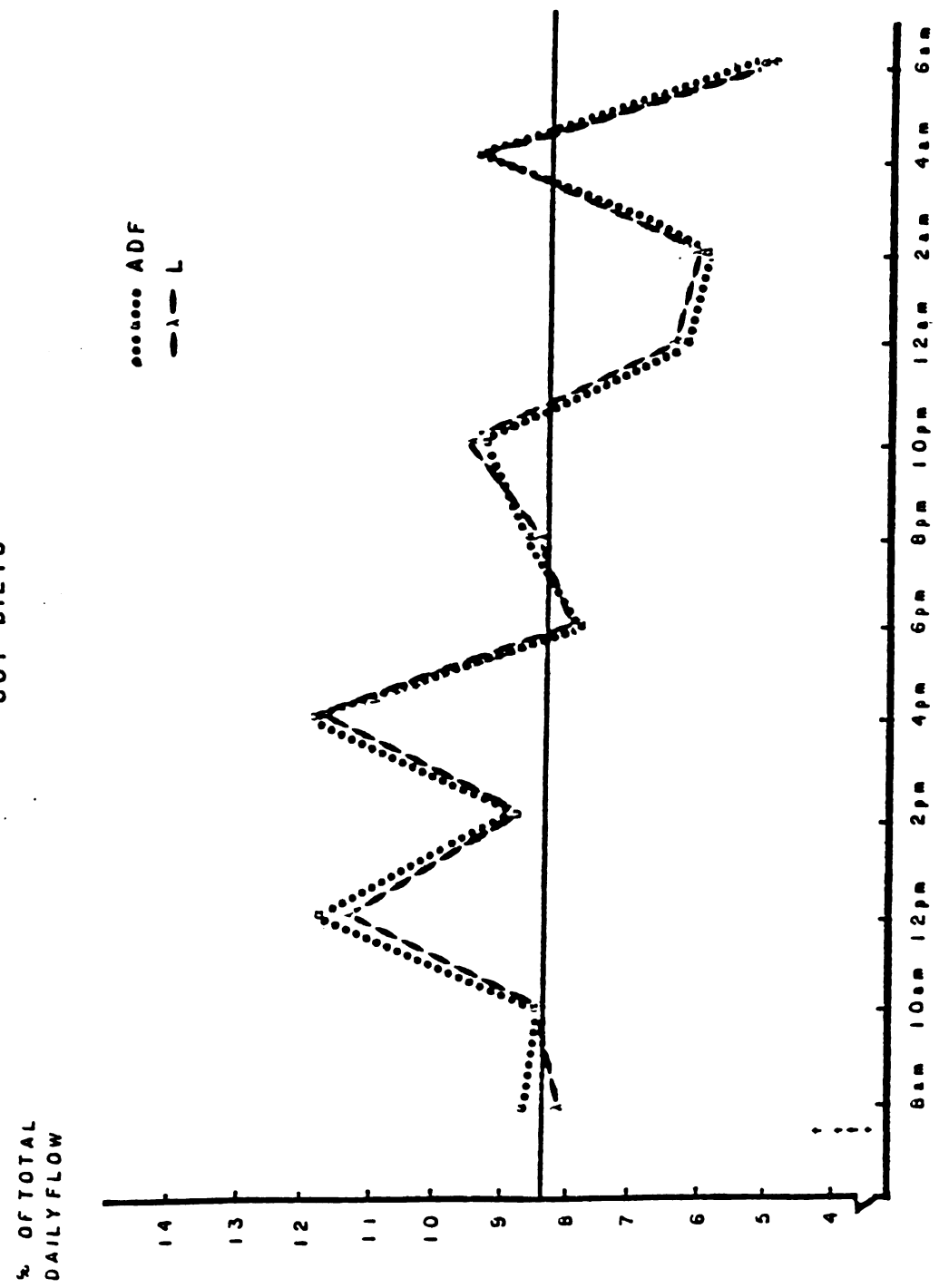

Figure 12 Flow patterns ADF, L, Soybean Meal Diets

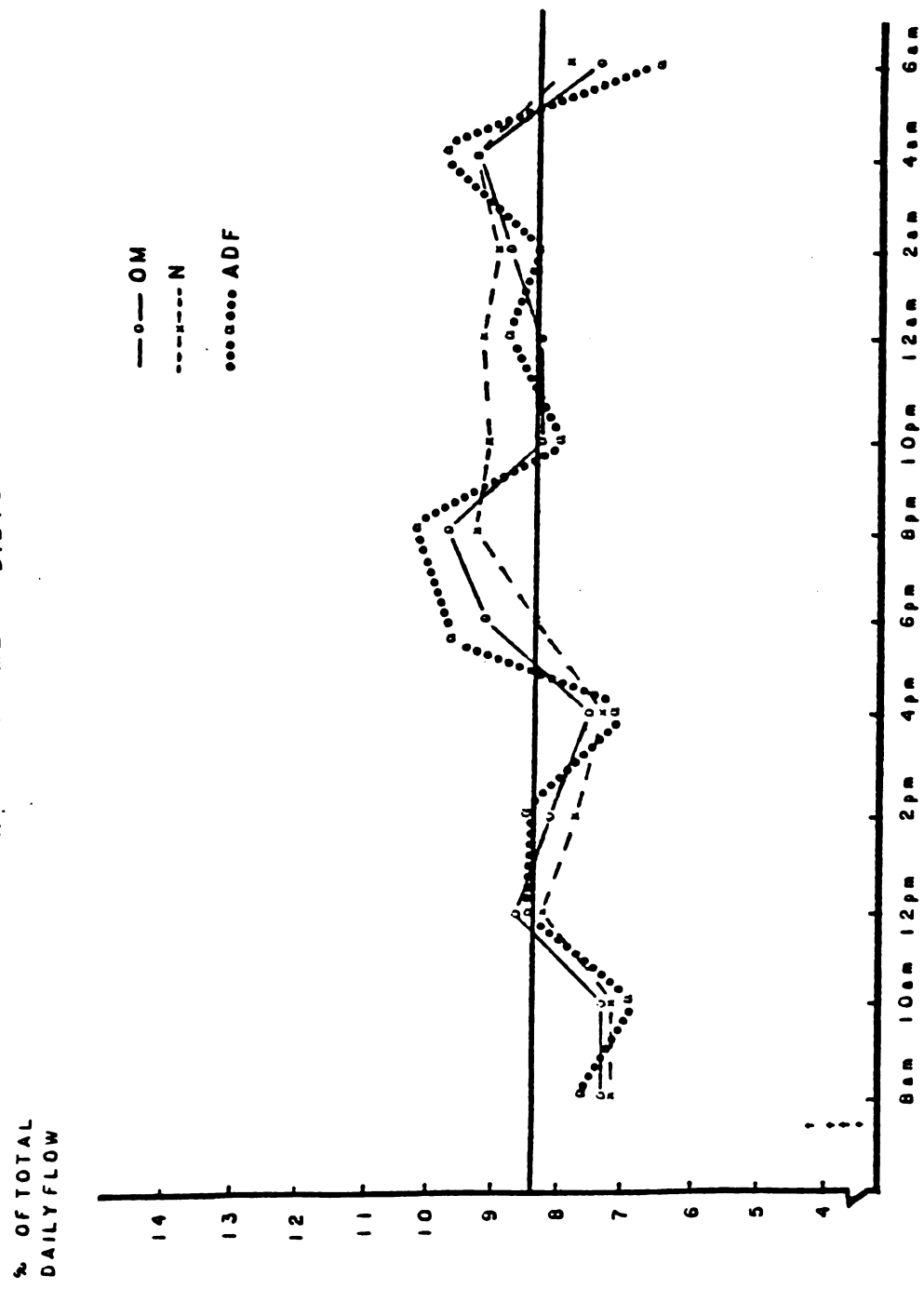

Figure 13 Flow patterns OM, N, ADF, Corn Gluten Meal Diets

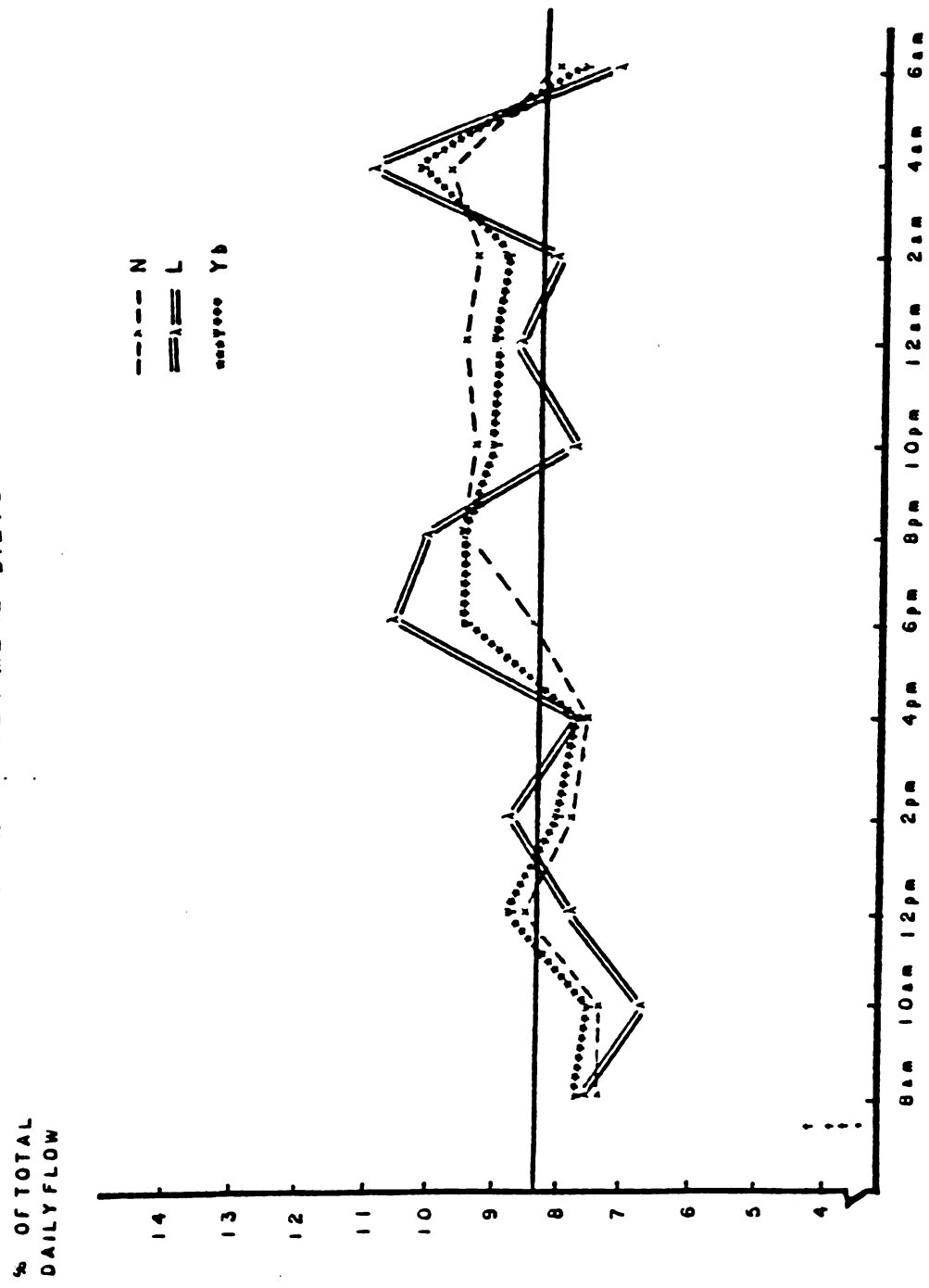

Figure 14 Flow patterns N, L, Yb, Corn Gluten Meal Diets

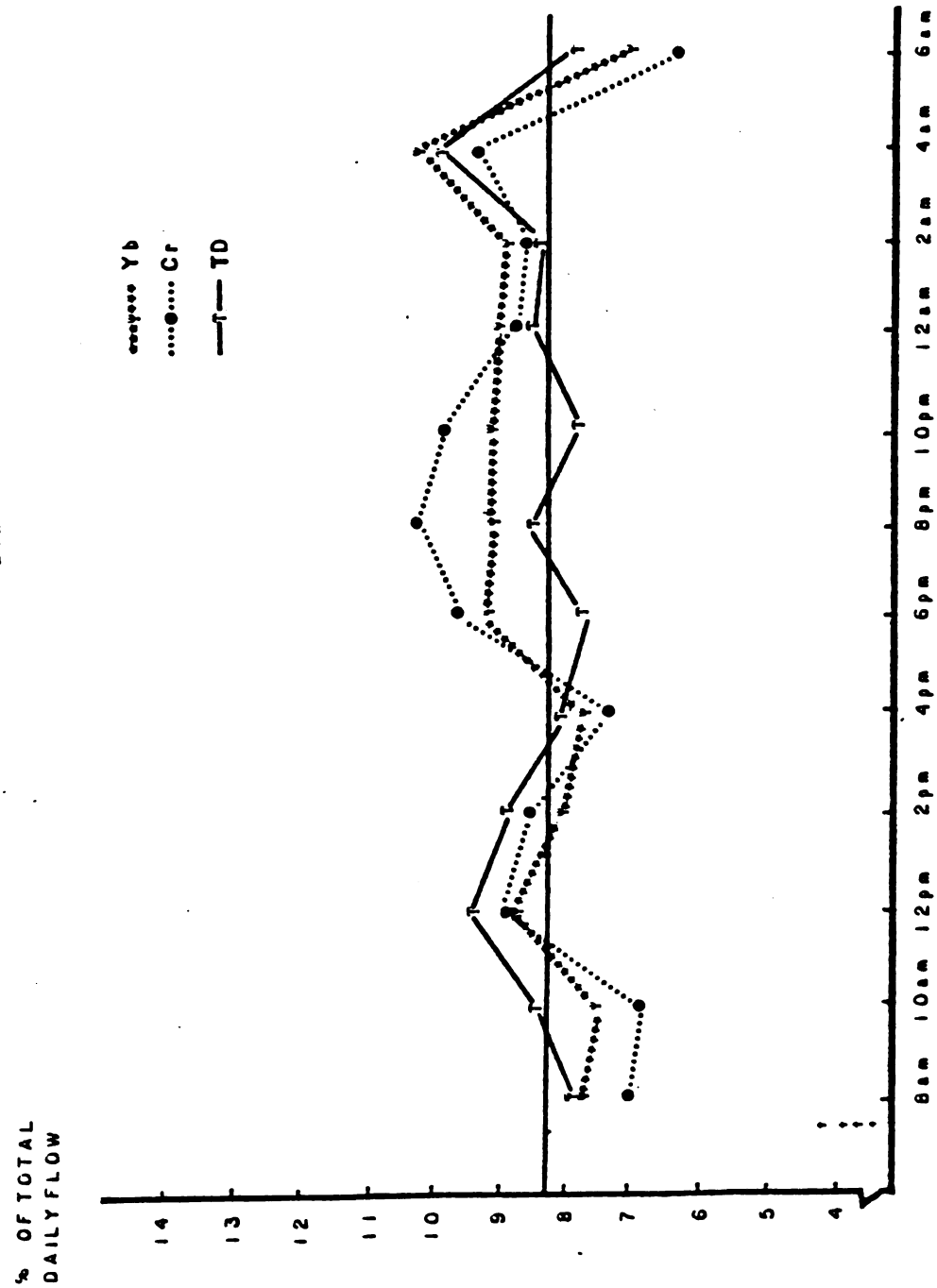

Figure 15 Flow patterns Yb, Cr, TD, Corn Gluten Meal Diets

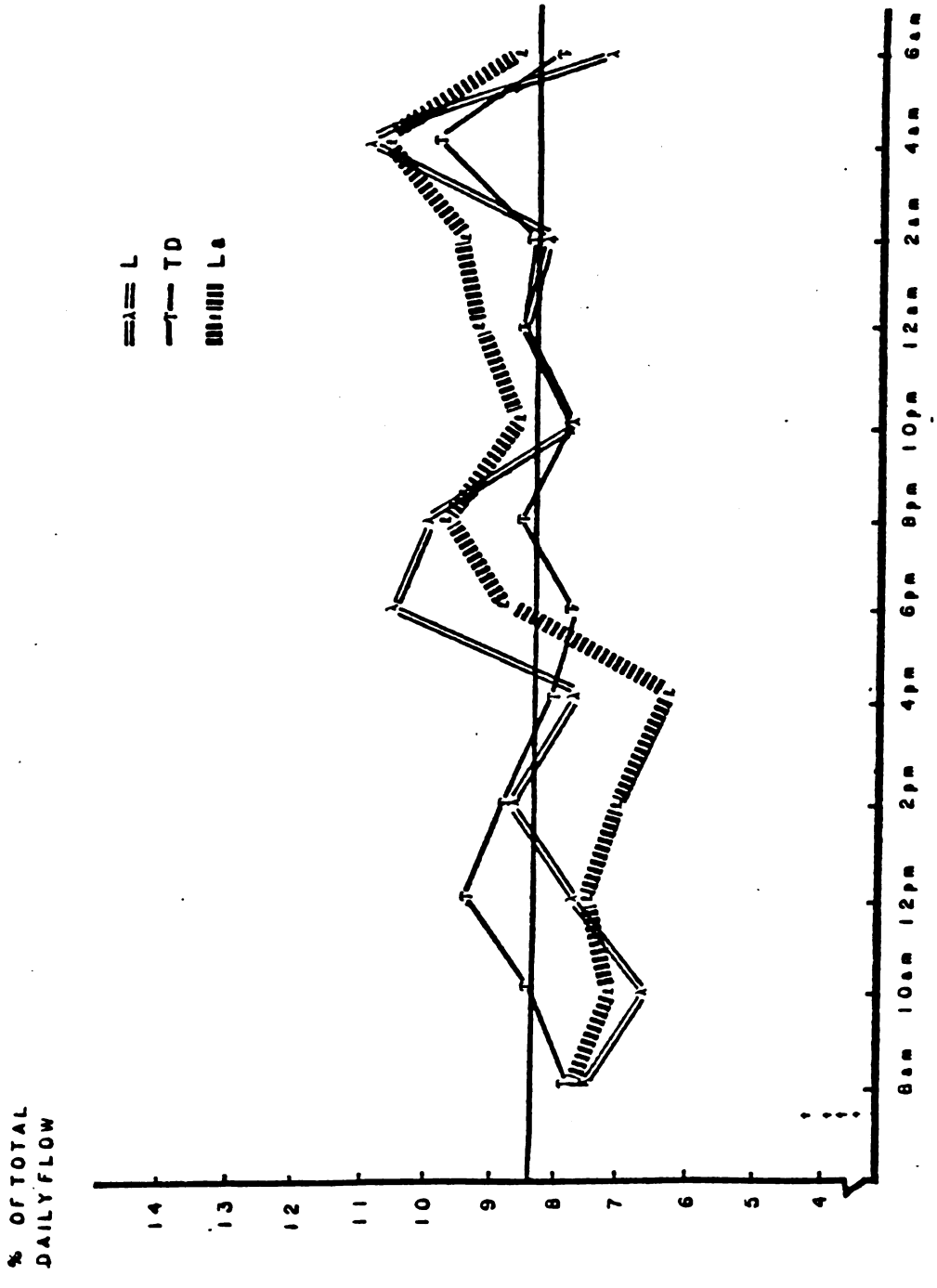

Figure 16 Flow patterns L, TD, La, Corn Gluten Meal Diets

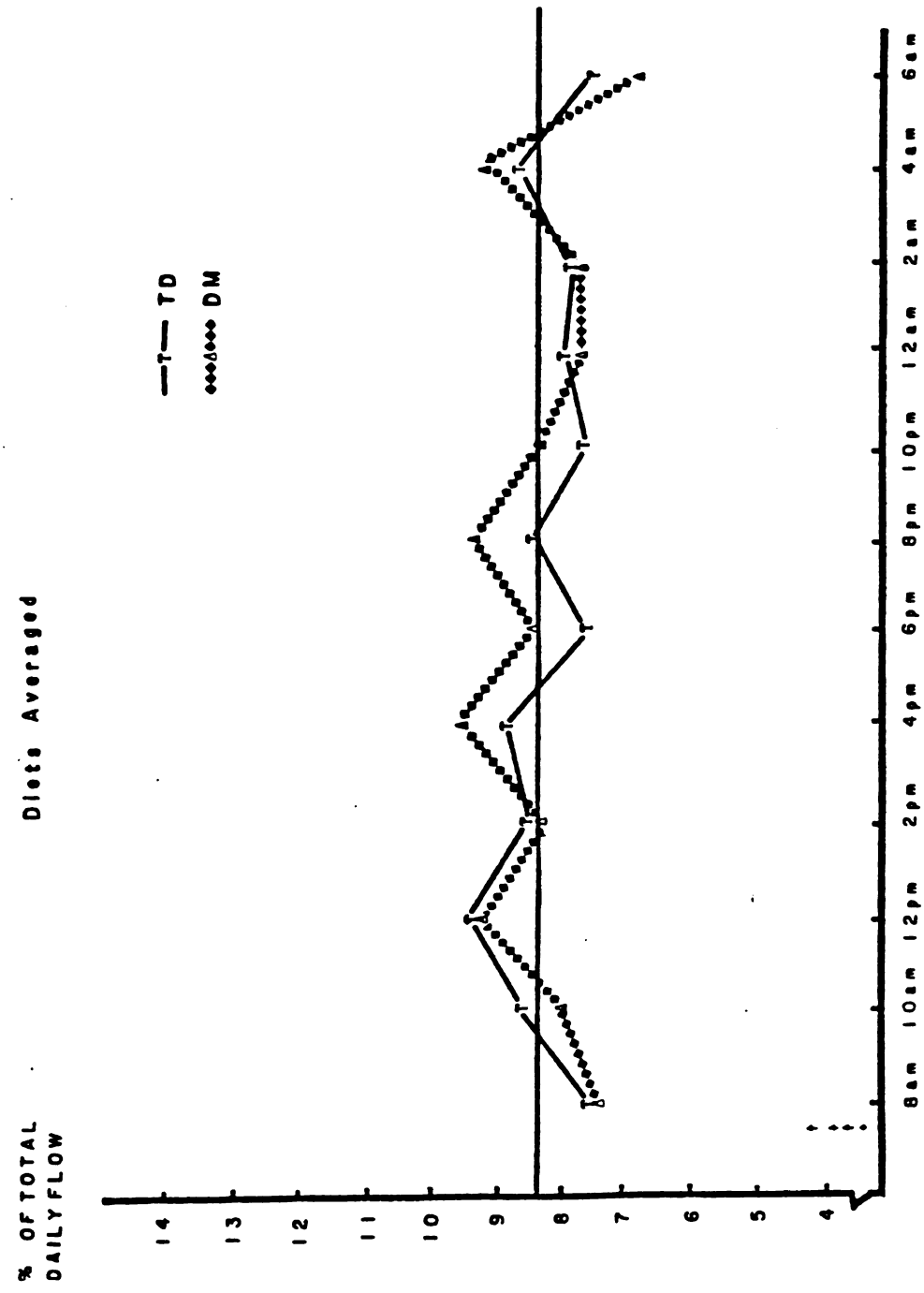

Figure 17 Flow patterns, TD, DM, averaged across diets and time periods

SUMMARY

Data in this thesis illustrate factors involved with digestive tract processing of dietary constituents and the importance of sound evaluation of data based on typical markers available to nutritionists. The following conclusions can be drawn from the data.

1. Estimates of apparent rumen digestibility of dietary constituents can be affected by the marker selected. Lignin may commonly be too high in duodenal samples due to high ADF levels. This results in high estimates of digestibility of dietary constituents in the rumen. Yb recovery may not be far from 100% with equal sample compositing; however, elevated ADF in samples contributes to high OM levels in duodenal samples and as a result, apparent ruminal digeston estimates of ADF and OM are too low.

   Due to variable Cr absorption from the digestive tract, its role as a suitable marker is very suspect. Variability of La content in duodenal samples results in high SEM values when it is used as a marker and duodenal recovery is usually high based upon PEG infusion data.

2. Estimates of total tract digestibility of dietary constituents do not vary significantly due to the markers selected. Fecal Cr levels, corrected for 5% total tract

absorption, provide adequate total tract digestibility estimates.

3. In corn silage based diets, total tract recovery of lignin averaged, across all other digesta marker estimates, diets and studies, 110% of intake, resulting in slightly elevated total tract digestibility estimates.
4. PEG based flow patterns of digesta passing the duodenal cannula in steers indicates the presence of a phasic pattern. The pattern varies with protein source and dietary constituent of interest. The circadian rhythms of digesta flow present may be related to rumination and or water intake patterns. The patterns of digesta flow may provide the potential to improve the efficiency of production of meat, milk and fiber. Feeding high quality dietary constituents prior to peak flow periods may maximize bypass and improve efficiency. Further analysis of flow patterns may prove beneficial for improving marker studies.
5. Based on our studies, Yb appears to represent the best marker for passage studies as compared to CrEDTA, lignin or lanthanum. It is easily quantified by specific and sensitive means. Flow patterns are not excessively variable as those for CrEDTA, lignin, or lanthanum. In general, standard errors of digestibility means are no larger and often smaller when Yb is used as a marker as compared to the other markers studied. Ytterbium has been shown to not be absorbed or toxic to gut tissues or to microbial flora to any great extent.

6. Ruminal and total tract digestion of corn silage diets supplemented with soybean, corn gluten meal and urea indicate equal nitrogen, organic matter and ADF digestion regardless of protein source.
7. Nitrogen contained in wet distillers grains is not as available ruminally as other protein sources evaluated. Nor is total tract digestion as complete as with the other protein sources evaluated.
8. Addition of urea to diets containing wet distillers grains resulted in a shift in site of digestion of a substantial portion of the organic matter, ADF and nitrogen to the lower tract. This may be due to more rapid rumen turnover of digesta due to urea addition stimulating water intake as a result of greater urinary output as described by Utley et al. (1970).

APPENDIX

Table 30 Experiment Two: Whole Digesta Flow, % of Total Daily

	Soybean Meal				Corn Gluten Meal			
	Animals							
Periods	1		2		2		1	
	813	819	820	550	813	819	820	550
Sampling Times								
12 AM	6.66	7.80	7.80	8.10	7.80	8.00	8.96	8.36
2 AM	7.60	6.72	6.92	9.44	7.92	7.76	7.62	8.26
4 AM	8.80	7.26	8.10	8.54	8.30	8.38	14.82	8.88
6 AM	6.06	7.26	6.84	8.76	7.48	7.72	7.36	9.08
8 AM	8.00	7.20	7.68	8.68	7.28	7.78	9.34	7.58
10 AM	10.18	8.58	7.55	7.48	7.68	7.10	7.28	7.68
12 PM	11.22	9.70	8.80	9.28	10.38	8.68	7.30	8.56
2 PM	8.92	8.92	9.92	7.92	8.02	12.40	8.34	9.94
4 PM	9.46	13.28	9.76	6.48	8.84	8.32	6.68	7.26
6 PM	7.62	7.50	8.68	8.74	8.98	8.40	8.04	7.56
8 PM	8.04	8.22	8.62	7.96	7.48	7.44	7.28	8.66
10 PM	7.44	7.74	9.08	8.68	9.72	8.02	6.98	8.18
Total, Kg/day:	86.56	88.50	104.60	105.80	89.77	86.20	106.66	85.63

Table 31 Experiment Two: Dry Matter Flow, % of Total Daily

	Soybean Meal				Corn Gluten Meal			
	Animals							
Periods	1		2		2		1	
	813	819	820	550	813	819	820	550
Sampling Times								
12 AM	5.66	6.22	8.34	9.60	7.68	7.54	8.84	8.82
2 AM	7.26	5.64	7.00	9.86	9.56	6.24	7.34	8.02
4 AM	10.92	8.28	6.88	7.40	11.44	7.36	14.16	7.22
6 AM	4.68	5.16	6.52	8.58	7.52	8.98	6.16	10.24
8 AM	8.26	6.46	5.90	9.08	5.52	7.44	8.82	7.12
10 AM	9.30	8.40	8.90	7.42	7.52	6.40	7.04	7.74
12 PM	10.34	9.61	6.34	9.02	18.04	6.60	6.32	9.38
2 PM	7.88	9.00	11.46	8.02	7.42	11.52	8.22	8.48
4 PM	10.06	14.70	10.42	5.98	8.30	7.72	8.26	6.48
6 PM	7.86	7.00	10.04	9.32	8.22	9.56	8.66	7.72
8 PM	8.84	10.96	9.68	7.34	8.92	10.88	9.74	9.30
10 PM	8.92	8.56	8.28	8.46	9.56	9.78	8.34	9.48
Total, Kg/day:	4.60	5.10	4.70	5.16	5.50	5.95	4.85	5.10
Intake/day, Kg:	7.15	7.06	6.98	7.00	6.93	6.90	7.02	7.00

Table 32 Experiment Two: Nitrogen Flow, % of Total Daily

	Soybean Meal				Corn Gluten Meal			
	Animals							
Periods	1		2		2		1	
	813	819	820	550	813	819	820	550
Sampling Times								
12 AM	7.06	7.86	9.60	9.48	8.02	9.10	9.50	10.08
2 AM	7.90	6.70	7.78	11.28	10.32	7.44	7.88	8.98
4 AM	8.76	7.48	7.46	8.86	11.30	8.34	14.38	7.42
6 AM	4.60	6.30	6.90	8.16	7.70	8.32	5.98	11.90
8 AM	7.36	6.90	6.36	8.88	5.44	7.86	9.12	6.70
10 AM	8.72	8.20	7.92	7.22	7.04	5.88	6.38	6.60
12 PM	9.44	8.62	6.86	7.68	19.06	6.32	6.04	8.06
2 PM	8.66	8.84	19.80	7.66	7.70	11.48	7.72	7.44
4 PM	10.28	11.72	10.64	5.44	8.40	7.18	5.80	5.70
6 PM	8.24	7.12	19.20	8.22	8.12	9.98	8.12	7.94
8 PM	9.72	10.90	10.40	7.52	7.42	19.04	9.94	9.14
10 PM	9.28	9.34	7.08	9.62	8.46	9.06	9.12	10.04
Total flow, g/day :	140.36	155.42	149.97	166.53	184.82	198.77	172.34	165.65
Intake, g/day:	145.72	153.38	149.54	146.84	145.70	147.38	143.31	135.68

Table 33 Experiment Two: Organic Matter Flow, % of Total Daily

	Soybean Meal				Corn Gluten Meal			
	Animals							
Periods	1		2		2		1	
	813	819	820	550	813	819	820	550
Sampling Times								
12 AM	5.44	6.08	8.50	9.82	7.72	7.58	8.58	8.90
2 AM	7.14	5.58	7.02	9.84	9.90	5.98	7.22	7.82
4 AM	11.66	8.64	6.66	7.24	11.94	7.30	15.10	6.88
6 AM	4.56	4.96	6.58	8.54	7.54	8.22	6.02	10.36
8 AM	8.14	6.50	5.64	9.16	5.32	7.82	8.68	7.12
10 AM	8.34	8.58	9.16	7.42	7.26	6.10	7.08	7.82
12 PM	10.26	9.62	6.00	9.08	7.88	6.80	6.28	9.54
2 PM	7.67	8.82	11.68	8.02	7.38	11.44	8.00	8.36
4 PM	10.06	14.68	10.60	5.96	8.44	7.68	6.26	6.30
6 PM	7.84	6.96	10.18	9.44	8.10	9.38	8.32	7.83
8 PM	8.86	10.96	9.86	7.18	8.98	11.82	9.74	9.44
10 PM	9.00	8.66	8.16	8.30	9.56	9.90	8.40	9.60
Total Flow, kg/day :	3.94	4.37	3.86	4.34	4.71	5.00	4.15	4.46
Intake, g/day calculated :	6.70	6.60	6.55	6.57	6.53	6.53	6.63	6.58

Table 34 Experiment Two: ADF Flow, % of Total Daily

	Soybean Meal				Corn Gluten Meal			
	Animals							
Periods	1		2		2		1	
	813	819	820	550	813	819	820	550
Sampling Times								
12 AM	3.64	4.48	8.50	10.32	7.02	7.08	8.46	10.64
2 AM	6.66	4.54	6.68	9.34	10.36	5.36	6.16	7.48
4 AM	14.26	9.28	5.30	6.80	13.48	5.92	15.16	5.42
6 AM	2.06	3.52	5.36	8.46	7.72	10.78	5.20	10.64
8 AM	8.32	6.14	3.92	10.16	3.80	6.54	8.90	6.60
10 AM	11.76	9.60	10.96	7.22	8.10	6.64	6.72	7.66
12 PM	12.30	11.20	3.06	10.36	7.00	6.14	5.24	9.58
2 PM	6.26	9.52	15.62	7.56	6.60	12.68	9.18	7.98
4 PM	9.82	15.88	12.78	4.42	9.64	7.54	6.38	6.36
6 PM	7.60	6.14	11.36	9.92	5.90	10.72	8.48	9.04
8 PM	7.86	11.68	10.04	6.22	11.00	10.04	11.84	9.30
10 PM	9.48	8.02	6.44	8.22	9.34	10.56	7.56	10.20
Total Flow, kg/day :	.98	1.09	.79	.86	1.14	1.47	.98	1.02
Intake, kg :	1.54	1.64	1.59	1.48	1.85	1.56	1.68	1.75

Table 35 Experiment Two: Lignin Flow, % of Total Daily

	Soybean Meal				Corn Gluten Meal			
	Animals							
Periods	1		2		2		1	
	813	819	820	550	813	819	820	550
Sampling Times								
12 AM	2.70	5.22	8.48	11.56	7.06	6.14	8.16	9.80
2 AM	6.04	5.82	6.98	9.88	10.24	4.72	6.36	8.32
4 AM	12.84	8.98	5.22	6.72	10.70	6.60	15.44	5.96
6 AM	3.14	3.60	5.60	8.40	8.08	14.16	6.18	11.92
8 AM	7.98	6.28	4.02	9.36	4.60	4.42	8.02	6.20
10 AM	11.68	10.84	10.34	7.40	7.82	7.56	6.44	7.68
12 PM	11.84	11.00	3.06	9.38	8.02	4.36	5.38	9.08
2 PM	7.00	9.80	14.20	7.90	4.42	12.12	9.92	7.48
4 PM	9.36	15.52	13.18	4.78	10.08	7.80	6.18	6.16
6 PM	7.86	6.88	11.72	8.92	6.46	12.16	9.02	9.16
8 PM	9.34	8.70	9.94	6.66	13.54	9.94	11.48	8.52
10 PM	10.22	7.38	6.26	9.08	9.96	10.04	7.44	9.70
Total Flow, g/day	235.29	248.52	171.75	206.73	192.55	347.49	244.94	268.01
Intake, g Calculated	205.88	202.00	157.75	147.01	153.80	152.50	200.92	213.75

Table 36 Experiment Two: Yb Flow, % of Total Daily

	Soybean Meal				Corn Gluten Meal			
	Animals							
Periods	1		2		2		1	
	813	819	820	550	813	819	820	550
Sampling Times								
12 AM	6.14	7.56	9.00	9.88	8.12	8.02	9.02	8.26
2 AM	7.80	6.26	7.34	12.48	9.86	6.84	7.76	8.04
4 AM	8.16	7.22	6.18	8.44	8.54	9.36	13.72	8.50
6 AM	3.82	5.44	6.30	8.18	8.04	9.08	6.48	9.34
8 AM	8.60	6.34	5.66	8.28	4.28	7.72	9.10	6.32
10 AM	8.40	8.17	6.66	8.74	8.74	6.00	6.74	7.30
12 PM	9.90	8.48	5.36	7.64	9.12	6.74	6.68	8.16
2 PM	8.44	9.40	8.98	8.01	7.92	11.46	7.80	8.26
4 PM	11.52	12.88	10.20	5.88	8.16	7.72	5.36	6.94
6 PM	8.28	7.62	11.50	7.88	7.76	9.52	9.24	9.06
8 PM	9.28	11.74	11.54	6.98	9.28	9.36	8.80	9.66
10 PM	9.66	8.92	11.26	7.58	10.16	8.18	9.30	10.16
Total Flow, g/day :	4.92	6.03	5.38	5.60	5.66	7.04	6.42	5.96
Intake, g/day Calculated :	5.98	5.68	4.95	6.79	5.25	5.61	6.20	5.62

Table 37 Experiment Two: Cr Flow, % of Total Daily

	Soybean Meal				Corn Gluten Meal			
	Animals							
Periods	1		2		2		1	
	813	819	820	550	813	819	820	550
Sampling Times								
12 AM	6.10	8.00	7.44	10.68	10.78	7.86	9.80	8.24
2 AM	6.24	5.58	5.96	12.22	9.52	7.10	7.32	8.90
4 AM	5.32	5.96	6.10	8.52	8.28	8.28	14.26	9.50
6 AM	3.54	5.18	5.08	8.14	6.16	7.28	5.78	8.94
8 AM	5.46	4.06	5.10	6.58	3.74	5.80	8.08	5.88
10 AM	9.26	6.94	5.92	7.00	7.72	6.20	6.18	5.60
12 PM	12.78	10.50	8.04	6.80	7.26	7.64	7.02	5.94
2 PM	10.70	10.24	10.48	8.60	8.74	13.12	8.48	7.84
4 PM	11.94	13.20	10.18	6.40	8.30	9.26	6.28	6.80
6 PM	9.06	9.48	11.76	8.02	6.78	8.80	9.74	8.86
8 PM	10.22	10.82	11.68	7.56	9.86	9.62	8.46	11.38
10 PM	9.28	10.04	12.24	9.50	11.86	8.96	8.48	12.08
Total Flow, g/day :	1.74	2.02	2.04	2.20	2.10	2.64	2.16	2.14
Intake, g/day Calculated :	2.28	2.25	2.02	2.67	2.18	2.22	2.53	2.24

Table 38 Experiment Two: La Flow, % of Total Daily

	Soybean Meal				Corn Gluten Meal			
	Animals							
Periods	1		2		2		1	
	813	819	820	550	813	819	820	550
Sampling Times								
12 AM	5.40	6.28	9.56	12.94	8.14	8.16	9.08	10.08
2 AM	6.90	6.30	7.82	11.62	10.66	6.82	7.24	7.88
4 AM	11.64	11.06	6.90	10.08	14.02	8.82	19.62	5.40
6 AM	3.22	4.84	6.06	10.09	7.70	10.14	6.12	15.06
8 AM	6.54	6.50	5.00	9.36	3.68	7.88	9.34	6.32
10 AM	6.74	7.96	6.22	8.08	7.52	6.60	6.38	8.88
12 PM	8.86	11.92	4.46	8.28	6.92	5.60	5.28	8.50
2 PM	7.80	7.66	8.50	6.62	6.40	10.30	6.68	7.56
4 PM	12.02	10.78	10.42	4.44	7.70	5.88	4.90	4.98
6 PM	9.78	5.70	12.76	7.96	6.48	8.66	7.76	7.40
8 PM	10.38	9.80	11.54	5.08	9.44	10.76	9.64	7.68
10 PM	10.68	11.20	11.76	6.32	11.32	10.38	7.96	10.24
Total Flow, g/day :	4.57	5.32	5.04	5.50	2.71	3.78	2.95	3.35
Intake, g/day Calculated :	4.25	4.01	5.25	5.83	2.32	2.76	1.80	2.07

Abbreviations Code for Tables 39, 40 and 41

A	animal numbers
ADF	acid detergent fiber, %, dry matter
ADIN	acid detergent insoluble nitrogen, mg/gr dry matter
CP	crude protein, %, dry matter
Cr	ppm chromium, dry matter
D	diets
S	soybean meal
U	urea
WDG	wet distillers grains
WDG-U	wet distillers grains and urea
DAN	duodenal ammonia nitrogen, % of total nitrogen
DM	dry matter of original material
DMI	dry matter intake
Lgn	lignin, %, dry matter
OM	organic matter, %, dry matter
P	experimental periods
Yb	ppm ytterbium, dry basis

Table 39 Feed Analysis Data: Experiment One

A	P	D	DMI	CP	OM	ADF	ADIN	Lgn	Yb	Cr
788	1	U	15.76	12.64	94.99	23.67	.85	2.81	340.8	573.0
789	1	WDG	14.68	13.46	94.89	19.94	1.94	2.72	335.92	489.0
790	1	WDG-U	15.17	13.08	94.89	22.24	1.08	3.14	335.84	553.4
791	1	S	14.55	13.25	94.45	20.34	.74	2.55	307.2	500.4
788	2	S	16.26	13.85	94.41	19.66	.79	2.20	312.7	538.5
789	2	WDG-U	15.72	13.18	94.45	23.80	1.13	2.84	327.1	528.2
790	2	WDG	15.20	13.47	94.90	22.64	1.38	3.10	307.1	474.9
791	2	U	16.24	12.34	94.57	22.52	.82	2.57	362.5	540.0
788	3	WDG	14.27	13.61	94.46	21.65	2.77	2.78	392.0	652.4
789	3	S	15.14	13.42	93.51	21.58	.93	2.15	410.4	604.7
790	3	U	15.09	13.54	94.10	22.92	1.09	2.65	347.0	596.9
791	3	WDG-U	14.70	13.32	94.92	22.20	2.77	2.86	337.9	595.5
788	4	WDG-U	14.35	13.82	94.66	21.72	1.92	3.37	369.1	595.3
789	4	U	14.29	13.99	93.88	20.78	.90	2.19	363.8	639.4
790	4	S	14.31	15.64	93.39	20.36	.76	2.53	365.7	571.3
791	4	WDG	13.88	14.18	94.92	21.05	2.82	2.58	406.6	425.7

Table 40 Duodenal Analysis Data: Experiment One

A	P	D	DM	CP	DAN	OM	ADF	Lgn	Yb	Cr
788	1	U	7.18	11.25	2.86	84.98	19.07	4.45	429.5	399.3
789	1	WDG	6.16	14.42	3.51	85.02	20.65	5.41	506.4	456.5
790	1	WDG-U	7.96	11.39	3.90	83.68	21.60	4.26	418.4	437.1
791	1	S	6.51	12.18	5.05	84.80	23.05	5.00	468.8	459.3
788	2	S	6.17	12.40	7.64	85.94	15.08	3.52	398.4	542.8
789	2	WDG-U	4.90	13.25	6.79	84.18	23.15	6.31	444.8	609.3
790	2	WDG	4.89	10.55	8.53	83.24	21.20	4.40	420.8	608.8
791	2	U	5.68	13.69	6.71	85.23	18.33	4.19	427.2	632.3
788	3	WDG	5.59	13.35	5.90	84.84	18.84	5.17	424.0	712.4
789	3	S	4.69	13.62	4.36	83.36	21.38	5.45	499.2	872.6
790	3	U	4.95	12.79	8.31	84.16	19.71	4.76	445.2	850.5
791	3	WDG-U	5.52	10.98	8.00	86.13	22.44	4.98	421.6	744.9
788	4	WDG-U	7.65	10.65	4.87	88.45	11.68	2.94	320.8	531.7
789	4	U	4.26	12.65	7.53	81.32	22.13	6.06	595.6	1057.4
790	4	S	6.12	11.67	7.63	85.11	18.14	3.85	448.4	727.0
791	4	WDG	6.38	10.93	5.83	87.69	15.52	4.46	443.8	423.2

Table 41 Fecal Analysis Data: Experiment One

A	P	D	CP	OM	ADF	ADIN	Lgn	Yb	Cr
788	1	U	12.85	87.25	34.19	2.74	10.49	1182.8	1375.5
789	1	WDG	16.40	87.32	29.37	3.48	9.81	991.2	1094.1
790	1	WDG-U	12.96	86.80	32.92	2.55	9.90	818.4	1235.8
791	1	S	15.38	86.51	32.41	2.84	9.92	1230.8	1238.0
788	2	S	14.04	88.90	33.35	2.27	9.49	1088.0	1704.5
789	2	WDG-U	14.16	88.38	33.08	2.40	8.89	1116.4	1893.7
790	2	WDG	13.12	88.75	29.99	1.96	7.63	1194.8	1953.0
791	2	U	11.99	88.74	35.97	1.90	9.49	1015.2	1917.3
788	3	WDG	13.39	89.30	32.29	2.50	7.96	977.6	1764.0
789	3	S	14.47	87.99	35.72	2.93	9.31	1131.6	2006.6
790	3	U	11.82	88.68	36.84	2.38	9.46	1037.6	1975.6
791	3	WDG-U	13.08	89.39	34.68	2.41	8.65	1103.2	1659.0
788	4	WDG-U	13.94	88.20	28.50	2.34	8.89	1218.0	1871.6
789	4	U	14.96	86.16	32.71	2.58	11.08	1598.8	3094.4
790	4	S	14.41	85.83	32.00	2.33	9.77	1622.4	2627.6
791	4	WDG	15.61	88.84	29.59	3.15	9.42	1159.0	1254.2

Table 42 Experiment Two: Cr Data

Cr as a marker. calculations based upon compositing duodenal digesta on an equal dry matter basis, duodenal Cr conc. 42.5%. fecal Cr Calculations of flow and digestibility of nitrogen, ADF and organic matter.

BLOCK NUMBER	ANIMAL NUMBER	DIET	PEG INTAKE (kg/day)	DIET Cr-I (mg/day)	DIET DM-I (kg/day)	DIET N-I (kg/day)	DIET ADF-I (kg/day)	DIET ADL-I (kg/day)	DIET OM-I (kg/day)	DUOD DM Cr-CHC (mg/kg)
1	813	SOY	0.52	2280.00	7.15	0.15	1.54	0.21	6.70	387.77
1	819	SOY	0.50	2250.00	7.06	0.15	1.64	0.20	6.60	409.53
1	820	CGM	0.53	2530.00	7.02	0.14	1.66	0.20	6.63	457.56
1	550	CGM	0.50	2240.00	7.00	0.14	1.75	0.21	6.56	430.57
2	813	CGM	0.52	2180.00	6.93	0.15	1.65	0.15	6.53	391.64
2	819	CGM	0.48	2220.00	6.70	0.15	1.56	0.15	6.53	458.53
2	820	SOY	0.53	2020.00	6.98	0.15	1.59	0.16	6.55	441.21
2	550	SOY	0.61	2670.00	7.00	0.15	1.48	0.15	6.57	438.18

Table 43 Experiment Two: Lignin Data

Lignin as a marker. calculations based on compositing duodenal digesta on an equal dry matter basis.
Calculations of flow and digestibility of nitrogen, ADF and organic matter.

BLOCK NUMBER	ANIMAL NUMBER	DIET	PEG INTAKE (kg/day)	DIET lignin-I (mg/day)	DIET DM-I (kg/day)	DIET N-I (kg/day)	DIET ADF-I (kg/day)	DIET ADL-I (kg/day)	DIET OM-I (kg/day)	DUOD DM Lignin-CHC (mg/kg)
1	813	SOY	0.52	205880.00	7.15	0.15	1.54	0.21	6.70	49200.00
1	819	SOY	0.50	202000.00	7.06	0.15	1.64	0.20	6.60	46000.00
1	820	CCM	0.53	200920.00	7.02	0.14	1.68	0.20	6.63	49700.00
1	550	CCM	0.50	213750.00	7.00	0.14	1.75	0.21	6.58	52200.00
2	813	CCM	0.52	153800.00	6.93	0.15	1.85	0.15	6.53	34700.00
2	819	CCM	0.48	152500.00	6.90	0.15	1.56	0.15	6.53	57200.00
2	820	SOY	0.53	157750.00	6.98	0.15	1.59	0.16	6.55	35000.00
2	550	SOY	0.61	147010.00	7.00	0.15	1.48	0.15	6.57	39700.00

Table 44 Experiment Two: La Data

La as a marker. calculations based upon compositing duodenal digesta on an equal dry matter basis.
Calculations of flow and digestibility of nitrogen, ADF and organic matter.

BLOCK NUMBER	ANIMAL NUMBER	DIET	PEG INTAKE (kg/day)	DIET La-I (mg/day)	DIET DM-I (kg/day)	DIET N-I (kg/day)	DIET ADF-I (kg/day)	DIET ADL-I (kg/day)	DIET OM-I (kg/day)	DUOD DM La-CNC (mg/kg)
1	813	SOY	0.52	4240.00	7.15	0.15	1.54	0.21	6.70	779.60
1	819	SOY	0.50	4010.00	7.06	0.15	1.64	0.20	6.60	1058.66
1	820	CGM	0.53	1800.00	7.02	0.14	1.68	0.20	6.63	387.74
1	550	CGM	0.50	2070.00	7.00	0.14	1.75	0.21	6.56	646.54
2	813	CGM	0.52	2320.00	6.93	0.15	1.85	0.15	6.53	483.07
2	819	CGM	0.48	2760.00	6.70	0.15	1.56	0.15	6.53	638.27
2	820	SOY	0.53	5250.00	6.96	0.15	1.57	0.16	6.55	1064.01
2	550	SOY	0.61	5830.00	7.00	0.15	1.48	0.15	6.57	1053.49

Table 45 Experiment Two: Yb Data

Yb as a marker, calculations based upon compositing duodenal digesta on an equal dry matter basis.
Calculations of flow and digestibility of nitrogen, ADF and organic matter.

BLOCK NUMBER	ANIMAL NUMBER	DIET	PEG INTAKE (kg/day)	DIET YB-I (mg/day)	DIET DM-I (kg/day)	DIET N-I (kg/day)	DIET ADF-I (kg/day)	DIET ADL-I (kg/day)	DIET OM-I (kg/day)	DUOD DM Yb-CNC (mg/kg)
1	813	SOY	0.52	5980.00	7.15	0.15	1.54	0.21	6.70	1071.04
1	819	SOY	0.50	5680.00	7.06	0.15	1.64	0.20	6.60	1201.36
1	820	CON	0.53	6200.00	7.02	0.14	1.68	0.20	6.63	1326.34
1	550	CON	0.50	5620.00	7.00	0.14	1.75	0.21	6.56	1173.24
2	813	CON	0.52	5250.00	6.93	0.15	1.85	0.15	6.53	1133.23
2	819	CON	0.48	5610.00	6.70	0.15	1.56	0.15	6.53	1175.73
2	820	SOY	0.53	4950.00	6.98	0.15	1.59	0.16	6.55	1142.23
2	550	SOY	0.61	6790.00	7.00	0.15	1.48	0.15	6.57	1085.76

Table 46 Experiment Two: Infusion Data, Period 1, Animal 813, Soybean Meal Diet

1813 CNC.INF. 130.66 (g/l) PEG

SAMPLE TIME	PEG CNC (mg/100ml)	INF.RT. (ml/min)	DUOD DM	YB CNC (ug/g)	CR CNC (ug/g)	LA CNC (ug/g)	CP CNC (g/100g)	NIT CNC (g/100g)	NH3-N (mg/100ml)	ADF CNC (g/100g)	ADL CNC (g/100g)	OM CNC (g/100g)	PEG FLW (g/hr)
12:00am	806.30	2.83	4.52	1156.50	407.80	947.20	23.76	3.80	5.56	13.76	2.44	82.30	22.1[illegible]
2:00am	716.10	2.85	5.08	1148.50	325.00	944.50	20.77	3.32	6.00	19.61	4.26	84.20	22.34
4:00am	570.90	2.59	6.59	799.60	185.00	1060.00	15.31	2.45	6.11	29.92	6.02	91.40	20.36
6:00am	381.90	2.83	4.10	870.60	286.60	683.00	18.77	3.00	6.00	7.42	3.44	83.60	22.17
8:00am	641.70	2.68	5.48	1114.00	250.70	787.00	17.00	2.72	6.00	21.56	4.94	84.40	21.02
10:00am	531.00	2.84	4.85	765.80	377.50	720.90	17.90	2.86	10.78	24.79	6.43	86.10	22.26
12:00pm	453.60	2.67	4.90	1023.50	468.40	850.70	17.41	2.79	9.33	25.44	5.66	85.00	20.93
2:00pm	616.30	2.89	4.70	1145.00	513.90	983.40	20.94	3.35	5.89	17.00	4.54	83.40	22.66
4:00pm	561.80	2.77	5.64	1225.00	450.10	1187.50	19.50	3.12	3.89	20.91	4.77	85.70	21.72
6:00pm	716.20	2.85	5.48	1125.00	436.20	1235.50	19.97	3.20	3.89	20.66	5.11	85.40	22.34
8:00pm	684.00	2.86	5.84	1121.50	441.90	1166.00	20.96	3.35	3.69	19.04	5.40	85.70	22.42
10:00pm	733.10	2.82	6.37	1157.50	393.90	1189.50	19.84	3.17	3.89	22.72	5.86	86.30	22.11
AVERAGE	659.41	2.79	5.30	1071.04	378.08	979.60	979.60	3.10	5.94	20.40	4.92	85.27	21.87
PER DAY		4017.60											524.94

Table 47 Experiment Two: Infusion Data, Period 1, Animal 819, Soybean Meal Diet

1619 CNC.INF. 130.66 (g/l) PEG

SAMPLE TIME	PEG CNC (mg/100ml)	INF.RT. (ml/min)	DUOD DM	YB CNC (ug/g)	CR CNC (ug/g)	LA CNC (ug/g)	CP CNC (g/100g)	NIT CNC (g/100g)	NH3-N (mg/100ml)	ADF CNC (g/100g)	ADL CNC (g/100g)	OM CNC (g/100g)	PEG FLW (g/hr)
12:00am	645.40	2.71	4.60	1435.50	509.60	1051.00	24.02	3.84	5.22	15.35	4.06	83.72	21.25
2:00am	747.80	2.70	4.84	1310.00	393.00	1165.00	22.62	3.62	6.89	17.16	5.03	84.66	21.17
4:00am	682.30	2.61	6.58	1030.00	286.00	1392.50	17.21	2.75	7.89	23.86	5.26	89.40	20.40
6:00am	673.40	2.65	4.09	1245.50	398.30	980.20	23.28	3.72	8.33	14.52	3.40	82.52	20.77
8:00am	656.10	2.53	5.18	1157.50	248.40	1045.50	20.29	3.25	7.89	20.16	4.72	85.94	19.83
10:00am	581.70	2.66	5.65	1144.00	326.90	984.90	18.55	2.97	11.11	24.28	6.27	87.30	20.65
12:00pm	472.80	2.44	5.72	1043.00	433.40	1291.00	17.04	2.73	8.22	24.80	5.57	85.62	19.13
2:00pm	579.40	2.75	5.81	1236.00	451.20	888.00	18.72	3.00	7.78	22.54	5.31	84.02	21.56
4:00pm	383.60	2.65	6.48	1035.50	356.10	764.60	15.18	2.43	5.11	22.98	5.14	85.52	20.77
6:00pm	579.40	2.72	5.39	1284.50	537.10	848.20	19.33	3.09	5.11	18.68	4.78	85.10	21.32
8:00pm	652.00	2.79	7.70	1265.50	391.20	932.00	18.93	3.03	2.89	22.66	3.86	85.61	21.87
10:00pm	664.50	2.72	6.38	1229.50	465.00	1361.00	20.75	3.32	3.11	19.92	4.19	86.66	21.32
AVERAGE	618.20	2.66	5.70	1201.33	399.68	1058.66	1058.66	3.15	6.63	20.58	4.80	85.51	20.86
PER DAY		3831.60											500.64

Table 48 Experiment Two: Infusion Data, Period 1, Animal 820, Corn Gluten Meal Diet

1820 CNC.INF. 130.66 (g/l) PEG

SAMPLE TIME	PEG CNC (mg/100ml)	INF.RT. (ml/min)	DUOD DM	YB CNC (ug/g)	CR CNC (ug/g)	LA CNC (ug/g)	CP CNC (g/100g)	NIT CNC (g/100g)	NH3-N (mg/100ml)	ADF CNC (g/100g)	ADL CNC (g/100g)	OM CNC (g/100g)	PEG FLW (g/hr)
12:00am	480.80	2.80	4.53	1337.00	488.90	616.60	23.64	3.78	3.56	19.13	4.61	82.30	21.65
2:00am	550.50	2.73	4.38	1397.50	444.40	600.60	23.84	3.81	4.22	16.93	4.38	84.20	21.40
4:00am	286.10	2.78	4.34	1283.50	449.30	844.40	22.56	3.61	6.76	22.63	5.51	91.40	21.79
6:00am	596.60	2.87	3.81	1392.50	418.50	604.00	21.59	3.45	5.89	17.05	5.06	83.60	22.53
8:00am	467.20	2.84	4.29	1367.50	408.60	645.90	23.00	3.68	6.89	20.40	4.60	84.40	22.26
10:00am	587.40	2.78	4.40	1266.00	391.60	552.20	20.12	3.22	8.78	19.27	4.62	86.10	21.79
12:00pm	616.40	2.94	3.94	1396.50	495.50	508.80	21.18	3.39	8.78	16.72	4.30	85.60	23.05
2:00pm	519.10	2.81	4.48	1258.00	461.00	495.80	20.87	3.34	6.56	22.58	6.10	83.40	22.03
4:00pm	666.40	2.90	4.26	1132.00	453.60	476.80	20.60	3.30	6.11	20.55	4.98	85.70	22.73
6:00pm	540.60	2.81	4.90	1411.50	501.10	545.70	20.82	3.33	4.44	19.78	5.26	85.40	22.03
8:00pm	580.40	2.70	6.06	1195.60	387.20	602.70	22.69	3.63	3.78	24.56	5.55	85.70	21.17
10:00pm	600.50	2.70	5.42	1478.50	453.70	581.40	24.33	3.89	3.11	18.34	4.51	86.30	21.17
AVERAGE	541.17	2.81	4.57	1326.34	446.12	589.74	589.74	3.54	5.74	19.83	4.99	85.29	21.99
PER DAY		4039.20											527.76

Table 49 Experiment Two: Infusion Data, Period 1, Animal 550, Corn Gluten Meal Diet

1550 CNC.INF. 121.44 (g/l) PEG

SAMPLE TIME	PEG CNC (mg/100ml)	INF.RT. (ml/min)	DUOD DM	TB CNC (ug/g)	CR CNC (ug/g)	LA CNC (ug/g)	CP CNC (g/100g)	NIT CNC (g/100g)	NH3-N (mg/100ml)	ADF CNC (g/100g)	ADL CNC (g/100g)	OM CNC (g/100g)	PEG FLW (g/hr)
12:00am	628.30	2.89	6.29	1093.50	391.60	751.60	23.20	3.71	7.11	24.17	5.84	88.12	21.06
2:00am	625.20	2.86	5.78	1172.00	465.30	645.80	22.64	3.62	6.89	18.69	5.45	85.33	20.84
4:00am	578.40	2.87	4.85	1373.00	550.60	491.20	20.82	3.33	6.56	15.32	4.34	83.30	20.91
6:00am	562.00	2.80	6.72	1065.00	365.20	965.20	23.56	3.77	7.44	20.81	6.11	88.28	20.40
8:00am	684.10	2.88	5.59	1036.40	346.30	583.80	19.12	3.06	6.89	18.58	4.58	87.38	20.96
10:00am	660.10	2.80	6.01	1099.50	302.80	753.30	17.30	2.77	9.56	19.81	5.21	88.09	20.40
12:00pm	672.80	3.16	6.54	1014.50	264.60	595.20	17.44	2.79	7.56	20.44	5.08	88.72	23.03
2:00pm	519.40	2.88	5.08	1139.50	387.50	586.60	17.82	2.85	6.44	16.48	4.63	86.14	20.98
4:00pm	728.60	2.94	5.32	1253.50	439.60	505.30	17.88	2.86	7.56	19.67	5.00	85.13	21.42
6:00pm	682.80	2.85	6.09	1370.00	479.70	628.70	20.84	3.33	8.89	23.42	6.22	88.87	20.77
8:00pm	606.10	2.89	6.40	1210.50	511.90	542.30	19.92	3.19	7.22	20.00	4.81	88.60	21.06
10:00pm	631.00	2.82	6.92	1251.50	532.60	709.50	21.49	3.44	6.00	21.55	5.37	88.38	20.55
AVERAGE	631.57	2.89	5.97	1173.24	419.81	646.54	646.54	3.23	7.34	19.91	5.22	87.20	21.03
PER DAY		4156.80											504.80

Table 50 Experiment Two: Infusion Data, Period 2, Animal 813, Corn Gluten Meal Diet

2813 CNC.INF. 130.66 (g/l) PEG

SAMPLE TIME	PEG CNC (mg/100ml)	INF.RT. (ml/min)	DUOD DM	YB CNC (ug/g)	CR CNC (ug/g)	LA CNC (ug/g)	CP CNC (g/100g)	NIT CNC (g/100g)	NH3-N (mg/100ml)	ADF CNC (g/100g)	ADL CNC (g/100g)	OM CNC (g/100g)	PEG FLW (g/hr)
12:00am	630.70	2.65	6.02	1068.00	537.50	523.30	21.97	3.52	8.67	18.96	3.22	86.26	20.77
2:00am	631.20	2.65	7.40	1061.20	380.80	549.50	22.64	3.62	8.33	22.41	3.75	88.60	20.77
4:00am	607.20	2.64	8.44	769.40	276.80	605.20	20.79	3.33	7.67	24.42	3.28	89.50	20.70
6:00am	709.00	2.85	6.16	1101.00	313.00	504.70	21.50	3.44	8.89	21.26	3.76	85.98	22.34
8:00am	654.20	2.60	4.64	798.80	258.80	330.20	20.70	3.31	11.11	14.30	2.92	82.75	20.36
10:00am	681.70	2.82	6.00	1195.50	391.90	472.70	19.64	3.14	8.00	22.26	3.64	82.56	22.11
12:00pm	498.10	2.62	4.74	1169.00	346.00	425.60	23.70	3.79	11.11	18.04	3.50	83.99	22.11
2:00pm	651.00	2.82	5.66	1101.00	451.00	426.30	21.85	3.50	7.56	18.46	2.09	85.28	22.11
4:00pm	587.00	2.83	5.88	979.10	414.20	442.90	20.57	3.29	7.33	23.24	4.11	84.26	22.19
6:00pm	581.00	2.82	5.61	970.30	315.40	389.20	20.72	3.32	5.89	14.86	2.75	84.36	22.11
8:00pm	705.40	2.80	7.30	1072.50	422.60	522.80	17.51	2.80	6.44	25.56	5.32	86.37	21.95
10:00pm	552.00	2.89	6.02	1093.00	474.20	584.40	20.78	3.32	6.22	20.22	3.28	85.60	22.66
AVERAGE	624.06	2.77	6.16	1033.23	381.85	483.07	483.07	3.36	8.10	20.33	3.47	85.46	21.68
PER DAY		3982.80											520.35

Table 51 Experiment Two: Infusion Data, Period 2, Animal 819, Corn Gluten Meal Diet

2819 CNC.INF. 119.50 (g/l) PEG

SAMPLE TIME	PEG CNC (mg/100ml)	INF.RT. (ml/min)	DUOD DM	YB CNC (ug/g)	CR CNC (ug/g)	LA CNC (ug/g)	CP CNC (g/100g)	NIT CNC (g/100g)	NH3-N (mg/100ml)	ADF CNC (g/100g)	ADL CNC (g/100g)	OM CNC (g/100g)	PEG FLW (g/hr)
12:00am	622.00	2.80	6.50	1258.00	462.40	688.20	25.19	4.03	6.22	23.16	4.75	84.38	20.08
2:00am	634.40	2.80	5.54	1297.50	505.00	694.40	24.87	3.98	7.10	21.19	4.41	80.40	20.06
4:00am	598.90	2.84	6.05	1506.00	505.60	763.20	23.68	3.79	8.88	19.83	5.24	83.28	20.36
6:00am	655.40	2.80	8.03	1195.50	358.90	716.60	19.31	3.09	9.10	29.54	9.20	76.85	20.08
8:00am	663.20	2.90	6.60	1226.50	345.50	672.40	22.04	3.53	8.99	21.62	3.46	88.24	20.79
10:00am	689.30	2.76	6.23	1109.50	428.70	655.10	19.18	3.07	11.11	25.53	6.89	79.87	19.79
12:00pm	567.10	2.81	5.24	1206.50	513.90	538.20	19.99	3.20	11.81	22.90	3.86	86.60	20.15
2:00pm	396.80	2.77	6.41	1177.00	505.00	567.70	20.81	3.33	74.37	27.11	6.14	83.37	19.86
4:00pm	595.90	2.79	6.40	1185.50	532.40	484.00	19.45	3.11	63.27	24.06	5.90	83.50	20.00
6:00pm	590.00	2.75	7.84	1180.00	408.40	575.80	21.79	3.49	51.06	27.67	7.43	82.36	19.72
8:00pm	680.60	2.74	10.08	1017.50	392.80	629.20	17.34	2.77	55.55	22.76	5.34	91.22	19.65
10:00pm	647.60	2.86	8.42	989.20	406.20	674.40	19.32	3.09	52.17	26.58	5.99	85.00	20.51
AVERAGE	611.77	2.80	6.95	1195.73	447.07	638.27	638.27	3.37	29.97	24.33	5.72	83.76	20.09
PER DAY		4034.40											482.11

Table 52 Experiment Two: Infusion Data, Period 2, Animal 820, Soybean Meal Diet

2820 CNC.INF. 135.91 (g/l) PEG

SAMPLE TIME	PEG CNC (mg/100ml)	INF.RT. (ml/min)	DUOD DM	YB CNC (ug/g)	CR CNC (ug/g)	LA CNC (ug/g)	CP CNC (g/100g)	NIT CNC (g/100g)	NH3-N (mg/100ml)	ADF CNC (g/100g)	ADL CNC (g/100g)	OM CNC (g/100g)	PEG FLW (g/hr)
12:00am	587.20	2.80	4.80	1235.00	386.20	1228.50	22.94	3.67	4.66	17.06	4.15	83.70	22.63
2:00am	660.40	2.80	4.55	1197.50	367.60	1197.00	22.14	3.54	4.99	15.96	3.64	82.40	22.83
4:00am	549.70	2.75	3.82	1028.20	382.80	1072.20	21.55	3.45	5.33	12.90	2.77	79.42	22.43
6:00am	647.60	2.72	4.41	1074.00	327.40	965.00	20.49	3.28	4.77	13.34	3.04	80.35	22.18
8:00am	567.60	2.70	3.45	1097.50	374.60	908.20	21.48	3.44	4.66	11.16	2.49	78.50	22.02
10:00am	588.20	2.70	5.30	857.00	287.40	748.00	17.71	2.83	6.44	20.60	4.24	84.43	22.02
12:00pm	495.80	2.71	3.24	966.80	548.90	752.90	21.56	3.45	5.66	8.06	1.76	77.78	22.10
2:00pm	456.90	2.76	5.19	896.20	395.80	793.80	17.04	2.73	4.66	22.82	4.52	83.66	22.51
4:00pm	466.30	2.78	4.80	1120.00	422.90	1072.00	20.34	3.25	3.55	20.52	4.62	83.50	22.67
6:00pm	508.00	2.75	5.08	1311.50	506.60	1361.50	18.24	2.92	3.44	18.94	4.26	83.28	22.43
8:00pm	519.30	2.73	5.04	1366.50	522.80	1277.50	21.44	3.43	3.44	17.39	3.75	83.71	22.26
10:00pm	438.20	2.45	4.10	1556.50	639.10	1391.50	17.02	2.72	3.11	13.00	2.76	80.88	19.98
AVERAGE	540.43	2.72	4.48	1142.23	430.18	1064.01	1064.01	3.23	4.56	15.98	3.50	81.80	22.17
PER DAY		3916.00											532.50

Table 53 Experiment Two: Infusion Data, Period 2, Animal 550, Soybean Meal Diet

2550 CNC.INF. 152.13 (g/l) PEG

SAMPLE TIME	PEG CNC (mg/100ml)	INF.RT. (ml/min)	DUOD DM	YB CNC (ug/g)	CR CNC (ug/g)	LA CNC (ug/g)	CP CNC (g/100g)	NIT CNC (g/100g)	NH3-N (mg/100ml)	ADF CNC (g/100g)	ADL CNC (g/100g)	OM CNC (g/100g)	PEG FLW (g/hr)
12:00am	632.60	2.80	5.78	1116.00	473.40	1435.50	19.90	3.18	5.22	19.74	4.62	85.94	25.56
2:00am	539.80	2.80	5.10	1372.50	527.40	1256.00	23.08	3.69	4.66	15.88	4.00	83.90	25.50
4:00am	590.00	2.80	4.22	1238.50	490.00	1451.50	24.14	3.86	4.77	15.40	3.64	82.14	25.56
6:00am	585.80	2.83	4.78	1034.50	404.30	1247.00	19.17	3.07	5.00	16.51	3.92	83.74	25.83
8:00am	607.20	2.90	5.10	989.00	308.60	1100.00	19.71	3.15	5.88	18.76	4.13	84.82	26.47
10:00am	651.80	2.69	4.84	1275.00	401.60	1159.00	19.59	3.13	5.99	16.29	3.79	83.94	24.55
12:00pm	552.80	2.63	4.74	920.00	321.00	978.50	17.21	2.75	6.33	19.28	4.17	84.68	25.83
2:00pm	671.90	2.93	4.94	1086.00	456.30	881.40	19.28	3.08	7.20	15.82	3.95	84.00	26.74
4:00pm	771.50	2.77	4.50	1066.00	455.20	790.80	18.34	2.93	7.33	12.38	3.20	83.55	25.26
6:00pm	577.20	2.77	5.20	917.20	366.70	797.40	17.81	2.85	6.77	17.84	3.84	85.10	25.28
8:00pm	601.40	2.65	4.44	1044.00	444.40	748.20	20.93	3.35	7.77	14.40	3.68	83.30	24.19
10:00pm	579.50	2.76	4.78	970.60	477.80	820.60	22.92	3.67	6.10	16.28	4.30	82.54	25.19
AVERAGE	613.46	2.79	4.87	1085.78	427.23	1055.49	1055.49	3.23	6.09	16.55	3.97	83.77	25.50
PER DAY		4023.60											612.11

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