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EFFECT OF DIETARY CONCENTRATE LEVELS ON IN SITU DRY MATTER
DISAPPEARANCE, NEUTRAL DETERGENT FIBER DISAPPEARANCE AND
DIGESTION KINETICS OF ALFALFA HAY, WHEAT STRAW AND STEAM
PROCESSED AND FLAKED MILO GRAIN

The University of Arizona

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by

Alejandro Ruben Urias

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COMMITTEE OF NUTRITIONAL SCIENCES (GRADUATE)

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In the Graduate College

THE UNIVERSITY OF ARIZONA

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As members of the Final Examination Committee, we certify that we have read
the dissertation prepared by Alejandro R. Urias

entitled Effect of Dietary Concentrate Levels on In Situ Dry Matter
Disappearance, Neutral Detergent Fiber Disappearance and
Digestion Kinetics of Alfalfa Hay, Wheat Straw and Steam
Processed and Flaked Milo Grain

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

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Final approval and acceptance of this dissertation is contingent upon the
candidate's submission of the final copy of the dissertation to the Graduate
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I hereby certify that I have read this dissertation prepared under my
direction and recommend that it be accepted as fulfilling the dissertation
requirement.

R. Spencer Swingle
Dissertation Director

January 20, 1986
Date

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SIGNED:

Alexandros R. Quas

"Dedicated to my wife, Nina."

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ABSTRACT

A replicated 3x3 latin square design (3 periods and 6 rumen fistulated steers) was utilized to investigate the effect of dietary concentrate levels (30, 60 and 90%) on the in situ disappearance of dry matter (ISDMD) and neutral detergent fiber (NDF) and kinetics of fiber digestion of alfalfa hay, wheat straw and steam processed and flaked milo (SPFM). Concentrate levels of 30 or 60% did not affect ($P>.05$) ISDMD or NDF disappearance from any of the substrates. However, ISDMD and NDF disappearance of all substrates were depressed ($P<.05$) by the 90% concentrate diet.

The degradation of the potentially digestible fiber in these substrates followed first order kinetics at all concentrate levels. Fiber digestion kinetics were not affected ($P>.05$) by dietary concentrate levels of 30 or 60%. Incubation of substrates in animals receiving the 90% concentrate diet resulted in lower potential extent of digestion ($P>.05$) and decreased rate of degradation ($P>.05$) for all substrates. However, digestion was not complete even after incubations of 168-h, and thus, it is possible that the potential extent of digestion was underestimated.

For all substrates lag time of digestion appeared to increase in the 90% concentrate diet. However, influx of NDF-like material into the bag during incubation makes the validity of the lag times obtained in situ questionable.

Ruminal pH was depressed in animals consuming the 90% concentrate diet and could be at least one of the factors responsible for the decreased ($P > .05$) fiber digestibility in this diet. Mean ruminal pH of 6.5 (30 and 60% concentrate diets) did not depress ($P > .05$) fiber digestibility while a mean pH of 6.2 resulted in a lower ($P > .05$) fiber digestibility in the rumen.

CHAPTER 1

INTRODUCTION

Fiber comprises a significant portion of the ruminant diet. Although large advances in the understanding of fiber composition and digestion have been made in recent years, there are still many areas where the knowledge is incomplete. In recent studies at the University of Arizona, performance of growing finishing steers was not adversely affected when wheat straw was substituted for alfalfa hay in conventional high concentrate diets. These results cannot be explained by our current understanding of the nutritional value of fibrous low quality roughages. The well documented low energy density of wheat straw coupled with the low fiber digestibility expected in high-concentrate diets should have resulted in depressed performance, particularly increased feed requirements. It is possible that the utilization of wheat straw in these types of diets is greater than expected. However, another more likely explanation is that the inclusion of low-quality roughages into these diets results in modifications in rumen environment such that the utilization of other diet components is improved through

changes in rate and extent of degradation and/or effects solid and liquid passage.

A first step towards examining the latter possibility is to characterize the effects of dietary concentrate level on the utilization of different ingredients in mixed diets. Partitioning of total fiber digestion to individual diet ingredients and compartmentalization to different segments of the ruminant digestive tract is complicated at best. Recent developments in digestion kinetics and modeling promise to be helpful in accomplishing this task. At this time there is basic agreement that fiber degradation in the rumen can be approximated as a first order reaction. However, there is uncertainty as to the proper methods for obtaining data to use in the kinetic model. This is especially true when ruminal conditions are affected, such as is the case of alterations in dietary concentrate levels. Furthermore, the development of these models has been with data obtained primarily under closed in vitro systems with constant incubation conditions. It is well known that the ruminal environment is highly dynamic and therefore, the first order model for fiber digestion which is applicable under in vitro conditions may not apply to the live animal. It is necessary that these problems be resolved before any useful applications of digestion kinetics can be made.

The objectives of this dissertation are to: 1) investigate the effect of concentrate level on ruminal utilization of fiber from low and high quality forages and a cereal grain under in situ conditions; and 2) to determine whether the current fiber digestion kinetic models are appropriate for in situ investigations.

CHAPTER 2

LITERATURE REVIEW

The term fiber is generally used loosely and in the course of this discussion will be used to describe those fractions of the plant which are not digested by mammalian enzymes. Conceptually, fiber is defined as those fractions of the plant which are associated with the cell wall. Anatomically cell walls include a number of different tissues which vary in function and chemical composition (Cutter, 1978; Fahn, 1982). Chemical make-up of plant cell walls is complex and they are composed of a number of different substances. Due to this complexity, and its importance in fiber utilization, a brief section has been included in the following discussion to further characterize the chemical nature of the plant cell wall. Analytical determination of plant cell walls can be accomplished as that portion of the total plant material which is not soluble in a neutral detergent solution (Goering and Van Soest, 1970).

The main scope of this discussion is to review the development and current understanding of concepts of fiber digestion kinetics as well as its association with plant structures, and factors affecting it. However, a brief

section dealing with the anatomy and structure of the plant is necessary in order to support the later discussion.

Plant Structure

Most forages used in livestock feeding are herbaceous, annual or perennial plants which lack secondary woody growth. Arrangement and characteristics of plant tissues vary between different plant species as well as within species at different stages of maturity. There is evidence that this may be related to, and perhaps limit, utilization of plant materials. This section provides an overview of plant anatomy, with a brief characterization of the types of tissues present in forage stems and leaves (Weir, Stocking and Barbour, 1970; Cutter, 1978; Fahn, 1982; Akin and Barton, 1983).

The Stem

A cross section of the alfalfa hay stem is shown in Figure 1 as an example for the general structures of the herbaceous dicotyledon stem (Fahn, 1982). The epidermis consists of several layers of tissue which vary, depending on the species, as to the size, number, and morphology of the cells. The outermost layer, the cuticle, is composed of a waxy polymer called cutin which is impermeable to gases and water. Regulated openings, the stomata, are scattered throughout the surface of the stem and serve primarily for gas exchange. Epidermal appendages such as hairs are often

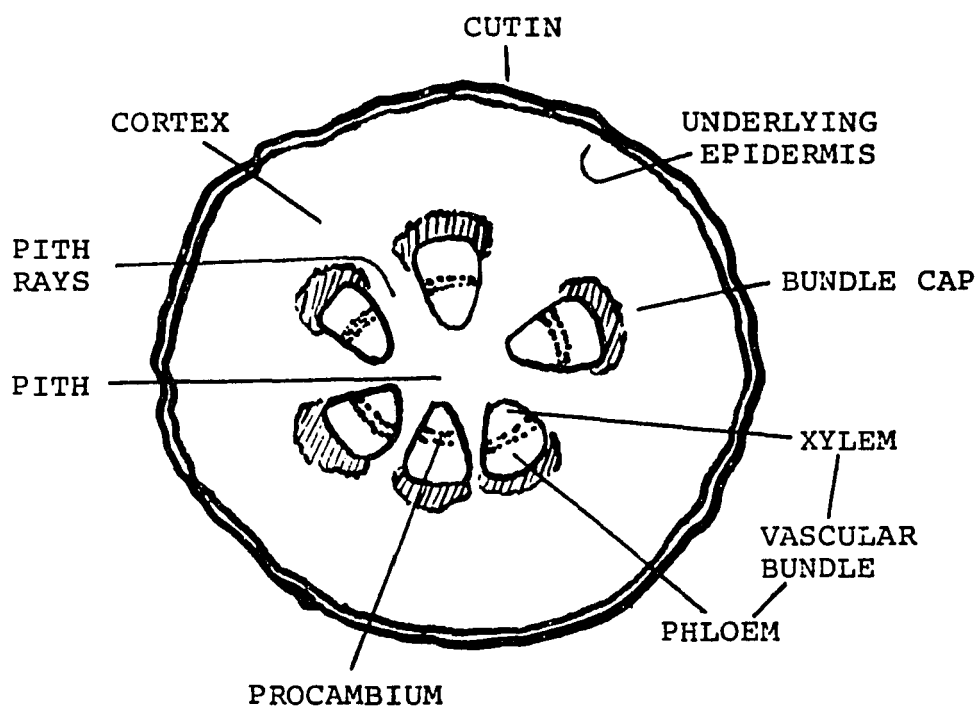


FIGURE 1. Cross section of an alfalfa hay stem (monocoty ledon) as described by Fahn, 1982.

present. The underlying epidermal cells are made up of symmetrical elongated cells characteristic of the plant series.

The cortex is defined by Fahn (1982) as that tissue located between the epidermal and vascular tissues (the steele). Three types of tissues may be found in this area: collenchyma, parenchyma and schlerenchyma. The arrangement of these tissues is not constant but varies between plant species and even within species at different stages of maturity.

Collenchyma is often the outermost tissue of the cortex in young stems where it may constitute a complete cylinder or occur in separate strands. Collenchyma cells are elongated with blunt or oblique ends. They possess thick cellulosic walls which thicken at the corners where cells meet. High amounts of hemicellulose and pectins are also found in these walls. Due to their chemical composition, which gives them great plasticity, collenchyma tissues serve for support allowing stem flexibility. In addition, collenchyma tissues possess living contents allowing them to function in plant nutrient production. Collenchyma type tissues are not generally found in monocotyledon species such as grasses.

Parenchyma tissues may comprise a considerable portion of the cortex of some stems, however they are not exclusive to this region. Parenchyma tissues may also be

found in pith, roots, leaves, endosperm of seeds and to a lesser extent, may be associated with the vascular bundles. Parenchyma cells are isodiametric (polyhedral) with thin cellulosic walls and occasional intercellular air spaces. They serve many important functions to the plant such as photosynthesis, respiration, secretion and storage. Although parenchyma cells usually contain primary walls, secondary lignified walls may be found in this type of tissue in some topographical locations, such as vascular xylem and occasionally pith parenchyma.

Sclerenchyma tissues function in support and protection of the stem. These tissues are distinguishable from collenchyma both in the lack of living contents and in possessing thick, lignified secondary cell walls. Two types of cells may be found in this tissue: fiber cells and sclerids. Fiber cells are elongated elements with pointed tips, narrow lumens and hard, thick secondary walls. Fibers are commonly found in bundles, usually in association with xylem and phloem, but occasionally also in bundle caps and cortex. Sclerids are isodiametric cells which may be branched and possess very thick, hard, lignified walls. They occur singly or in groups sometimes associated with xylem and phloem, but more commonly in parenchymatous tissues (i.e., pith and cortex of stem and petiole).

The innermost layer of the cortex in young dicotyledon stems contains many large starch granules (the

starch sheath), which disappear from older stems. In general, the herbaceous stem consists of a parenchymal matrix containing vascular bundles scattered throughout and arranged in a circular fashion (Figure 1).

The steele is the vascular cylinder enclosed by the cortex and is composed of non-vascular tissue (the pericycle), vascular tissue and pith (Figure 1). The steele may vary from a solid to a hollow tube. Arrangement of vascular tissues differs among various plant groups, and sometimes even within the same species. The vascular tissue is organized into vascular bundles, usually arranged around the pith in a circular fashion. Each bundle contains xylem toward the center of the stem and phloem toward the external surface. In some plants the xylem is both internal and external to the phloem. The cambium is a narrow band of dense meristematic tissue which separates, and gives origin to, phloem and xylem.

The phloem is a complex structure which serves primarily in the transport of organic nutrients throughout the plant. Xylem functions to transport water and inorganic solutes throughout the plant. The xylem functional units are tube-like structures made up of tracheids and vessel elements, which are dead tissues with lignified cell walls. Usually, sclerenchyma fiber cells are associated with these structures. The pith consists of parenchymatous tissue in a loose arrangement, often containing thick-walled cells, and

serves mainly in the storage and radial transport of nutrients. This tissue may develop early in the plant life and later stop growing while the rest of the plant develops, creating a hollow pith. Such is the case of most grasses.

Grasses (monocotyledons) possess tissues similar to that of the dicotyledons previously described, however, their arrangement is generally different. As an example, a cross section of a typical grass stem (wheat) is shown in Figure 2. Collenchyma is absent from this stem. Vascular bundles are structurally organized as in dicotyledons. However, instead of being arranged in a circular fashion, vascular bundles are found scattered throughout the parenchymal matrix in a less rigid arrangement.

The Leaf

The upper surface of the leaf is referred to as the adaxial surface, while the lower surface is called the abaxial surface. The two, although similar, often differ as to the number, type and degree of development of structures present (e.g., hairs, stomata, thickness of cuticle). The epidermis is similar in arrangement to the stem epidermis. The outer impermeable layer (cuticle) is made of waxy cutin and contains scattered stomata. The underlying epidermis, like that of the stem, is normally a single layer of symmetrical elongated cells which are characteristic of the plant. The veins, which are distributed throughout the

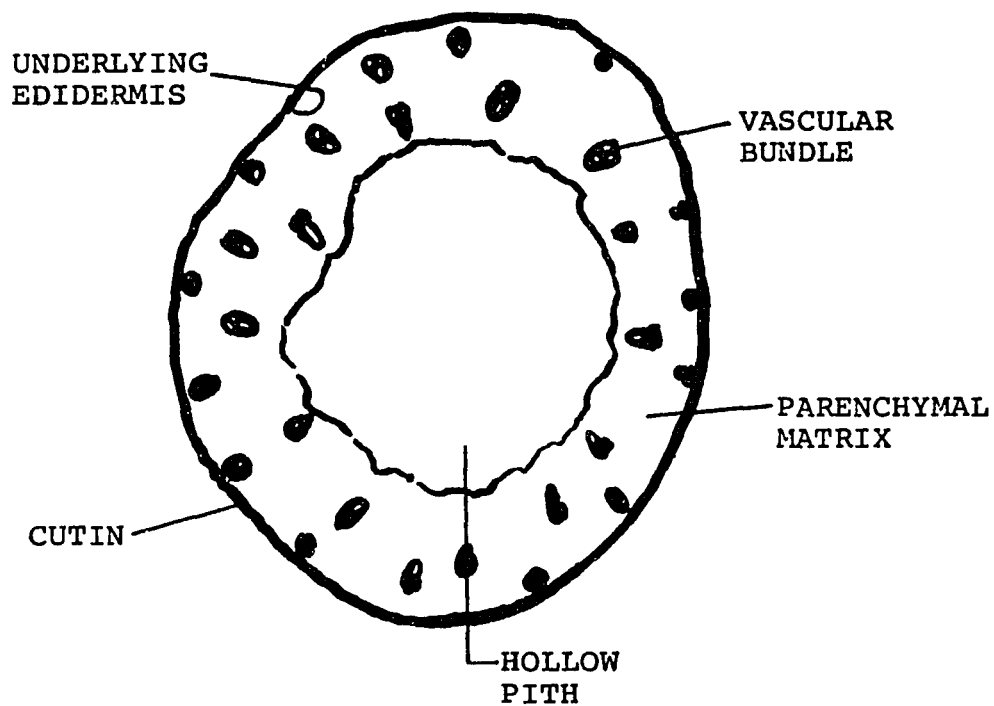


FIGURE 2. Cross section of a wheat grass stem (dicotyledon) as described by Weir et. al., 1970.

leaf, contain vascular tissue which has the same anatomical arrangement as stems. The mesophyll which lies between the upper and lower epidermis contains two parenchyma tissue: 1) the upper paladize parenchyma (closer to the adaxial surface), and 2) the lower spongy parenchyma. Although slightly different in their structure, both of these tissues function in the synthesis and storage of plant food. These tissues have characteristics similar to those described for the stem parenchyma.

The Cell Wall

According to Cutter (1972) a primary wall is formed first during the development of the plant, followed by a secondary wall as the plant matures. Cell wall formation begins with the deposition by cell organelles of the cell plate, which in higher plants gives rise to the middle lamella. The middle lamella, which is composed of pectin substances, is a thin layer sometimes called intercellular substance which holds together the primary walls of adjacent cells. The primary wall is the first to be formed by the cell and is deposited on both sides of the middle lamella by the contiguous cells. Chemically it consists mainly of cellulose and hemicellulose. All meristematic cells have primary walls as do many mature cells which still have living contents. Secondary walls are formed after the cells have completed their elongation, and are considered to

provide mechanical support. Where the secondary wall is formed, it is deposited on the inner side of the existing wall, next to the cell lumen. It consists of cellulose and other polysaccharides, but hemicelluloses are of less importance than in the primary wall. Lignin is usually deposited during secondary wall formation in both primary and secondary walls. In some tissues the secondary wall may be stratified and composed of several layers, while in others it may be absent (Weir et. al., 1970). Secondary walls are present (such as sclerids and fibers) in cells which are non-living at maturity.

Akin and Barton (1983) summarize the structural polysaccharides which make up the plant cell walls and divide them into fiber polysaccharides (cellulose) and matrix polysaccharides (hemicellulose and pectins).

Hemicellulose is generally poly B, 1-4, D, xylose with branches of arabino-, gluco-, and/or galactopyranose. Based on HPLC analyses of extracted hemicellulose Akin and Barton (1982) point out that the xylose is the predominant component of hemicellulose. However, the relative proportions of the various sugars vary between different grass species, even those with similar amounts of hemicellulose.

Pectin substances make up the intercellular structures and are composed of chains of galacturonic acid, galactans and arabinans. Pectins are mixed and branched

polysaccharides chains bound to hemicellulose through hydrogen bonding.

Cellulose is composed of β , 1-4, D-glucopyranose units which combine to form cellulose microfibrills which presumably are held together by hydrogen bonding and appear to vary according to species as to their degree of crystallinity (packing).

Lignin is a polymer of phenyl-propane units intimately associated with the structural carbohydrates (perhaps by covalent bonding) binding as to prevent microbial attack.

Current Concepts of Fiber Digestion Kinetics

The overall process of digestion in the rumen can be visualized as the product of two competing events: the rate at which a substrate is degraded and its residence time in this compartment. Description and understanding of these events should allow the recognition and selection of conditions which would maximize the utilization of substrates in the rumen.

It is generally accepted that the limiting factor in the utilization of roughages is their high fiber content. At least in theory, fiber digestion kinetics permits a more precise description of its mode and rate of degradation. The following section deals with the development of current concepts of fiber degradation kinetics.

Original interest in determining rates of forage fiber digestion seems to have originated from the theories proposed by Crampton, Donefer and Lloyd (1960). These researchers proposed that the effective nutritive value of a forage is determined jointly by its maximum voluntary intake when it constitutes the entire ration, and its ultimate yield of digestible energy.

Crampton et. al. (1960) further proposed that in ruminant animals fed all-roughage diets, hunger is associated with a reduction in rumen load. Second, that rates of reduction of rumen loads vary depending on the composition of the forage being consumed. Therefore, those forages which are reduced at faster rates will support higher intakes. Following these assumptions, Crampton, et. al. (1960) proposed their nutritive value index (NVI), which was designed to combine observed voluntary intake and digestibility to a single value to rank forages relative to a standard forage of high quality (See insert for determination of NVI). These researchers found NVI to be more effective than either voluntary intake or digestibility alone in ranking forages in relation to performance among forage-fed cattle. It should be kept in mind that these assumptions would apply only in those cases in which the fiber content (or bulk) of the roughage is the factor controlling voluntary intake. Van Soest (1964, 1965) points out that other factors besides fiber may control voluntary

intake (VI) and that positive correlations between VI and lignin or VI and total fiber are possible. For example, intake of mature fescue grass is often higher than that of immature plants even though cell wall content increases with maturity. Furthermore, Van Soest (1965) showed some evidence that cell walls are not a factor controlling intake until their level exceeds 55-60% of the dry matter, and therefore these theories would only apply to those cases.

CALCULATION OF NUTRITIVE VALUE INDEX.
Crampton et. al. (1960).

Designed to serve as an estimator of the nutritive value of roughages relative to a standard roughage. It was based on 5 roughages (three legumes and two grasses) in which voluntary intake and digestibility by beef cattle was known. Since legumes generally demonstrate higher levels of intake, the standard forage was defined in terms of these. Intake was expressed on a per unit of metabolic body weight (Kg.75). The average intake (rounded value) of all legumes was 80g/Kg.75 and is arbitrarily assigned a value of 100. The relative VI of any forage was calculated as follows:

- 1) Metabolic body is calculated (i.e., Wt. Kg.75)
- 2) Calculate the expected intake of the standard forage i.e., [Wt. Kg.75] x [80g/Kg.75]
- 3) Divide the observed intake of the test forage by the calculated intake of the standard forage and multiply by 100 to calculate the VI index.

The in vivo dry matter digestibility of the forages which showed a VI index of 100 or better averaged 70.0%. Therefore, the NVI of the standard forage would be:

$$\text{NVI} = [\text{VI index}] \times [\text{DMD}] = 100 \times 70 = 70$$

NVI for the test forages would be calculated in a similar manner.

Crampton et. al. (1960) based NVI on known voluntary intake and digestibility of forages and therefore this measure suffered from the time and labor requirements of the digestion trials. To overcome this problem, these researchers suggested the values could be derived from in vitro data.

Doenefer, Crampton and Lloyd (1960) determined in vitro cellulose digestibility at 3-, 6-, 12-, 24- and 48-h for the sample feeds utilized by Crampton et. al. (1960). The 12-h in vitro cellulose digestibility was highly correlated with voluntary intake, while the 24-h value was better correlated with the observed in vivo dry matter digestibilities for these forages. No improvement in the correlation values were obtained when the 12x24 values were used. These workers then proposed the 12-h in vitro cellulose digestibility value could be used for the calculation of the NVI (a regression equation was published). Similar results were published by Johnson et. al. (1962).

Chalupa and Lee (1966) designed a series of experiments in order to develop a system for estimating forage digestibility and intake from in vitro cellulose digestibility. Twenty-five grasses for which digestibility and/or voluntary intake had been determined were used in these studies. Incubations were carried out for 6-, 12-, 18-, 24-, 30-, 36-, 42-, and 48-h. It was concluded that

voluntary intake was best correlated with the 18-h in vitro cellulose disappearance while the 30-h value was a better estimator of in vivo digestibility. NVI was best correlated with the 18x30-h values. The reasons for the discrepancies between these results and those of Doenefer et. al. (1960) are not clear.

Further discrepancies can be found in the studies conducted by Reid et. al. (1960) with nylon bags. These workers reported a high correlation between 24- and 48-h disappearance values and in vivo digestibility values, but failed to find a relationship between 4-, 8-, 12-, 20-, 32- and 48-h disappearance values and voluntary intake.

It is possible to theorize from these studies, as proposed by Van Soest (1982), that while the long term incubation values (e.g., 24- or 30-h) are an estimation of total in vivo digestibility, the short term values (e.g., 12- and 18-h) are an estimation of the rate of degradation since they are better correlated with in vivo intake. Furthermore, the short term incubation values are located in the portion of the digestion curve which is rapidly changing and should indicate rate of degradation. The lack of agreement between these studies as to which time predicts these rates may be due to the fact that neither the 12- or 18-h value, is a good estimator of the overall degradation rate. Thus, a need had been established for techniques which could be used to better describe the digestion

processes and enable prediction of digestibility and intake.

Wilkins (1969) postulated that cellulose digestibility reached a maximum after 6-d incubation in vitro. In these studies, no further digestibility was obtained when the 6-d residues were re-incubated in vitro for an additional 6-d. Similar results were obtained when nylon bags were suspended in the rumen for 4-d. Wilkins (1969) concluded that not all of the cellulose present in roughages could be digested in the rumen.

Waldo (1969) noted the work of Wilkins (1969) and indicated that while the disappearance of total cellulose could not be explained with any mathematical function, the disappearance of the potentially digestible cellulose fraction was first order.

Gill, Conrad and Hibbs (1969) designed experiments to: 1) correlate in vitro cellulose disappearance with feed intake in cows consuming high dry matter legume grass silage and 2) to test the theories proposed by Waldo (1969). The forages tested represented four different cuttings in which voluntary intake had been measured and digestibility determined in two cows. In vitro incubations were carried out for 0-, 6-, 12-, 24-, 36-, and 48-h.

These workers assumed that the 48-h in vitro residue defined the potentially digestible fiber present in these samples (Figure 3). Following these assumptions, these workers indicate that as postulated by Waldo (1969), the

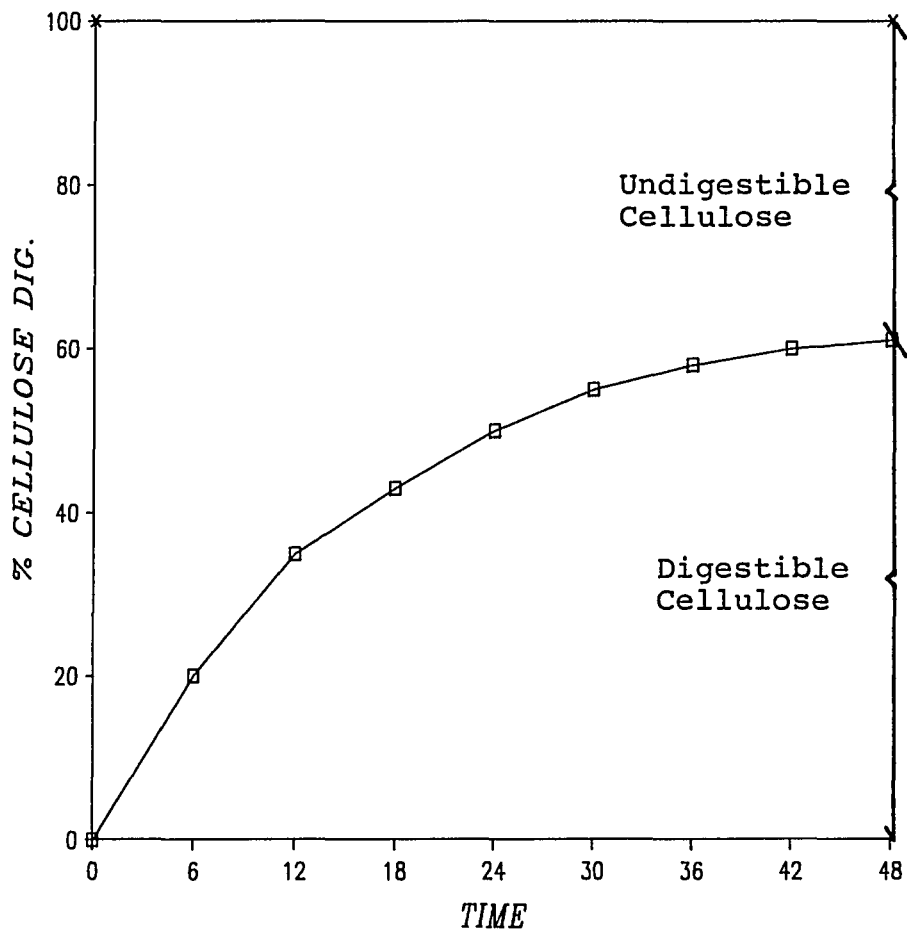


FIGURE 3. Cumulative cellulose digestion curve for a roughage (Gill et. al., 1969).

disappearance of the potentially digestible portion was first order with respect to the amounts of this fraction present at any time. Therefore, the plot of the natural logarithm of the amounts of remaining potentially digestible fiber (y axis) versus time, yielded a straight line. The slope (k) of the regression line represented the disappearance rate of the substrate from the in vitro system and thus rate of digestion. The units of this system were mg of cellulose digested per hour. If the amount of cellulose present was converted to percent of the total potentially digestible cellulose and plotted as indicated before, the value could then be called the relative rate of cellulose digestion (Figure 4). In these studies, these rates were not correlated with the 12-, 24- and 48-h incubation values, but were strongly correlated with voluntary intake when the forage was fed alone. The correlations were completely nullified when concentrates were fed.

The postulations made by these workers imply several characteristics of cellulose digestion. First, there are potentially digestible and undigestible portions in the cellulose fraction of roughages, both of which can be defined by the 48-h in vitro residue. Second, cell contents, which are almost completely available, do not influence the rate at which cellulose is digested. Third, degradation of cellulose begins immediately upon its

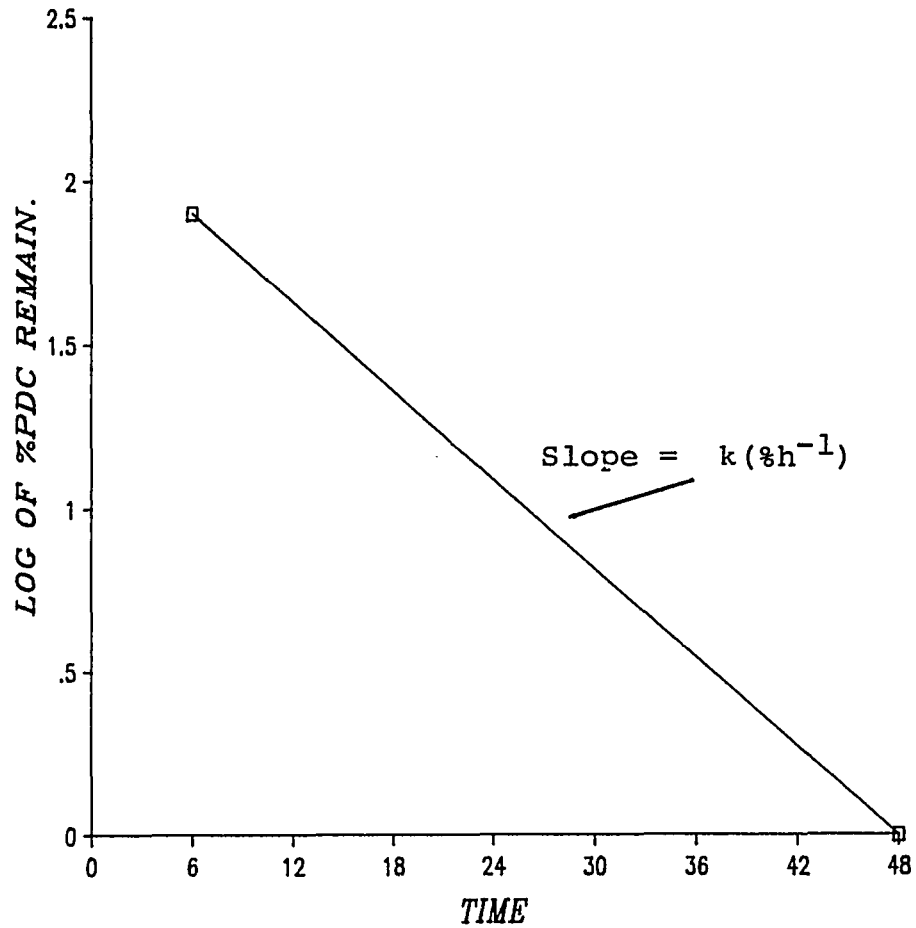


FIGURE 4. Plot of the logarithm of the percent potentially digestible cellulose remaining versus time. The first order model for fiber digestion (Waldo, 1969; Gill et. al., 1969).

entering the ruminal compartment at an overall rate which is dependent on a constant fractional rate and the amounts of the digestible fraction present in the sample. In other words, the reaction is first order with respect to the amounts of potentially digestible fiber present in the sample and therefore obeys the formula $R = k[A]$, where R is the overall rate, k is the fractional rate constant and A is the amount of potentially digestible fiber present at any time.

Smith et. al. (1971) set up a series of experiments to corroborate: 1) that the disappearance of cell walls as determined by the detergent system of analyses (NDF) also obeyed first order kinetics; 2) that rate of cell wall digestion is not as well related to the lignification of fiber as it is to the cell contents; and 3) that the similarity of the lignin:cellulose ratios in the theoretical undigestible residues strongly supports the extent of digestion limiting role of lignin. Three ground whole grasses and three hays and their isolated cell walls were incubated with rumen fluid for 6-, 12-, 18-, 24-, 30-, 48- and 72-h. Cell solubles of the roughages ranged from 31 to 67% and lignin from 2 to 7%. Digestibilities of isolated cell walls were not different to those predicted from whole samples and chemical composition. This led the authors to conclude that cell wall digestibility predicted from whole incubated samples and chemical composition were accurate.

Digestibility of samples approached the asymptote after 72-h of incubation. Thus, digestion was considered essentially complete at this time and the undigestible residue was defined by these values. These workers found that the lignin:ADF and lignin:cellulose ratios of the undigested samples had a 3.3- to 3.6-fold range respectively, while the 72-h in vitro residues had a 1.2- to 1.3-fold range respectively (Table 1). Smith et. al. (1971) indicated that this points toward lignin having a role in limiting the extent of digestion, rather than the rate of digestion. However, recoveries of lignin of 100% had to be assumed in order to attain these results.

Linearity of the semilog plots of the remaining digestible cell walls on time and the high correlation of these values ($r = .98$) indicated first order digestion kinetics of these forages, in spite of their range in composition. Rates were calculated by regressing the log 10 of the percent potentially digestible cell walls versus time. The regression coefficient (b) multiplied by 2.303 (to convert to ln) and then by 100 gave the digestion rate in percent digested per hour.

Differences in rate of digestion were observed between these roughages. Fiber from more mature forages was digested more slowly, while the immature hays had the fastest rates (Table 1). lignin:ADF ratios and rates of disappearance were not

TABLE 1. Chemical composition, in vitro digestibility and L/C ratios of forage samples as reported by Smith et. al., 1971.

HAY	CELL WALL		L/C x 100		CELL SOL. % of DM
	DIG. %/h	INITIAL % LIGNIN	INITIAL	RESIDUAL	
Rye, 23 cm tall	27.03	4.0	10.0	77.0	67.0
Alfalfa, prebud	11.38	17.0	32.0	80.0	61.0
Alfalfa, 1/2 b	10.31	19.0	36.0	80.0	55.0
Alfalfa	8.75	19.0	35.0	76.0	52.0
Orchadgrass	7.81	9.0	20.0	79.0	40.0
Timothy, mature	5.65	10.0	20.0	63.0	31.0

It is interesting to note that in these studies the correlations between the lignin:cellulose ratios or significant but those between cell solubles and rate of digestion were positive ($r = .77$) and significant ($P < .05$). However, these authors concluded that similarities in digestibility between isolated cell walls and whole plant cell walls strongly precludes the possibility of cell solubles per se contributing to the rate of cell wall digestion.

This paper appears to support the theories of Waldo (1969) and Gill et. al. (1969) discussed earlier, and indicates that digestibility of both cellulose, as determined by the method of Crampton and Maynard (1938) and cell walls, as determined by the detergent system of analyses, follow first order kinetics. However, Smith et. al. (1969) proposed that the undigestible portion of the roughage be defined by the 72-h residue instead of the 48-h residue as suggested by Gill et. al. (1969). Furthermore, Smith et. al. (1971) indicated that the digestibility curve approached but did not reach the asymptote by 72-h. Tauskey, Ellis and Matis (1972) observed the same problem and suggested that even though digestion continued after 72-h, the maximum theoretical asymptotic digestibility would not exceed 105% of this value and therefore this could be used to define the undigestible residue. Furthermore, these workers proposed that since digestion does not begin

immediately after the substrate reaches the rumen, an initial lag phase should be introduced into the model. They suggest that introduction of a time delay component into the model results in more accurate (lower error mean squares) description of the reaction.

Mertens and Van Soest (1972) indicated that when the 72-h in vitro residue did not define the undigestible residue since degradation continues past this time. Instead, these workers proposed that a mathematical technique would be used to define the undigestible residue. This approach basically assumes that the degradation curve is a true hyperbola and predicts its asymptote by determining the y-intercept of a double reciprocal regression of $1/\text{time}$ vs. $1/\text{undigested residue}$. This is similar to the Lineweaver - Burke plot of Michaelis - Menten enzyme kinetics. At $1/t=0$ ($t = \text{infinite}$), $1/UR$ is maximal and conversely extent of digestion is maximal. To test these hypothesis, these workers incubated four grass samples for periods of 3- to 96-h to calculate the extent of digestion by the mathematical technique and compare these results to the same samples incubated for up to 166-h. The calculation of extent of digestion by inclusion of points up to 30-h resulted in non-linear regressions. However, calculations using the points between 30- and 96-h agreed well with the results obtained at 166-h. The validity of this technique remains to be documented.

Mertens (1977) proposed, in accordance with the suggestions of Tuskey et. al. (1972), that the inclusion of a discrete lag phase would improve the first order model. Mertens (1977) defined the lag phase of digestion as that period of initial fermentation when digestion either does not occur, or progresses at a greatly reduced rate. The lag phase is shown in Figure 5, as the initial non-linear segment of the curve, and would be calculated by solving the regression equation for x when y is 100%.

Mertens (1977) further stated that the first order kinetics model is dependent on the definition of the undigestible residue, and when the digestion curve is taken to infinite time (Mertens and Van Soest, 1972) the resulting curve significantly departs from linearity. Instead two phases are observed in the digestion curve (logarithmic transformation). The first portion of the curve represents a rapidly digesting fraction and the second portion a slower digesting fraction. Both of these fractions obey first order kinetics with respect to the amounts of each present and each would have its own fractional rate (k_1 and k_2). The rapidly and slowly digesting fractions of the potentially digestible fiber are illustrated in Figure 6.

The practical importance of the rapidly and slowly digesting fractions is still not clear. Their quantification has not been defined in exact terms and should be crucial to the solution of the model. The

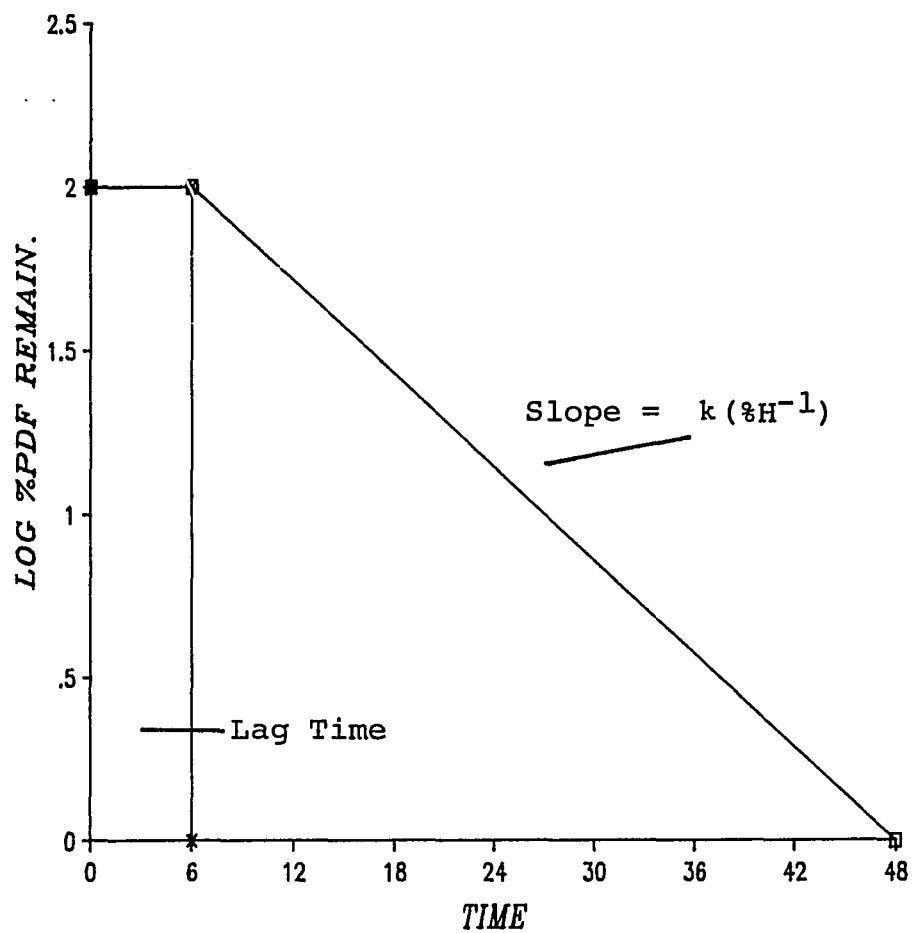


FIGURE 5. First order model of fiber digestion with the incorporation of a discrete lag phase (Tuskey et. al., 1972).

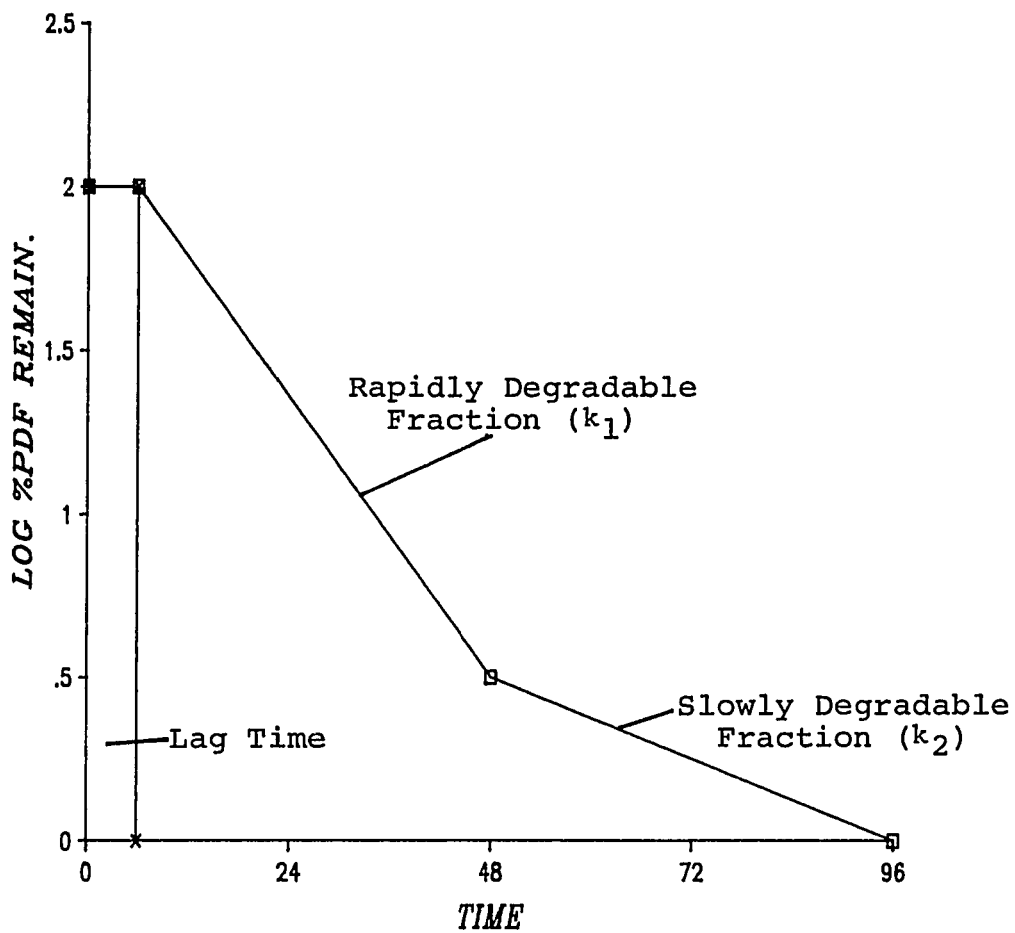


FIGURE 6. First order model of fiber digestion with a discrete lag phase, a rapidly digesting fraction and a slowly digesting fraction (Mertens, 1977).

observations of Mertens (1977) also raise a number of questions as to the mode of digestion of these fractions. Whether the digestion of these two fractions occurs concurrently or sequentially cannot be established from studies to-date. If digestibility of the slowly digesting fraction occurs after the rapidly digesting fraction has been exhausted its contribution to the overall fiber digestibility should be very small or non-existent, since it would occur after a time in which most material has left the digestive tract.

Fiber Digestion Kinetics as
Related to Plant Structure and Composition

Digestion kinetics parameters appear to be closely related to the types and composition of tissues found within the plant. Akin et. al. (1973) used the scanning electron microscope (SEM) to study the rate and extent of degradation of the different tissues found in the leaves of coastal bermuda grass (tropical grass) and "Kentucky 31" fescue grass (temperate grass). Intact leaves were incubated in vitro in a buffered medium with strained rumen fluid for 6-, 12-, 24-, 48- and 72-h.

Evaluation of the tissues remaining after 6-h incubations revealed little apparent degradation in the coastal bermuda grass; epidermal tissue essentially remained undisrupted and only slight degradation of the mesophyll and phloem was evident. Conversely, fescue leaves showed

detectable degradation, with more of the mesophyll and some of the underlying epidermal tissue being degraded. By 12-h, degradation appeared to occur at the same anatomical regions in both grasses. Mesophyll, phloem and underlying epidermal tissues appeared extremely degraded in both grasses. Schlerenchymal tissues, xylem and cuticle tissues appeared to resist degradation. Tissue degradation was more extensive in the fescue grass at all times. The authors speculate that the more extensive degradation of the lignified tissues of fescue grass may be due to the different types of lignins between these forages.

Akin, Burdick and Michaels (1974) studied the bacterial attachment to intact bermuda during digestion using SEM. It was found that during digestion the physical association of the bacteria with the plant cell wall varied with the tissue type. Mesophyll and parts of the phloem structures were degraded prior to the other tissues and without bacterial attachment to the cell walls. On the other hand, rumen bacteria appeared to attach to the thick walls of the lignified peripheral schlerenchyma and bundle sheaths prior to their degradation. In degrading the epidermis, the bacteria prefer the underlying tissues of this structure and will not attack the cuticle, which is separated during the process.

Akin and Amos (1975) conducted similar studies with fescue leaves. As with bermuda leaves (Akin et. al., 1974),

bacteria initially and preferentially attacked the mesophyll and phloem of fescue leaves which were rapidly degraded. Bacterial attachment to these tissues was not necessary for their degradation. However, those tissues which were degraded only after long periods of incubation (vascular bundles and peripheral sclerenchyma) apparently required bacterial attachment prior to their degradation. Bacteria such as *R. Albus* have been isolated which digest with extracellular enzymes, while others such as *B. Succinogens* and *B. Ruminicola* require attachment prior to degradation.

The observations of Akin and coworkers pertained primarily to intact leaf structures and it was uncertain whether they applied to the whole plant. Brazle and Harbers (1977) collected 16 samples of alfalfa and separated leaves and stems. Digestibilities were determined in vitro for 12-, 24-, 48- and 72-h and for 12-, 24- and 36-h in situ. Residues were studied using SEM. Samples incubated for 48-h in water showed no structural changes indicating that changes observed after incubation were due to bacterial action or rumen motility.

Digestibilities obtained by the two techniques are shown in Table 2. Cuticle was randomly attacked at the adaxial leaf surface causing cuticle sloughing off and degradation of the underlying epidermis and mesophyll after 12-h. At this time leaves varied from partially degraded mesophyll and vascular tissue to completely degraded

TABLE 2. Digestibility of stems and leaves of alfalfa hay by the nylon bag and in vitro techniques. Brazle and Harbers, 1977.

TISSUE	TECHNIQUE	
	NYLON BAG (24 h)	IN VITRO (48 h)
LEAF	78-89%	70.6%
STEM	34-46%	38.5%

mesophyll with only adaxial and abaxial cuticle and small amounts of vascular tissue remaining. Modes of digestion for leaves were similar for all incubation times with longer times resulting in an increased degree of hydrolysis. After 24-h in the rumen, cuticle of stems was sloughed off and the underlying dense matrix (collenchyma and parenchyma) was partially hydrolyzed. By 48-h the dense matrix was completely hydrolyzed exposing the fiber cells (sclerenchyma) which define the outer limits of the vascular tissues (bundle cap). After 72-h most of the vascular tissue remained except for small amounts near the lumen. The same patterns were observed in extracted fecal material. These workers conclude that the alfalfa stem cortex was rapidly degraded while the vascular tissues were essentially non-degradable. The authors further indicate that since most of the lignin is contained in the vascular tissue, their observations supported the postulations of Smith et. al. (1971) that lignin has a role in limiting the extent of digestion rather than as inhibitor of the rate of digestion.

The above discussion, although based on observations made with the electron microscope on intact structures, presents some interesting points. It would appear that the amounts of rapidly degradable and slowly degradable cell walls is directly related to the microanatomy of the plant. First, in both stems and leaves the cuticle appears

undegraded and presents a barrier for microbial attack. Removal of this layer would be necessary for degradation of at least the underlying epidermal tissues and factors which disrupt the cuticle could conceivably have an effect on either the lag phase or rate of digestion. Cutin plus those highly lignified tissues which remain after long incubation times (xylem, metaxylem and vascular cambium) correspond to the undegradable cell walls.

Second, bacteria seem to preferentially attack the mesophyll tissues of the leaf and cortex tissues of the stem which are similar in cell wall structure (collenchyma and parenchyma). Since these tissues are rapidly degraded during initial stages of incubation, it is possible to speculate that they correspond to the rapidly degradable portion of the digestion curve proposed by Mertens (1977). The more lignified tissues with thick walls (sclerenchyma) which are degraded only after prolonged incubations would then represent the slowly degradable portion of the digestion curve. These tissues require bacterial attachment for degradation and are apparently degraded only after the more available tissues have been hydrolyzed.

These hypotheses would indicate that the overall digestion curve is not the summation of the curves of rapidly and slowly degradable portions occurring at the same time. Instead, digestion of the rapidly degradable fraction would precede digestion of the slowly degradable portion.

This is contrary to the assumptions made by Mertens (1977) in his model of rumen digestion since he implies that both processes occur simultaneously. This model is discussed in the following section. These hypotheses also have implications in the determination of the fractional rate constants (k) and the application of fiber digestion kinetics in models designed to predict total rumen digestion. If the degradation of the rapidly and slowly digesting fractions are subsequent events, the determination of the quantities and fractional rate constants of the slowly degradable portion is only important in those situations in which the residence time in the rumen is longer than the time required for the rapidly degradable portion to be exhausted. However, in situations in which the rate of digestion is decreased (e.g., due to concentrate additions) there would be no contribution to the overall digestion from the slowly degradable portion and therefore, it can be ignored. It is necessary that this question be resolved before practical applications of fiber digestion kinetics data can be made.

Applications of Fiber Digestion Kinetic Data

Applications of fiber digestion kinetic data could be widespread. Combining fiber digestion kinetic data with feed passage data in complete models could be used for prediction of feed intake, especially in those cases where

high cell wall content is the factor limiting consumption. It could also allow a more precise prediction of the total utilization feedstuffs from laboratory measures. Furthermore, this technique could be extremely useful in those situations where a more precise description of the ruminal degradation reaction is desired for the understanding of the mechanisms of digestion and its controlling factors. The following section merely attempts to illustrate some of the possible applications of fiber kinetic data. For more detailed derivations the reader is referred to the original papers being discussed.

Waldo, Smith and Cox (1972) proposed that a complete rumen model should relate rate of passage, rate of digestion, extent of digestion, daily intake, rumen composition and rumen fill. These workers attempted to describe with a mathematical model the disappearance of chemically determined fiber components, such as lignin-free cellulose and hemicellulose. Lignin was considered essentially undigestible. In this model, cellulose was considered to be composed of two fractions: potentially digestible (PDC) and undigestible (UC). Second, it was accepted that disappearance of PDC followed first order kinetics with respect to the amounts present at any time (Waldo, 1969; Gill et. al., 1969).

The basic assumptions of this model can be described by the following equations:

$$(1) \quad dA/dt = -k_1A - k_2A$$

$$(2) \quad dB/dt = -k_2B$$

where:

A = Potentially digestible cellulose remaining

B = Undigestible cellulose

k_1 = Rate of digestion

k_2 = Rate of passage

t = time (h)

Equation (1) simply states that the rate of change in PDC in the rumen, at a point in time (t) is negative (decreases) and is dependent on the rate of digestion ($-k_1A$) and the rate of passage ($-k_2A$) which are competing processes. On the other hand, disappearance of the UC (Equation 2 above) can only occur due to passage out of the ruminal compartment.

The basic equations can now be mathematically manipulated to attain a meaningful equation. A noncalculus solution to the problem is sought.

Equation 1 can be rearranged as in (1.1).

$$(1.1) \quad dA/dt = -k_1A - k_2A = A[-(k_1 + k_2)]$$

We now define A_0 as the PDC originally present in the sample and B_0 as the UC. Then, to determine the amounts of A and B remaining at any one time, we can use:

$$(3) \quad A = A_0 * e^{-(k_1 + k_2)t}$$

$$(4) \quad B = B_0 * e^{-k_2t}$$

These are the functions which describe the logarithmic curve of a first order reaction. Equation 3 is expressed in terms of two competing processes (passage and digestion).

Next, T is defined as the sum of the fractions A and B at any given time, $T = A + B$. If (a) represents the fraction of A in T, and (b) is the fraction of B in T, then at zero, a and b would be calculated as:

$$(5) \quad a = A_0 / (A_0 + B_0)$$

$$(6) \quad b = B_0 / (A_0 + B_0)$$

It is now possible to calculate the cellulose remaining (f) after a period of time. If we express (f) as a fraction of the original amounts of PDC and UC present we obtain:

$$(7) \quad f = T / (A_0 + B_0) = A + B / A_0 + B_0$$

By substituting Equations 3 and 4 into Equation 8 we can obtain the fraction (f) of the original material ($A_0 + B_0$) remaining in the rumen after a period of time (mean residence time):

$$(8) \quad f = [A_0 * e^{-(k_1 + k_2)t} + B_0 * e^{-k_2 t}] / [A_0 + B_0]$$

Equation 8 simply predicts the amount of the original feeding present in the rumen after a time (t) postfeeding. After an animal has been in a feeding regime for a period of time, a steady state in ruminal dry matter content is reached. At this state, Equation 8 theoretically predicts ruminal fill. The remainder of the manipulations only serve to simplify Equation 8.

From Equation 8:

$$(8.1) f = [A_0 * e^{-(k_1+k_2)t}] / [A_0+B_0] + [B_0 * e^{-k_2t}] / A_0+B_0$$

$$(8.2) f = [e^{-(k_1+k_2)t}] [A_0 / (A_0+B_0)] + [e^{-k_2t}] B_0 / (A_0+B_0)$$

Then substituting Equations 5 and 6 into Equation 8.2 we obtain:

$$(9) f = a * e^{-(k_1+k_2)t} + b * e^{-k_2t}$$

The predictive ability of this model depends on the successful determination of A_0 and B_0 , as well as the fractional rate constants k_1 and k_2 . This model does not provide for rapidly and slowly degradable fractions (as proposed by Mertens, 1977) and assumes that digestion begins immediately after the feed reaches the ruminal compartment. To this author's knowledge this model has not been extensively tested against live animal measures.

Mertens (1977) expanded the model of Waldo et. al. (1972). Rate of passage estimates were made by using the two compartment model of Hungate (1966) which estimates turnover time. This model was selected due to its easy interpretation.

The following modifications to Waldo's model (1972) were proposed by Mertens (1977): 1) inclusion of all fiber components by separation of the digestible fiber into slowly digesting and rapidly digesting portions, both of which obey first order kinetics; 2) the combination of these two fiber pools to estimate digestion of fiber in the rumen; 3) the introduction of a discrete lag phase of digestion into the

model; 4) introduction into the model of variables which would attempt to predict total tract digestibility.

Since this model is simply an extension of the model of Waldo et. al. (1972) discussed earlier, its detailed mathematical derivation will not be considered in this discussion. However, for the purpose of illustrating possible applications of fiber digestion kinetic measurements, this model is described in general terms below.

The following definitions are necessary:

C = rapidly degradable fraction

D = slowly degradable fraction

E = undergradable fraction

CWC = cell wall contents

CW = cell walls

k_1 = Fractional digestion rate for rapidly degradable fraction

k_2 = Fractional digestion rate for slowly degradable fraction

k_3 = rate of passage

T = fraction of fiber remaining at time t

By definition, the total amount of fiber remaining at time t, can be obtained by:

$$(10) \quad T = C + D + E$$

Based on the first order model, Equations 11, 12 and 13 would then describe digestion in the rumen at a point in time:

$$(11) \quad dC/dt = -(k_1 + k_3)C$$

$$(12) \quad dD/dt = -(k_2 + k_3)D$$

$$(13) \quad dE/dt = -k_3E$$

Then, by a noncalculus solution similar to that described for the previous model, the total fraction of cell walls which can be digested in the rumen can be obtained by:

$$(14) \quad C/T[k_1/(k_1+k_2)] + D/T[k_2/k_2+k_3]$$

This equation describes the potentially digestible fiber which is disappearing from the rumen. Then assuming that the lag phase is that period of time in which passage occurs but digestion does not, we can describe the fractions of $[C/T]$ and $[D/T]$ at the beginning of digestion by e^{-k_3L} . Incorporating the lag component into Equation 14 we obtain:

$$(15) \quad [C/T][k_1/k_1+k_3][e^{-k_3L}] + [D/T][k_2/k_2+k_3][e^{-k_3L}]$$

Equation 15 describes the fraction of the potentially digestible cell wall which is digested in the rumen. This is perhaps the most useful part of Mertens (1977) model since it would allow digestion to be compartmentalized into ruminal and lower tract components in those situations in which a greater understanding of the digestion processes is desired.

Since the above equation only predicts digestibility in the ruminal compartment, the remainder of this model

requires that the fraction of total digestion occurring in the rumen (R) be known. Furthermore, to predict total apparent dry matter digestibility (ADMD) it assumes (as suggested by Goering and Van Soest, 1970) that CWC digestibility is 98% and endogenous dry matter loss is a constant 12.9% units. The complete model is shown in Equation 16:

$$(16) \text{ ADMD} = \{ [\text{CW}][\text{C}/\text{T}][k_1/k_1+k_3][e^{-k_3L}] + [\text{CW}][\text{D}/\text{T}][k_2/k_2+k_3][e^{-k_3L}] \} / \text{R} + .98(\text{CWC}) - 12.9$$

Other more complicated models have been proposed (Mertens and Ely, 1979) which attempt to incorporate particle size pools and their change due to lag phases, mastication, degradation and passage.

It is central to the validity of these models, first, that the first order model of fiber digestion describe the digestion process within the rumen and second, that kinetic parameters of passage and digestion can be measured with a degree of precision under a variety of dietary and intake conditions. The majority of the data discussed so far has been developed under in vitro conditions which do not necessarily reflect the situation in the rumen in vivo. More research needs to be conducted to validate these theories and models.

Factors Affecting Fiber Digestion Kinetics

It has been established that under in vitro conditions those factors which affect forage digestibility (i.e., species, stage of maturity) will affect lag phase of digestion as well as potential extent and rate of digestion (Smith et. al., 1971). Other factors affecting digestion kinetics have not been studied extensively.

It is generally accepted that additions of concentrate to ruminant diets will lower dietary fiber digestibility. However, the mechanisms by which this occurs are still under debate. Effects of concentrate addition on fiber digestion kinetics have been reported in two studies. Mertens and Loften (1978) studied the effects of wheat (high solubility) and corn (low solubility) starch additions on in vitro digestion kinetics of four forages. Alfalfa hay and coastal bermuda, fescue and orchard grasses with starch added at four levels (0, 40, 60, and 80%) were incubated in vitro for 0-, 6-, 12-, 18-, 24-, 48-, 72- and 96-h.

In these studies starch type did not affect any of the digestion parameters. Across starch additions, individual forages differed in their kinetic parameters. For each roughage type, duration of lag phase increased and extent of digestion decreased with each starch addition from 0 to 80%. However, rate of degradation was not affected by starch addition. The decrease in potentially digestible cell walls reported could be an artifact of the manner in which this

fraction was determined in these studies. Since increases in lag time would essentially shift the digestion curve to the right, maintaining a constant rate of degradation while holding a constant end point would result in decreased potentially digestible fiber.

Miller and Muntifering (1985) conducted similar studies in situ. Bags containing fescue grass were incubated for 6-, 12-, 18-, 24-, 48- and 72-h in rumen fistulated steers consuming diets which contained 0, 20, 40, 60 and 80% corn grain. Rate of fiber digestion was not affected by concentrate addition, and lag time of digestion was increased only slightly (not significant) at the 80% level of concentrate addition. However, addition of 80% concentrate to these diets resulted in decreased potential extent of digestion. As in the previous study, end point of digestion was held constant and therefore the decrease in extent of digestion observed in these studies could be an artifact of this restriction.

System and mathematical factors which affect the potentially digestible cell walls merit further investigation. Since their definition is central to the first order model, its determination would affect calculations of the fractional rates as well as lag time.

CHAPTER 3

MATERIALS AND METHODS

A replicated 3x3 latin square design (3 periods and 6 steers) was used to study the effects of dietary concentrate levels (30, 60 or 90%) on in situ disappearance of neutral detergent fiber (NDF) from alfalfa hay, wheat straw and steam processed and flaked milo (SPFM).

Six rumen fistulated okie-type steers were used in these experiments. Three of the steers used (square A) were older animals (approximately 8 years old) with an average weight during the experiment of 670 kg, while the other three (square B) were younger animals (approximately 2 years old) with a mean body weight during the experiment of 430 kg. Steers were housed in individual stalls and fed the appropriate diet twice daily at 0700- and 1800-h. Throughout the experiment, steers in square A consumed 1.5% of body weight while those in square B consumed approximately 2.3% of body weight. Composition of the 30, 60 and 90% concentrate diets is shown in Table 3.

Each period in the latin square consisted of an adaptation period of at least 21-d followed by a period which in situ disappearance and pH measurements were taken. Bags for in situ measurements were constructed of a dacron

TABLE 3. Ingredient composition of experimental diets.

ITEM	Concentrate Level (%)		
	30	60	90
	----- %, as mixed -----		
Chopped Alfalfa Hay	34.28	22.12	5.53
Chopped Wheat Straw	38.08	21.76	5.44
Steam Flaked Milo	21.95	51.94	80.72
Urea	0.84	0.72	0.59
Molasses	6.11	6.02	5.94
Tallow	1.77	1.75	1.72
Dicalcium-Phosphate	0.94	0.59	0.30
Limestone	-	0.23	0.85
Salt	0.44	0.44	0.43
	100.00	100.00	100.00
Vitamin A	3300 IU/kg	3300 IU/kg	3300 IU/kg
Monensin	30 g/ton	30 g/ton	30 g/ton

material with an approximate pore size of 50 microns. A single piece of material (19x20 cm) was cut with a hot soldering iron to prevent fraying of the material at the edges. The material was then folded in half and glued with silicone rubber along the edge (glue line approximately 1-cm wide) rounding the corners as to avoid pocket formation. Bags were then sewn with nylon thread along the midline of the glue material to reinforce the seams.

Prior to the experiments, a sufficient amount of each substrate was ground through a 1-mm screen (Wiley mill) to last for the entire study. Approximately 6-g of substrate were weighed into previously dried (100 C) and tared bags. Bags were then closed approximately 7-cm from the top using nylon cord and tied to a weighted chain. Chains were placed into the ventral sac of the fistulated steers immediately before the morning feeding. All substrates were incubated simultaneously at each concentrate level. Duplicate bags for each substrate were then removed after 6-, 12-, 24-, 36-, 48-, 60-, 72-, 84-, 96-, 120- and 168-h of incubation.

Immediately after removal from the rumen, bags were washed under tap water until the rinse was clear. Bags were dried in a forced-air oven for 48-h at 45C and then in a vacuum oven for an additional 48-h at 60C. Residues were removed from each bag and analyzed for NDF according to the method of Goering and Van Soest (1970). Dry matter and NDF

disappearances were calculated as percent loss during incubation.

On the day after removal of the last set bags, pH measurements were taken hourly for 18-h starting prior to the a.m. feeding and then again prior to the next a.m. feeding (24-h). Approximately 250 ml of fluid were extracted from the liquid phase of the rumen using a plastic hose attached to a vacuum system. The plastic hose was inserted into the rumen through a special cannulae with a small opening (2.54-cm I.D.) and a quick release cap in an effort to minimize disturbance to rumen environment due to aeration. The pH was determined immediately after sample removal using a combination pH electrode.

In situ dry matter disappearance (ISDMD), NDF disappearance and pH data were analyzed using analyses of variance for a double latin square design (Steel and Torrie, 1960).

Kinetic parameters for NDF digestion were obtained assuming that the degradation curve could be described by a first order reaction (Waldo, 1969; Gill et. al., 1969; Mertens, 1977). Analyses of variance for the regression lines were done according to Meter and Wasserman (1974). Comparisons among slopes were made according to Steel and Torrie (1960) and Snedecor and Cochran (1978).

CHAPTER 4

RESULTS AND DISCUSSION

Effects of Dietary Concentrate Levels on In Situ Disappearance of Dry Matter and Neutral Detergent Fiber

In situ dry matter disappearances for alfalfa hay, wheat straw and SPFM at the different concentrate levels are illustrated in Figures 7, 8 and 9, respectively (Appendix Tables A.1, A.2 and A.3). For each substrate, ISDMD values were not different ($P > .05$) between the 30 and 60% concentrate diets but were depressed ($P < .05$) for the 90% concentrate diet at all incubation times, except that in the case of SPFM there was no difference ($P > .05$) due to diet at 6-h of incubation. Disappearance curves for NDF paralleled those of ISDMD in all cases (Figures 10, 11 and 12 and Appendix Tables A.1, A.2 and A.3).

Negative NDF disappearances were observed during the early incubation times, at all concentrate levels, for alfalfa hay and SPFM. Negative NDF digestibilities for wheat straw were only observed in those bags which were incubated in steers consuming the 90% concentrate diet. Since negative disappearances are not possible, there must have been an influx of NDF into the bags during the incubations. Migration of material from the medium into the

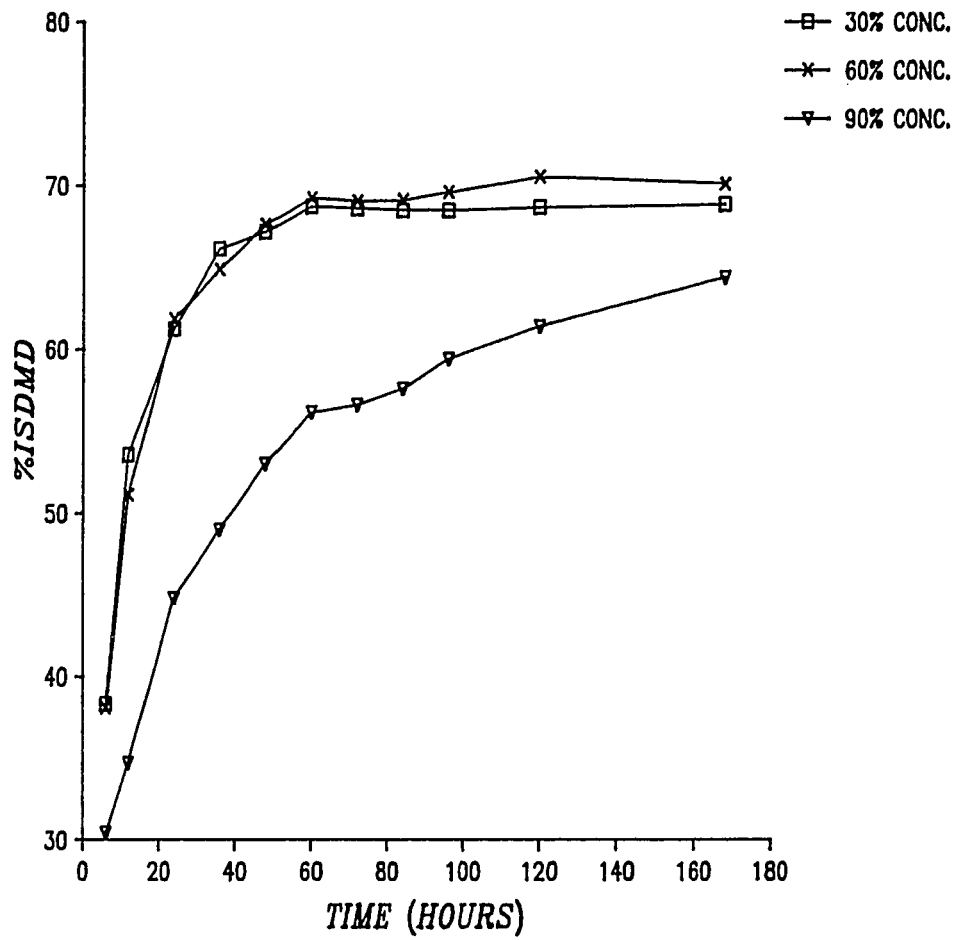


FIGURE 7. In situ dry matter disappearance of alfalfa hay as affected by dietary concentration levels.

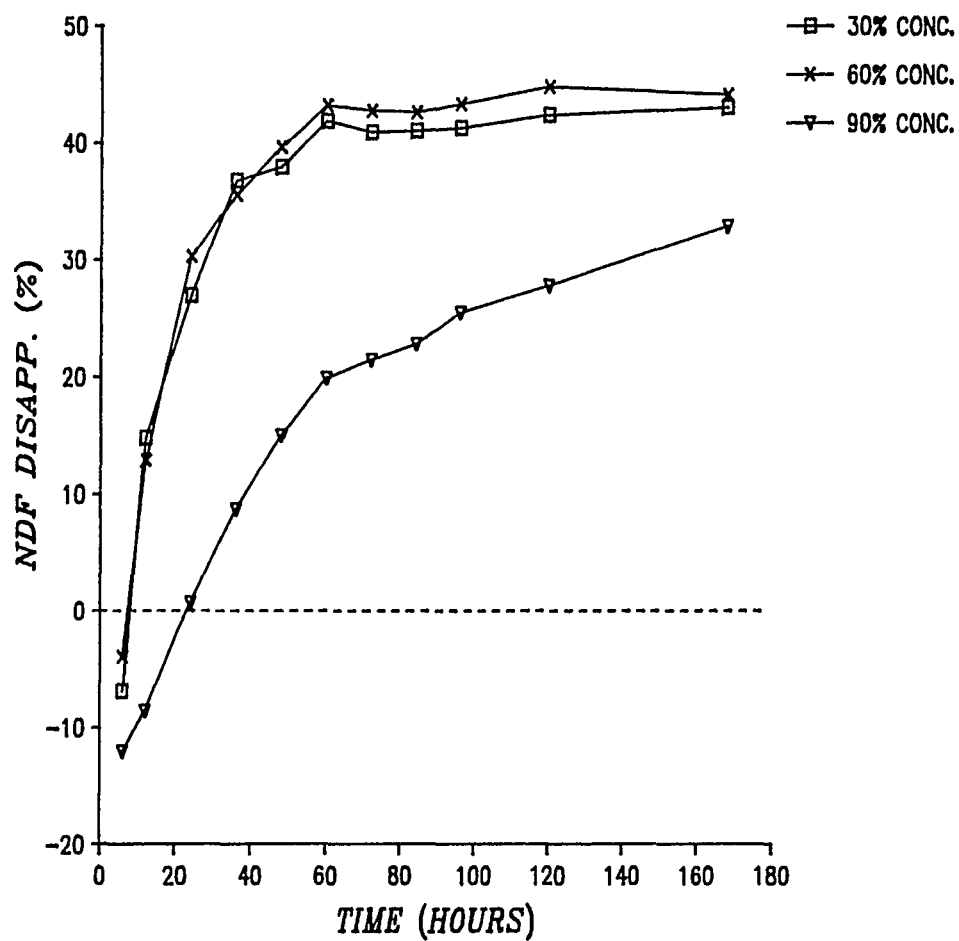


FIGURE 8. In situ dry matter disappearance of wheat straw as affected by dietary concentration levels.

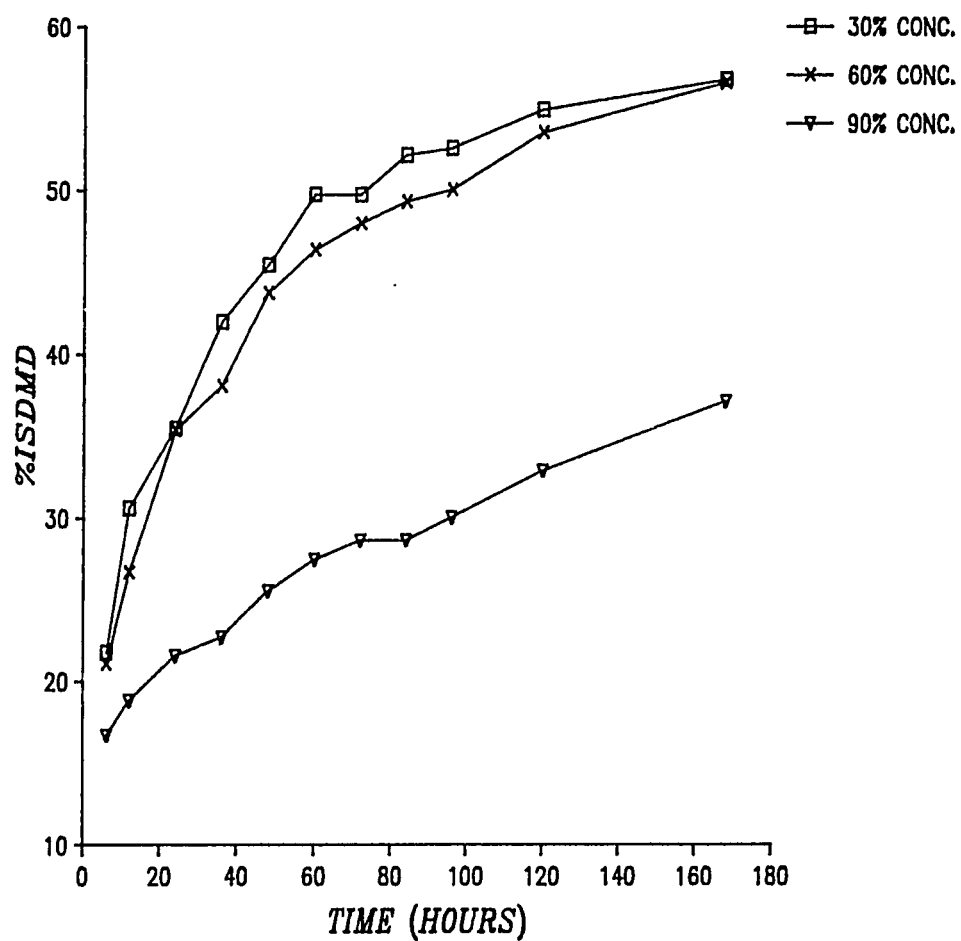


FIGURE 9. In situ dry matter disappearance of steam processed and flaked milo as affected by dietary concentrate levels.

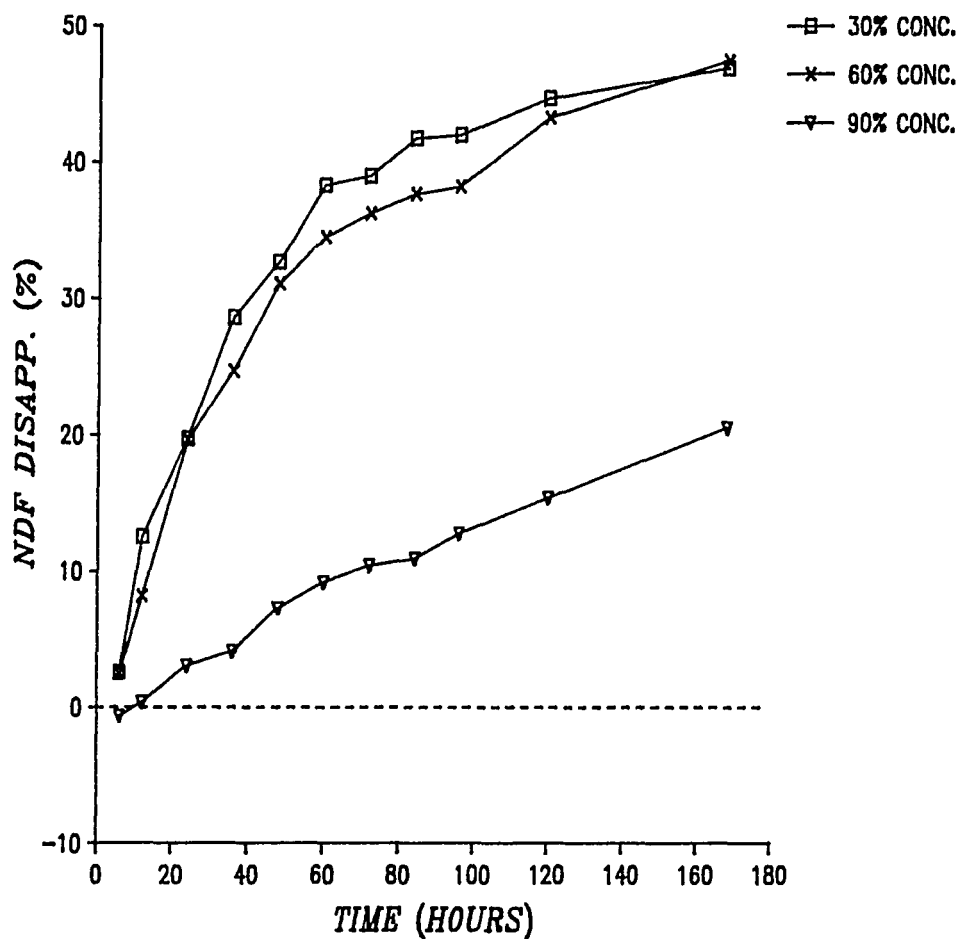


FIGURE 10. Disappearance of alfalfa hay neutral detergent fiber as affected by dietary concentrate levels.

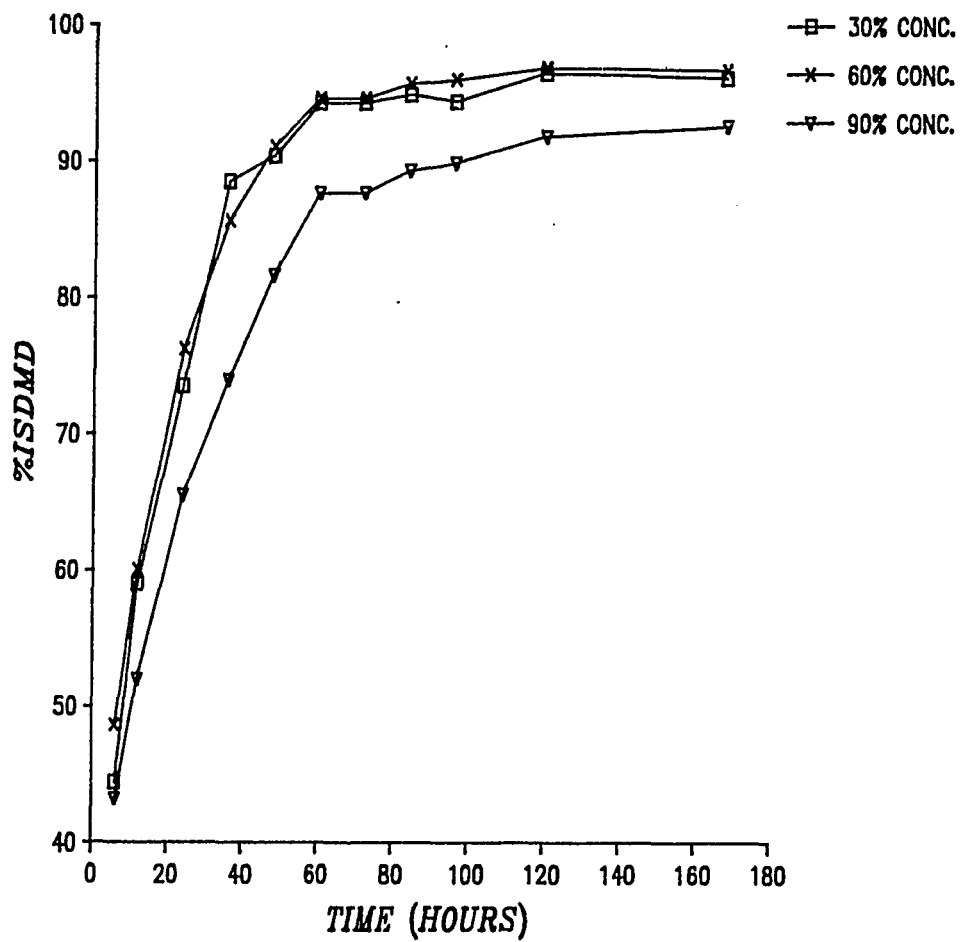


FIGURE 11. Disappearance of wheat straw neutral detergent fiber as affected by dietary concentrate levels.

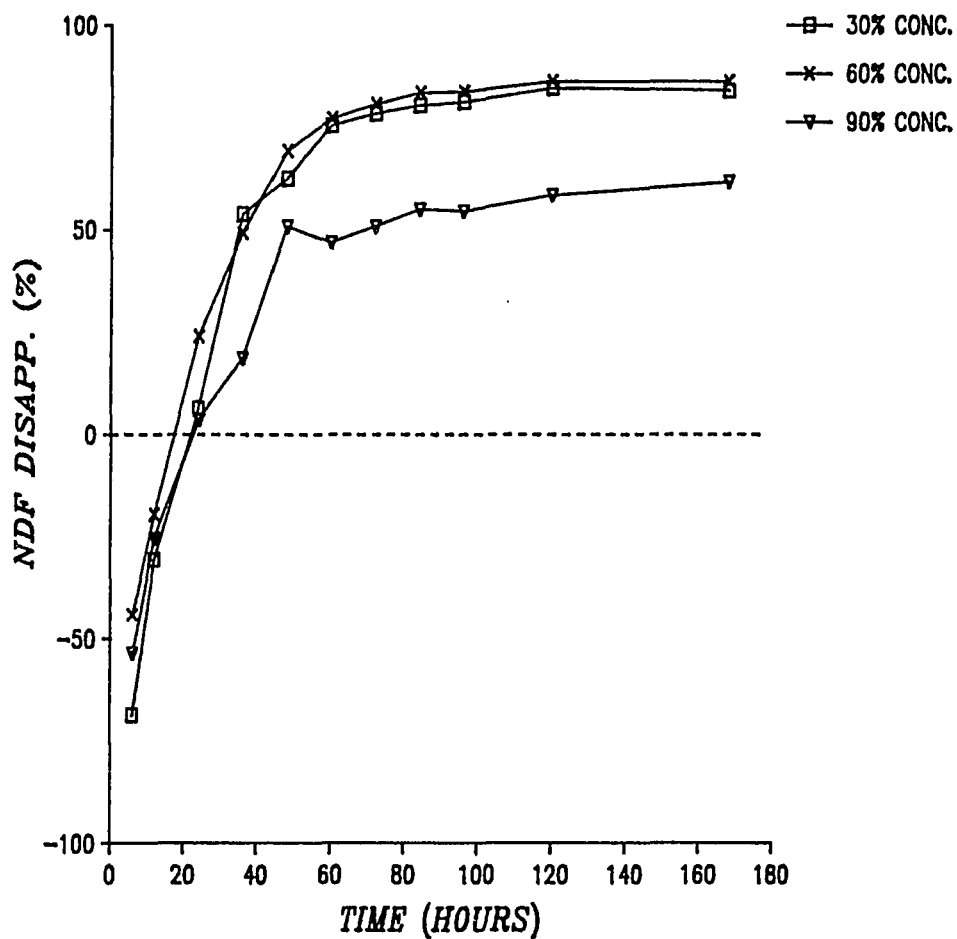


FIGURE 12. Disappearance of steam processed and flaked milo neutral detergent fiber as affected by dietary concentrate levels.

bag has been documented by other workers (Akin et. al., 1983; Uden and Van Soest, 1984). In an effort to classify and quantify the incoming material several assumptions were made. First, it was assumed that cell content digestibility was 98% (Van Soest, 1982). Second, that the negative disappearances which were observed at 6-h were a measure of the NDF-like material which migrated into the bag. Furthermore, it was assumed that this quantity was constant throughout the incubations, which may not be valid. Using these assumptions, adjusted NDF disappearance for each substrate at each concentrate level was calculated by adding the negative value observed at 6-h (NDF correction factor) to the observed NDF disappearance values for all incubation times. Calculated dry matter disappearances (CDMD) were obtained as the sum of digestible cell walls ($DCW = \%CW * CWD$, where $\%CWD$ is equivalent to the adjusted NDF disappearances) plus the digestible cell contents ($DCC = \%CC * \%CCD$, where $\%CCD = .98$). The results of these calculations, at all concentrate levels, are shown in Appendix Tables A.4 - A.6, A.7 - A.9 and A.10 - A.12 for alfalfa hay, wheat straw and SPFM, respectively.

The CDMD is in all cases larger than the observed ISDMD. The difference (CDMD - ISDMD) could be due to an influx of cell content-like material into the bag and/or the digestibility of cell contents being less than the estimated 98%. To illustrate this point cell contents digestibilities

were also calculated from the adjusted NDF disappearance, and the percent cell contents and NDF in the substrates (Appendix Tables A.4 through A.12). Across concentrate levels, the calculated cell content digestibilities range in value for alfalfa hay, wheat straw and SPFM were 82-90, 69-77 and 86-91, respectively.

This analysis simply illustrates that the dry matter and NDF digestibilities obtained by the in situ technique are confounded by migration into the bag of material which is both cell content- and NDF-like in nature and/or digestibilities of cell contents being less than 98%. The exact contribution of each of these factors to the measured ISDMD and NDF disappearances cannot be obtained from this study, since their accurate quantification is not possible.

Table 4 summarizes the NDF correction factors and the calculated cell content digestibilities across diets and concentrate levels. Values for cell content digestibility were obtained by averaging the calculated values obtained between 48- and 168-h. Values calculated from 6- to 36-h were increasing rapidly, indicating that cell content digestibility was not yet complete and thus, these values were deleted from the analysis.

Across substrates, the 90% concentrate diet had the lowest cell content digestibility. It is possible that the influx of neutral detergent soluble material (i.e., cell content-like) was higher in this diet. Rumen contents of

TABLE 4. Summary of NDF correction factors (NDFCF) and calculated cell content digestibilities (CCD) for NDF types and concentrate levels. Values represent means of the values observed in between 48- and 168-h of incubation.

Item	Concentrate Level (% of diet)			
	30	60	90	x _f (a)
Corrected for NDF flux				
Alfalfa Hay				
NDFCF	6.89	3.97	12.05	7.64
CCD	86.55	89.22	78.61	84.79
Wheat Straw				
NDFCF	0	0	0.66	0.22
CCD	73.39	72.33	64.09	69.94
Steam Processed and Flaked Milo				
NDFCF	68.68	44.07	53.63	55.46
CCD	85.09	89.73	85.29	86.70
X _c (b)				
NDFCF	25.19	16.01	22.11	8.64 ^(c)
CCD	81.68	83.76	76.00	80.48 ^(c)

- (a) Means for fiber type across concentrate levels.
 (b) Means for concentrate level across fiber types.
 (c) Means across fiber types and concentrate levels.

steers fed the 90% concentrate diet were homogeneous and showed no distinct liquid and solid phases as was observed for the other two diets. This may allow a greater number of very fine particles to come in contact with the bag, and therefore present greater opportunity for influx. A second possibility is that the ruminal digestibility of cell contents is, in fact, depressed by concentrate additions.

Across diets, wheat straw showed the lowest cell content digestibility and NDF correction factor. Since the wheat straw had slowest degradation rates, and the highest bulk density of the substrates there was a greater amount of residue remaining in those bags at all times. The residue remaining in the bag, through a mass action effect, may increase the quantity of NDF soluble material which can be trapped in the bag and also make it more difficult to wash influx material from the bag. However, it is possible that the digestibility of cell contents from this roughage was less than from the other substrates. Wheat straw is a low quality roughage for which the nature of the cell contents is not well characterized.

Steam processed and flaked milo grain showed the largest negative NDF disappearances during the initial incubation times, while wheat straw showed the smallest, again suggesting a mass action effect. Due to the higher density of SPFM, bags containing this substrate appeared to be nearly empty at the beginning of the incubations, while

those which contained wheat straw were quite full. In the case of SPFM, the NDF correction factor appears somewhat exaggerated since it is expressed as percent of the original fiber in the substrate, which is much smaller in the case of the milo grain. However, if the NDF correction factors are calculated and expressed as mg/100 mg of total substrate the values for SPFM, alfalfa hay and wheat straw are 8.4, 3.6 and 0.2, respectively.

If we accept the premise that digestibility of cell contents is 98%, across substrates and dietary treatments, substrate digestibility appears to be underestimated by the in situ technique by about 8.9 percentage units.

This analysis indicates that the influx of material into the bag and the calculated cell content digestibilities were not constant but were affected by the substrate being incubated and the dietary level of concentrate fed to the fistulated steer. This is important if the in situ technique is to be used for comparisons across substrates (e.g., roughage types) and dietary regimens (e.g., concentrate levels). Furthermore, these factors have implications in the calculation of fiber digestion kinetic parameters as discussed later.

Effects of Dietary Concentrate Levels on Ruminal pH

It has been demonstrated that addition of concentrate to ruminant diets lowers ruminal pH. It has

also been shown that lower pH of the incubation media is one of the factors which inhibit fiber degradation in vitro (Terry et. al., 1969). However, in vitro systems are buffered at a constant pH throughout the incubation period, while rumen pH in vivo is not constant, but shows cyclical patterns (Figure 13).

On each diet, ruminal pH was lowest about 2- to 3-h post-feeding and then gradually increased until the p.m. feeding (12-h). This pattern was essentially repeated following the p.m. feeding. Ruminal pH was lower ($P < .05$) on the 90% concentrate diet than on the 30 or 60% concentrate diets at all sampling times. Minimum pH was reached sooner on the 90% concentrate diet, most likely due to its rapid consumption and fermentation. Rumen pH for steers fed the 30 and 60% concentrate diets was different ($P < .05$) only at 2- and 3- hours post-feeding. Between feedings, mean pH for the 30, 60 and 90% concentrate diets were 6.55, 6.52 and 6.15, respectively. Thus, lower pH observed in the 90% concentrate diet could have contributed to the lower fiber digestibility observed in this diet.

Terry, Tilley and Outen (1969) showed that rate of fiber degradation in vitro decreased when the pH was lowered from 6.8 to 6.0. In the present study, a decrease in mean pH from 6.5 to 6.1 will reduce the rate of degradation significantly. Mould, Orskov and Mann (1983) suggested that

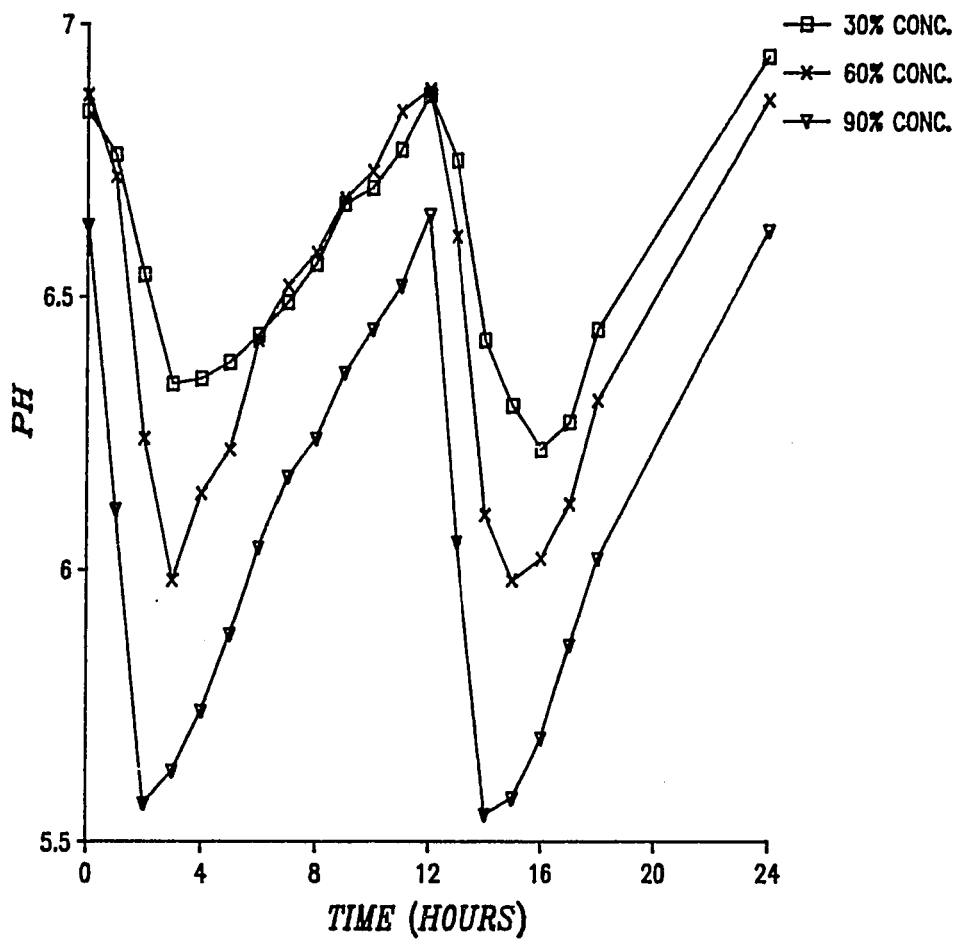


FIGURE 13. Effects of dietary concentrate levels on ruminal pH. Animals were fed immediately after the 0- and 12-h samplings.

the pH optimum for cellulolytic activity in the rumen is 6.7.

However, the overall picture cannot be explained simply with mean pH. Ruminal pH was lower than the mean values 50% of the time. Range in pH for the 30, 60 and 90% concentrate diets was 6.34-6.87, 5.98-6.88 and 5.57-6.65, respectively. Furthermore, rumen pH on the 60% concentrate diet was below the mean pH of the 90% concentrate diet for about 25% of the time (3-h between feedings) without a detrimental effect on fiber digestion. Conversely, pH on the 90% concentrate diet was above the mean for the other two diets about 20% of the time. Thus, from this study it is not possible to determine if there is a critical pH value for depressing fiber digestion. Furthermore, it would appear that the effect of ruminal pH on cellulolytic activity should be related not only to mean pH but also to the magnitude of the depression in ruminal pH, and the amount of time which the ruminal pH is below a critical level.

Effects of Dietary Concentrate Levels on Fiber Digestion Kinetics

The "uncorrected" NDF disappearances were used to investigate the effects of dietary concentrate additions on the kinetics of fiber digestion from the different substrates. Kinetic data were calculated for each substrate at each concentrate level using NDF disappearances averaged across steers for each substrate at each incubation time.

Analyses of variance for the NDF disappearance data showed that effects due to steers and periods were not significant ($P > .05$).

The first order model for fiber digestion presented by other workers (Waldo, 1969; Gill et. al., 1969; Smith et. al., 1971, 1972; Mertens, 1977) was used to describe the degradation curves. In principle, this model assumes that the curve of the potentially digestible fiber remaining at each incubation time can be described by a logarithmic function $f(x) = \log_e x$, or $x = e^y$.

For the initial analyses, potentially digestible fiber was defined as the maximum NDF digestibility observed for each substrate at each concentrate level. Next, the \log_{10} was obtained for the percent potentially digestible fiber remaining at each incubation time and regressed against time to obtain the kinetic parameters. Since \log_{10} was used for the logarithmic transformation of the data, degradation rates (percent/h) can be obtained by multiplying reaction rates (k) by 2.303 (to transform into a ln scale) and then by 100. This transformation will be necessary before this data can be utilized for prediction purposes in a complete rumen model such as that proposed by Mertens (1977).

Lag times were calculated from the regression lines as that point of x where y equals 2.0 (i.e., 100% of the potentially digestible fiber present, $\log_{10} 100 = 2.0$).

In order to define the rapidly and slowly degrading portions of the digestion curve proposed by Mertens (1977), the data were serially fitted (regressed) by Hudson's least squares method for estimating the joint point of two regression regimens, as described by Schwarz (1978).

The kinetic parameters obtained for alfalfa hay, wheat straw and SPFM are shown in Tables 5, 6, and 7 for all concentrate levels. In each case the first order model was appropriate as judged by the high r values and the low standard error of the estimate obtained when the log of the potentially digestible fiber remaining was regressed versus time.

None of the kinetic parameters for alfalfa hay and SPFM were different ($P > .05$) between the 30 and 60% concentrate diets. However, when these substrates were incubated in steers consuming 90% concentrate diets, rate of digestion ($P < .05$) while the lag phase increased. In contrast, each level of concentrate addition resulted in a decreased ($P < .05$) rate of digestion for wheat straw. However, extent of digestion decreased ($P < .05$) and lag phase increased for wheat straw only in the 90% concentrate diet. Alfalfa hay was the only substrate to show definite rapid and slowly degradable fractions, and then only for the 30 and 60% concentrate diets (Table 5). In all other cases, the overall curve could not be partitioned into two phases

TABLE 5. Digestion kinetic parameters of alfalfa hay NDF as affected by dietary concentrate levels and inclusion or deletion of lag phase.

Item	All Points Included			lag Phase Deleted		
	Concentrate level (% of diet)					
	30	60	90	30	60	90
<u>Rapidly Degradable Fraction</u>						
Slope (k_1)	-0.0276 ^a	-0.0257 ^a	-0.0085 ^b	-0.0272 ^a	-0.0255 ^a	-0.0082 ^b
Lag Phase (h)	7.60	7.15	20.43	7.03	6.92	17.86
PED (c)(%)	43.04 ^a	44.75 ^a	32.86 ^b	43.04 ^a	44.75 ^a	32.86 ^b
Y-Intercept	2.210	2.183	2.174	2.191	2.176	2.146
R ²	0.966	0.981	0.990	0.949	0.971	0.985
SEE (d)	0.121	0.085	0.034	0.139	0.097	0.035
Inc. Time (h)	6-60	6-60	6-120	12-60	12-60	24-120
<u>Slowly Degradable Fraction</u>						
Slope (k_2)	-0.0081 ^a	-0.0048 ^a	-	-0.0081 ^a	-0.0048 ^a	-
Y-Intercept	1.305	1.022	-	1.305	1.022	-
R ²	0.916	0.954	-	0.916	0.954	-
SEM (d)	0.107	0.056	-	0.107	0.056	-
Inc. Time (h)	72-168	71-168	-	72-168	72-168	-

a,b Slopes in the same line with different superscripts differ significantly (P<.05).

(c) PED = Percent potentially digestible NDF.

(d) SEE = Standard error of the estimate.

TABLE 6. Digestion kinetic parameters of wheat straw NDF as affected by dietary concentrate levels and inclusion or deletion of lag phase.

Item	All Points Included			lag Phase Deleted		
	30	Concentrate level (% of diet)		30	60	90
		60	90			
Slope (k_1)	-0.0110 ^a	-0.0084 ^b	-0.0052 ^c	-0.0110 ^a	-0.0084 ^b	-0.0052 ^c
Lag Phase (h)	0.69	-1.04	11.29	0.69	-1.04	12.28
PED ^(c) (%)	46.87 ^a	47.38 ^a	20.50 ^b	46.87 ^a	47.38 ^a	20.50 ^c
Y-Intercept	2.008	1.991	2.058	2.008	1.991	2.064
R ²	0.990	0.997	0.988	0.990	0.977	0.986
SEE ^(d)	0.044	0.051	0.023	0.044	0.051	0.024
Inc. Time (h)	6-120	6-120	6-120	6-120	6-120	12-120

a,b Slopes in the same line with different superscripts differ significantly ($P < .05$).

(c) PED = Percent potentially digestible NDF.

(d) SEE = Standard error of the estimate.

TABLE 7. Digestion kinetic parameters of steam processed and flaked milo NDF as affected by dietary concentrate levels and inclusion or deletion of lag phase.

Item	All Points Included			lag Phase Deleted		
	Concentrate level (% of diet)					
	30	60	90	30	60	90
Slope (k_1)	-0.0196 ^a	-0.0205 ^a	-0.0164 ^b	-0.0190 ^a	-0.0206 ^a	-0.0164 ^b
Lag Phase (h)	17.72	15.51	23.07	15.93	15.58	24.48
PED ^(c) (%)	84.70 ^a	86.34 ^a	61.72 ^b	84.70 ^a	86.34 ^a	61.72 ^b
Y-Intercept	2.347	2.319	2.379	2.302	2.321	2.414
R ²	0.991	0.991	0.993	0.964	0.983	0.989
SEE ^(d)	0.090	0.065	0.047	0.103	0.077	0.051
Inc. Time (h)	6-96	6-96	6-96	24-96	24-96	24-96

a,b Slopes in the same line with different superscripts differ significantly ($P < .05$).

(c) PED = Percent potentially digestible NDF.

(d) SEE = Standard error of the estimate.

since the semilog plots of percent potentially digestible to fit a line to the slowly degrading fraction.

The lag phase of digestion is defined as that initial period of time during an incubation in which no digestion occurs. It has been shown (Akin and Barton, 1983) by microscopic examination of plant tissues that at least in vitro there is an initial period of time during which little degradation takes place. This is the time required by microorganisms to affix and colonize fiber particles. Thus, it would appear that the lag phase is a valid component of digestion.

When a lag phase is incorporated into the fiber digestion kinetic model, the NDF disappearance values obtained before the conclusion of the lag phase should be deleted from the regression line. Since, by definition, no change in the potentially digestible fiber occurs during this time, failure to delete these points would bias the regression line, decreasing the rate of degradation and resulting in a poorer fit (Tauskey et. al., 1972; Mertens, 1977).

Thus, a lag phase was incorporated into the previously described regression lines. Observed points recorded before the conclusion of the previously calculated lag times were deleted from the data and the regression lines were recalculated. These results are shown in Tables 5, 6, and 7. Incorporation of a lag phase did not improve the fit of these lines, since the standard errors of the

estimate and r^2 values did not improve (Neter and Wasserman, 1974). Furthermore, neither lag time or digestion rate were changed significantly ($P > .05$) by this procedure. This suggests that lag times were overestimated in this study since fiber degradation appeared to be in progress before their conclusion.

It is interesting to note that the longest lag times were obtained in those cases in which NDF digestibilities at 6-h were the most negative (i.e. Largest NDF correction factor). If the three substrates were ranked in order of their NDF correction factors, SPFM would be first, alfalfa hay second and wheat straw third (Table 4), which is exactly the same order which is obtained if substrates were ranked by their lag times. Influx of NDF-like material into the bag would make digestibilities appear negative and shift the digestion curves in time. In other words, it would take longer for NDF digestibilities to become positive which would increase the lag time of digestion. From this observation it must be concluded that the lag times measured in situ are overestimated, and at least partially, an artifact of the influx of NDF insoluble material into the bag during incubation. This is especially critical when making comparisons across concentrate levels and substrate types. As described earlier, influx of material is probably not constant and would be affected by experimental conditions. Thus, the longer lag times observed in the 90% concentrate

diet, compared with other diets, and with SPFM, compared with other substrates, may not be completely real, since influx of material appeared to be the largest in these cases.

These postulations also seem to be supported by other studies. In in vitro studies conducted by Mertens and Loften (1980) the longest lag times reported were 5.2-h for fescue grass NDF when the substrate consisted of 80% starch and 20% fescue grass. In contrast, Miller and Muntifering (1985) reported in situ lag time for fescue grass of 11.5-h when incubated in steer consuming an 80% concentrate diet. The latter is nearly identical with the lag time obtained in this study for wheat straw in the 90% concentrate diet (11.3-h). These limited observations suggest that the lag times determined in situ are longer than those determined in vitro.

Influx of material in an in vitro system can be measured and therefore, the technique would appear to be better in determining lag times. However, incubation conditions in a closed in vitro system are constant (i.e. buffered at pH 7.0) and not affected by concentrate levels as they are in vivo. In this study it has been demonstrated that the ruminal pH shows a cyclical pattern after feeding and is depressed by higher concentrate levels. Thus the validity of the lag times obtained in vitro may also be questioned.

In complete digestion models, designed to predict total tract digestibility (See Mertens, 1977) lag time is considered to be that initial period during digestion in which passage, but not digestion, occurs. This component of the model is calculated as e^{-k_3L} and functions to decrease the amount of fiber which is available at the beginning of digestion ($PDF * e^{-k_3L}$). Overestimation of the lag time would cause total ruminal digestibility to be underestimated. It is necessary that further studies be conducted to characterize and quantify the lag time of digestion before any practical applications of this type of data can be successfully made.

The definition of the potential extent of digestion is critical to the determination of the rate of digestion, since the first order model basically functions to describe the changes of this fraction over time. Contrary to what is commonly done (Gill et. al., 1969; Smith et. al., 1971, 1972; Mertens, 1980) this data clearly shows that the potentially digestible fiber cannot be defined at a set point in time (i.e. 48- or 72-h). Instead the end-point for fiber digestion appears to be dynamic and is dependent on the substrate and ruminal conditions.

To illustrate the effects of underestimating the potentially digestible fiber, the kinetic data was recalculated with the potential extent of digestion defined as the maximal NDF digestibility value observed for each

substrate across concentrate levels (Tables 8, 9, 10 and Appendix Tables A.1, A.2 and A.3). Support for these assumptions can be found in the works of Akin et. al. (1973, 1974, 1975, 1979) and Brazle and Harbers (1977) which indicate that the potentially digestible material in a plant is due to the relative amounts, and composition of the different tissues it contains. Since these factors would be constant for a given fiber source, it can be argued that its potentially digestible fiber in a sample is also constant and can be defined as the maximal possible digestibility regardless of the diet used for its determination. Furthermore, it is apparent from Figures 10 and 11 that potential extents of digestion has not been reached for wheat straw at any concentrate level or alfalfa hay in the 90% concentrate diet, since the curves are still increasing even after 168-h.

Definition of potentially digestible fiber as the maximum extent at each concentrate level or as the maximum across concentrate levels did not affect ($P > .05$) kinetic parameters for the substrates at 30 and 60% concentrate, since these two values did not differ significantly. However, in the 90% concentrate diet the disappearance observed after 168-h were less ($P < .05$) than the maximum observed across concentrates, resulting in significant ($P < .05$) change in kinetic parameters. For each substrate, degradation rates were at least 50% slower when the

TABLE 8. Digestion kinetic parameters of alfalfa hay NDF as affected by dietary concentrate levels and definition of potentially digestible NDF.

Item	Concentrate Level (% of diet)		
	30	60	90
<u>PED As Maximum Value Within Concentrate Level</u>			
Slope (k_1)	-0.0276 ^{a,c}	-0.0257 ^{a,c}	-0.0085 ^{b,c}
Lag Phase (h)	7.60	7.15	20.43
PED (e)(%)	43.04	44.75	32.86
Y-Intercept	2.210	2.183	2.174
R ²	0.966	0.981	0.990
SEE (f)	0.121	0.085	0.034
Inc. Time (h)	6-60	6-60	6-120
<u>PED As Maximum Value Across Concentrates</u>			
Slope (k_1)	-0.0217 ^{a,c}	-0.0257 ^{a,c}	-0.0048 ^{b,d}
Lag Phase (h)	5.91	7.15	19.90
PED (e)(%)	44.75	44.75	44.75
Y-Intercept	2.128	2.183	2.055
R ²	0.976	0.981	0.956
SEE (f)	0.079	0.085	0.041
Inc. Time (h)	6-60	6-60	6-120

a,b Slopes in the same line with different superscripts differ significantly (P<.05).

c,d Slopes in the same column and belonging to the same item with different superscripts differ significantly (P<.05).

(e) PED = Percent potentially digestible NDF.

(f) SEE = Standard error of the estimate.

TABLE 9. Digestion kinetic parameters of wheat straw NDF as affected by dietary concentrate levels and definition of potentially digestible NDF.

Item	Concentrate Level (% of diet)		
	30	60	90
<u>PED As Maximum Value Within Concentrate Level</u>			
Slope (k_1)	-0.0110 ^{a, d}	-0.0084 ^{b, d}	-0.0052 ^{c, d}
Lag Phase (h)	0.69	-1.04	11.29
PED ^(f) (%)	46.87	47.38	20.50
Y-Intercept	2.008	1.991	2.058
R ²	0.990	0.977	0.988
SEE (g)	0.044	0.051	0.023
Inc. Time (h)	6-120	6-120	6-120
<u>PED As Maximum Value Across Concentrates</u>			
Slope (k_1)	-0.0104 ^{a, d}	-0.0084 ^{b, d}	-0.0015 ^{c, e}
Lag Phase (h)	-0.05	-1.04	5.47
PED ^(f) (%)	47.38	47.38	47.38
Y-Intercept	1.995	1.991	2.008
R ²	0.988	0.977	0.993
SEE (g)	0.045	0.051	0.006
Inc. Time (h)	6-120	6-120	6-168

a, b Slopes in the same line with different superscripts differ significantly (P<.05).

c, d Slopes in the same column and belonging to the same item with different superscripts differ significantly (P<.05).

(e) PED = Percent potentially digestible NDF.

(f) SEE = Standard error of the estimate.

TABLE 10. Digestion kinetic parameters of steam processed and flaked NDF as affected by dietary concentrate levels and definition of potentially digestible NDF.

Item	Concentrate Level (% of diet)		
	30	60	90
<u>PED As Maximum Value Within Concentrate Level</u>			
Slope (k_1)	-0.0196 ^{a,c}	-0.0205 ^{a,c}	-0.0164 ^{b,c}
Lag Phase (h)	17.72	15.51	23.07
PED (e)(%)	84.70	86.34	61.72
Y-Intercept	2.347	2.319	2.379
R ²	0.991	0.991	0.993
SEE (f)	0.090	0.065	0.047
Inc. Time (h)	6-96	6-96	6-96
<u>PED As Maximum Value Across Concentrates</u>			
Slope (k_1)	-0.0177 ^{a,c}	-0.0205 ^{a,c}	-0.0077 ^{b,d}
Lag Phase (h)	17.45	15.51	24.09
PED (e)(%)	86.34	86.34	86.34
Y-Intercept	2.309	2.319	2.186
R ²	0.973	0.991	0.961
SEE (f)	0.101	0.065	0.053
Inc. Time (h)	6-96	6-96	6-96

a,b Slopes in the same line with different superscripts differ significantly (P<.05).

c,d Slopes in the same column and belonging to the same item with different superscripts differ significantly (P<.05).

(e) PED = Percent potentially digestible NDF.

(f) SEE = Standard error of the estimate.

potential extent of digestion was defined as the maximum value across concentrates rather than as the maximum value for each concentrate level and by definition, potentially digestible fiber increased significantly. Lag time was changed considerably only in the case of wheat straw in the 90% concentrate diet (Table 9). Thus, it is clear that overestimation of the potentially digestible fiber would underestimate the rate of degradation.

Other studies (Mertens and Loften, 1980; Miller and Muntifering, 1985) which have examined the effects of concentrate levels on fiber digestion kinetics have not shown the decrease in rate of degradation observed in this study. However, in those studies the maximum level of concentrate was 80% and the potential extent of digestion was defined at 72- or 96-h. From the present study, it is clear that both of these end-points underestimate the potential extent of digestion when high concentrate levels are included in the diet. Therefore, in the studies of Mertens and Loften (1980), and Miller and Muntifering (1985) the failure to observe depressions in fiber degradation due to concentrate levels was most likely due to improper determination of the potentially digestible fiber. For future experiments it is recommended that incubations be carried out well past 168-h in order to define the true potential extent of digestion. This is especially critical in those experiments in which the rates of degradation are

expected to be affected.

It would be extremely useful to the determination and applications of kinetic parameters if it could be demonstrated that the potential extent of digestion is a function of the plant tissues present in a sample and independent of diet used for determination as suggested earlier. This would allow potential extent of digestion for a substrate to be determined once greatly reducing the amount of work necessary for determination of digestion kinetic parameters.

Fiber types differ ($P < .05$) in their kinetic parameters at equal concentrate levels (Table 11). Rate of fiber degradation was more rapid ($P < .05$) for alfalfa hay than for wheat straw at all concentrate levels. Degradation rate for SPFM fiber was intermediate between the two roughages in the 30 and 60% concentrate diets, but more rapid ($P < .05$) than the degradation of alfalfa hay fiber in the 90% concentrate diet. This indicates that concentrate level has less effect on rate of fiber degradation from SPFM than from the other substrates. At all concentrate levels lag time was the shortest for wheat straw NDF and longest SPFM fiber. Potential extent of digestion was in all cases largest for SPFM. Potential extent of digestion was similar for the alfalfa and wheat straw in the 30 and 60% concentrate diets. However, potential extent of digestion was lower for the wheat straw than for alfalfa hay in the

TABLE 11. Effect of dietary concentrate levels on fiber digestion kinetics of alfalfa hay, wheat straw and steam processed and flaked milo.

Item	Concentrate Level (% of diet)								
	30			60			90		
	Alfalfa Hay	Wheat Straw	SPFM	Alfafa Hay	Wheat Straw	SPFM	Alfafa Hay	Wheat Straw	SPFM
Slope (k_1)	-0.0276 ^a	-0.0110 ^b	-0.0196 ^c	-0.0257 ^a	-0.0084 ^b	-0.0205 ^c	-0.0085 ^a	-0.0052 ^b	-0.0164 ^c
Lag Phase (h)	7.60	0.69	17.72	7.15	-1.04	15.51	20.43	11.29	23.07
PED ^(c) (%)	43.04	46.87	84.70	44.75	47.38	86.34	32.86	20.50	61.72
Y-Intercept	2.210	2.008	2.347	2.183	1.991	2.319	2.174	2.058	2.379
R ²	0.966	0.990	0.991	0.981	0.977	0.991	0.990	0.988	0.993
SEE ^(d)	0.121	0.044	0.090	0.085	0.051	0.065	0.034	0.023	0.047
Inc. Time (h)	6-60	6-120	6-96	6-60	6-120	6-96	6-120	6-120	6-96

a,b Within concentrate levels, means on the same line with different superscripts differ significantly.

(c) PED = Percent potentially digestible NDF.

(d) SEE = Standard error of the estimate.

90% concentrate diet. Again, this suggests that the effects of concentrate addition are not constant across fiber types.

In summary, rate of fiber degradation was decreased ($P < .05$) in 90% concentrate diet when compared with 30 and 60% concentrate diets. Digestion kinetics of wheat straw fiber appear to be the most affected by concentrate additions, while SPFM fiber was the least affected.

CHAPTER 5

APPENDIX A

TABLE A.1 In situ dry matter disappearance (%) and neutral fiber disappearance (%) of alfalfa hay as affected by dietary concentrate levels.

Incubation Time (h)	ISDMD				NDFD			
	Concentrate Level (% of diet)							
	30	60	90	SE	30	60	90	SE
6	38.33 ^a	38.10 ^a	30.48 ^b	1.57	-6.89	-3.97 ^a	-12.05 ^b	1.97
12	53.57 ^a	51.14 ^a	34.73 ^b	1.65	14.81	12.89 ^a	-8.54 ^b	2.77
24	61.23 ^a	61.83 ^a	44.81 ^b	2.96	26.97	30.28 ^a	0.74 ^b	4.61
36	66.11 ^a	64.86 ^a	49.00 ^b	2.86	36.77	35.53 ^a	8.72 ^b	4.58
48	67.16 ^a	67.60 ^a	53.01 ^b	3.11	37.91	39.61 ^a	14.99 ^b	5.33
60	68.89 ^a	69.20 ^a	56.15 ^b	3.09	41.87	43.17 ^a	19.88 ^b	5.19
72	68.56 ^a	68.99 ^a	56.61 ^b	3.04	40.88	42.69 ^a	21.43 ^b	4.88
84	68.44 ^a	69.07 ^a	57.58 ^b	2.72	41.02	42.57 ^a	22.78 ^b	4.38
96	68.44 ^a	69.57 ^a	59.40 ^b	2.57	41.27	43.29 ^a	25.46 ^b	4.46
120	68.65 ^a	70.51 ^a	64.38 ^b	2.45	42.38	44.75 ^a	27.76 ^b	4.59
168	68.83 ^a	70.07 ^a	64.38 ^b	1.31	43.04	44.05 ^a	32.86 ^b	2.55

a,b Within each main subheading, means in the same line with different superscripts differ significantly (P<.05)

TABLE A.2 In situ dry matter disappearance (%) and neutral fiber disappearance (%) of wheat straw as affected by dietary concentrate levels.

Incubation Time (h)	ISDMD				NDFD			
	Concentrate Level (% of diet)							
	30	60	90	SE	30	60	90	SE
6	21.77 ^a	21.08 ^a	16.70 ^b	0.84	2.53 ^a	2.50 ^a	-0.66 ^b	0.68
12	30.64 ^a	26.71 ^a	18.84 ^b	1.42	12.53 ^a	8.18 ^a	0.34 ^b	1.81
24	35.49 ^a	35.39 ^a	21.57 ^b	1.99	19.73 ^a	19.62 ^a	3.04 ^b	2.31
36	41.97 ^a	38.07 ^a	22.69 ^b	2.29	28.59 ^a	24.66 ^a	4.08 ^b	2.31
48	45.44 ^a	43.75 ^a	25.56 ^b	3.74	32.63 ^a	31.04 ^a	7.28 ^b	4.69
60	49.75 ^a	46.37 ^a	27.49 ^b	2.95	38.27 ^a	34.44 ^a	9.20 ^b	3.76
72	49.73 ^a	47.98 ^a	28.66 ^b	3.41	38.93 ^a	36.17 ^a	10.43 ^b	4.18
84	52.17 ^a	49.31 ^a	28.66 ^b	2.93	41.71 ^a	37.62 ^a	10.93 ^b	3.76
96	52.27 ^a	50.04 ^a	30.06 ^b	3.43	41.96 ^a	38.17 ^a	12.76 ^b	4.40
120	54.93 ^a	53.54 ^a	32.90 ^b	3.05	44.63 ^a	43.22 ^a	15.38 ^b	4.19
168	56.77 ^a	56.58 ^a	37.12 ^b	2.58	46.87 ^a	47.38 ^a	20.50 ^b	3.74

a,b Within each main subheading, means in the same line with different superscripts differ significantly ($P < .05$)

TABLE A.3 In situ dry matter disappearance (%) and neutral fiber disappearance (%) of steam processed and flaked milo as affected by dietary concentrate levels.

Incubation Time (h)	ISDMD				NDFD			
	Concentrate Level (% of diet)							
	30	60	90	SE	30	60	90	SE
6	44.43	48.61	43.24	1.73	-68.63	-44.07	-53.63	12.51
12	59.05 ^a	60.05 ^a	51.97 ^b	1.33	-30.50	-19.50	-25.37	10.81
24	73.52 ^a	76.23 ^a	65.54 ^b	1.74	6.38	23.94	3.64	5.17
36	88.51 ^a	85.63 ^a	73.96 ^b	2.00	53.75 ^a	49.17 ^a	18.62 ^b	3.80
48	90.39 ^a	91.07 ^a	81.61 ^b	2.16	62.51 ^a	69.27 ^a	50.79 ^b	7.04
60	94.30 ^a	94.60 ^a	87.66 ^b	1.29	75.50 ^a	77.21 ^a	46.81 ^b	4.41
72	94.30 ^a	94.60 ^a	87.66 ^b	1.29	78.43 ^a	80.69 ^a	50.79 ^b	4.78
84	94.88 ^a	95.71 ^a	89.32 ^b	1.24	80.45 ^a	83.52 ^a	54.92 ^b	5.01
96	94.37 ^a	96.00 ^a	89.88 ^b	0.98	81.32 ^a	83.84 ^a	54.46 ^b	4.03
120	96.49 ^a	96.87 ^a	91.85 ^b	0.99	84.70 ^a	86.34 ^a	58.45 ^b	5.17
168	96.12 ^a	96.68 ^a	92.61 ^b	1.00	83.93 ^a	86.21 ^a	61.72 ^b	4.89

a,b Within each main subheading, means in the same line with different superscripts differ significantly (P<.05)

TABLE A.4. Comparison of actual dry matter disappearance (%) and calculated dry matter disappearance (%) and calculated cell content digestibility (%) for alfalfa hay incubated in steer consuming 30% concentrate diet.

Incubation Time (h)	Percent			
	Calculated ISDMD	Observed ISDMD	Unexplained Difference	Calculated CCD(1)
<u>Corrected For NDF Index (6.9 Units)</u>				
6	51.8	38.3	13.5	72.5
12	62.0	53.6	8.5	82.0
24	67.8	61.2	6.5	85.6
36	72.4	66.1	6.5	86.1
48	72.9	67.2	5.8	87.1
60	74.8	68.9	5.9	86.8
72	74.3	68.6	5.8	87.1
84	74.4	68.4	5.9	86.7
96	74.5	68.4	6.1	86.5
120	75.0	68.7	6.4	85.9
168	75.3	68.8	6.5	85.7

(1) CCD = Cell content digestibility calculated from the observed ISDMS and corrected digestible cell walls.

TABLE A.5. Comparison of actual dry matter disappearance (%) and calculated dry matter disappearance (%) and calculated cell content digestibility (%) for alfalfa hay incubated in steer consuming 60% concentrate diet.

Incubation Time (h)	Percent			
	Calculated ISDMD	Observed ISDMD	Unexplained Difference	Calculated CCD(1)
<u>Corrected For NDF Index (3.8 Units)</u>				
6	51.8	38.1	13.7	72.1
12	59.8	51.1	8.6	81.7
24	68.0	61.8	6.1	86.4
36	70.4	64.9	5.6	87.5
48	72.3	67.6	4.7	89.0
60	74.0	69.2	4.8	88.9
72	73.8	69.0	4.8	88.9
84	73.7	69.1	4.7	89.2
96	74.1	69.8	4.5	89.5
120	74.8	70.5	4.3	89.9
168	74.4	70.1	4.4	89.7

(1) CCD = Cell content digestibility calculated from the observed ISDMS and corrected digestible cell walls.

TABLE A.6. Comparison of actual dry matter disappearance (%) and calculated dry matter disappearance (%) and calculated cell content digestibility (%) for alfalfa hay incubated in steer consuming 90% concentrate diet.

Incubation Time (h)	Percent			
	Calculated ISDMD	Observed ISDMD	Unexplained Difference	Calculated CCD(1)
<u>Corrected For NDF Index (12.9 Units)</u>				
6	51.8	30.5	21.3	57.7
12	53.5	34.7	18.7	62.6
24	57.8	44.8	13.0	73.4
36	61.6	49.0	12.6	74.2
48	64.6	53.0	11.5	76.2
60	66.9	56.2	10.7	77.7
72	67.6	56.6	11.0	77.2
84	68.2	57.6	10.7	77.9
96	69.5	59.4	10.1	78.9
120	70.6	61.4	9.2	80.6
168	73.0	64.4	8.6	81.7

(1) CCD = Cell content digestibility calculated from the observed ISDMS and corrected digestible cell walls.

TABLE A.7. Comparison of actual dry matter disappearance (%) and calculated dry matter disappearance (%) and calculated cell content digestibility (%) for wheat straw incubated in steer consuming 30% concentrate diet.

Incubation Time (h)	Percent			
	Calculated ISDMD	Observed ISDMD	Unexplained Difference	Calculated CCD(1)
<u>Corrected For NDF Index (0 Units)</u>				
6	34.4	21.8	12.6	60.2
12	41.0	30.6	10.4	66.8
24	45.9	35.5	10.4	66.9
36	51.8	42.0	9.8	68.7
48	54.4	45.4	9.0	71.0
60	58.2	49.8	8.5	72.7
72	58.6	49.7	8.9	71.3
84	60.5	52.2	8.3	73.1
96	60.7	52.6	8.1	73.7
120	62.4	54.9	7.5	75.5
168	63.9	56.8	7.2	76.5

(1) CCD = Cell content digestibility calculated from the observed ISDMS and corrected digestible cell walls.

TABLE A.8. Comparison of actual dry matter disappearance (%) and calculated dry matter disappearance (%) and calculated cell content digestibility (%) for wheat straw incubated in steer consuming 60% concentrate diet.

Incubation Time (h)	Percent			
	Calculated ISDMD	Observed ISDMD	Unexplained Difference	Calculated CCD(1)
<u>Corrected For NDF Index (0 Units)</u>				
6	34.4	21.1	13.3	58.2
12	38.2	26.7	11.4	63.7
24	45.8	35.4	10.4	66.9
36	49.1	38.1	11.1	64.8
48	53.4	43.8	9.6	69.1
60	55.7	46.4	9.3	70.2
72	56.8	48.0	8.8	71.6
84	57.8	49.3	8.5	72.6
96	58.1	50.0	8.1	73.7
120	61.5	53.5	8.0	74.1
168	64.3	56.6	7.7	74.9

(1) CCD = Cell content digestibility calculated from the observed ISDMS and corrected digestible cell walls.

TABLE A.9. Comparison of actual dry matter disappearance (%) and calculated dry matter disappearance (%) and calculated cell content digestibility (%) for wheat straw incubated in steer consuming 90% concentrate diet.

Incubation Time (h)	Percent			
	Calculated ISDMD	Observed ISDMD	Unexplained Difference	Calculated CCD(1)
<u>Corrected For NDF Index (0.7 Units)</u>				
6	32.7	16.7	16.0	50.0
12	33.4	18.8	14.5	54.4
24	35.2	21.6	13.6	57.2
36	35.9	22.7	13.2	58.5
48	38.0	25.6	12.4	60.7
60	39.3	27.5	11.8	62.7
72	40.1	28.7	11.4	63.7
84	40.4	28.7	11.8	62.7
96	41.7	30.1	11.6	63.3
120	43.4	32.9	10.5	66.5
168	46.8	37.1	9.7	69.0

(1) CCD = Cell content digestibility calculated from the observed ISDMS and corrected digestible cell walls.

TABLE A.10. Comparison of actual dry matter disappearance (%) and calculated dry matter disappearance (%) and calculated cell content digestibility (%) for steam processed and flaked milo incubated in steer consuming 30% concentrate diet.

Incubation Time (h)	Percent			
	Calculated ISDMD	Observed ISDMD	Unexplained Difference	Calculated CCD(1)
<u>Corrected For NDF Index (68.6 Units)</u>				
6	83.2	44.4	38.8	52.3
12	89.0	59.1	29.9	62.8
24	94.5	73.5	21.0	73.3
36	101.7	88.5	13.2	82.5
48	103.0	90.4	12.6	83.1
60	105.0	94.3	10.7	85.4
72	105.4	94.3	11.1	84.9
84	105.7	94.9	10.8	85.2
96	105.8	94.4	11.5	84.5
120	106.4	96.5	9.9	86.4
168	106.2	96.1	10.1	86.1

(1) CCD = Cell content digestibility calculated from the observed ISDMS and corrected digestible cell walls.

TABLE A.11. Comparison of actual dry matter disappearance (%) and calculated dry matter disappearance (%) and calculated cell content digestibility (%) for steam processed and flaked milo incubated in steer consuming 60% concentrate diet.

Incubation Time (h)	Percent			
	Calculated ISDMD	Observed ISDMD	Unexplained Difference	Calculated CCD(1)
<u>Corrected For NDF Index (44.1 Units)</u>				
6	83.2	48.6	34.6	57.3
12	86.9	60.1	26.9	66.4
24	93.5	76.2	17.2	77.7
36	97.3	85.6	11.6	84.3
48	100.3	91.1	9.2	87.1
60	101.5	94.6	6.9	89.9
72	102.0	94.6	7.4	89.2
84	102.5	95.7	6.8	90.0
96	102.5	96.0	6.5	90.3
120	102.9	96.9	6.0	90.9
168	102.9	96.7	6.2	90.7

(1) CCD = Cell content digestibility calculated from the observed ISDMS and corrected digestible cell walls.

TABLE A.12. Comparison of actual dry matter disappearance (%) and calculated dry matter disappearance (%) and calculated cell content digestibility (%) for steam processed and flaked milo incubated in steer consuming 90% concentrate diet.

Incubation Time (h)	Percent			
	Calculated ISDMD	Observed ISDMD	Unexplained Difference	Calculated CCD(1)
<u>Corrected For NDF Index (53.6 Units)</u>				
6	83.2	43.2	40.0	50.9
12	87.5	52.0	35.5	56.2
24	91.9	65.5	26.3	67.0
36	94.1	74.0	20.1	74.3
48	99.0	81.6	17.3	77.6
60	98.4	87.7	10.7	85.4
72	99.0	87.7	11.3	84.7
84	99.6	89.3	10.3	85.9
96	99.5	89.9	9.6	86.6
120	100.1	91.9	8.3	88.3
168	100.6	92.6	8.0	88.6

(1) CCD = Cell content digestibility calculated from the observed ISDMS and corrected digestible cell walls.

TABLE A.13. Effects of dietary concentrate additions on ruminal PH.

Sampling Time ^(d) h	Concentrate Level (% of diet)			SE
	30	60	90	
0	6.84 ^a	6.87 ^a	6.63 ^b	.07
1	6.76 ^a	6.72 ^a	6.11 ^b	.11
2	6.54 ^a	6.24 ^a	5.57 ^c	.07
3	6.34 ^a	5.98 ^a	5.63 ^c	.05
4	6.35 ^a	6.14 ^a	5.74 ^b	.07
5	6.38 ^a	6.22 ^a	5.88 ^b	.09
6	6.43 ^a	6.42 ^a	6.04 ^b	.12
7	6.49	6.52	6.17	.11
8	6.56	6.58	6.24	.11
9	6.67	6.68	6.36	.11
10	6.70 ^a	6.73 ^a	6.44 ^b	.07
11	6.77 ^a	6.84 ^a	6.52 ^b	.06
12	6.87 ^a	6.88 ^a	6.65 ^b	.06
13	6.75 ^a	6.61 ^a	6.05 ^b	.12
14	6.42 ^a	6.10 ^a	5.55 ^c	.08
15	6.30 ^a	5.98 ^a	5.58 ^c	.06
16	6.22 ^a	6.02 ^a	5.69 ^b	.11
17	6.27 ^a	6.12 ^a	5.86 ^b	.14
18	6.44 ^a	6.31 ^a	6.02 ^b	.12
24	6.94 ^a	6.86 ^a	6.62 ^b	.08

a, b, c Means in the same line with different superscripts differ significantly (P<.05)

d Animals were fed immediately after the 0- and 12-h samplings

CHAPTER 6

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