EVALUATION OF KINETIC MODELS OF RUMINANT INTAKE AND DIGESTIBILITY UTILIZING TROPICAL GRASSES

By

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For Lillian, Michael, Joshua and Sara

(They packed.)

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EVALUATION OF KINETIC MODELS OF RUMINANT INTAKE AND DIGESTIBILITY UTILIZING TROPICAL FORAGES

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Three studies were conducted with the following goals: 1) to compare ten cultivars of tropical grasses, harvested at different maturities, as to their intake and digestibility by sheep, and to provide a set of forage samples with known *in vivo* values, for the evaluation of laboratory methods to predict forage quality; 2) to evaluate a model of forage cell wall digestion and to compare *in vitro* estimates of the parameters of the model with *in situ* derived estimates; and 3) to evaluate an integrated model of ruminal cell wall digestion and intake.

In Experiment 1, six cultivars of digitgrass (*Digitaria decumbens*), three cultivars of bahiagrass (*Paspalum notatum*) and one cultivar of bermudagrass (*Cynodon dactylon*) were harvested at different stages of regrowth (two to eight weeks), artificially dried, and fed to sheep in a digestibility and intake trial. Animal variation in organic matter intake (OMI) and neutral detergent fiber intake (NDFI) was best removed by powers of body weight that were closer to 1.0 than to .75. Digitgrasses grown in

the same area as the bahiagrasses were superior in OMD ($P<.01$), with a mean digestibility of 63.4 versus 58.7 percent. Digestible organic matter intake per metabolic weight (DOMI75), $g/kg^{.75}$, for the three genera varied from 26.4 to 50.2. No consistent regrowth effects, either within genera or within species, were noted. This may have been because there was severe insect damage to some of the grasses between the two and four-week cuttings. The experimental digitgrass X124-4 and 'Slenderstem' digitgrass maintained higher DOMI75 across all regrowths. The overall correlation (r) between organic matter intake and digestibility of these grasses was .47. Digestibilities of organic matter and neutral detergent fiber were closely related ($r = .96$) as were OMI and NDFI ($r = .94$).

In Experiment 2, two bermudagrass hays, one high quality (HQ) and one low quality (LQ), were fed *ad libitum* to wethers in a digestibility and intake trial. Samples of hays, orts and feces from this trial were digested *in vitro* and *in situ*, in two fistulated steers fed these hays in a crossover design. Samples were removed at 16 times ranging from 2 to 129 hours and analyzed for neutral detergent fiber (NDF). NDF residue over time was fitted to an exponential decay curve, generating estimates of potentially digestible cell wall (D, %), indigestible cell wall (U, %), digestion rate constant (k_1, hr^{-1}) and digestion lag time (L, hr). All differences between hays and method of digestion occurred in the estimates of D and U. The HQ hay had a higher fraction of D than did the LQ hay. *In situ* digestion provided higher estimates of D than did *in vitro* digestion. Application of the estimates of U in hay, orts, and feces to the intake and excretion data of the digestion trial resulted in positive digestibilities of U in both hays, irrespective of method of digestion, with a range of 14.3 to 18.1 percent.

In Experiment 3, sixty hay samples from Experiment 1, were subjected to *in vitro* NDF digestion for 12, 24, 36, 48, 60 and 96 hours. Parameters D, U, k_1 and L were determined. The rumen NDF passage rate constant (k_2) was estimated using these parameter estimates, an assumed rumen NDF fill of 16.03 g per kg body weight, and *in vivo* NDFI per kg of body weight, under two assumptions: that digestion lag observed *in vitro* does not occur *in vivo*, and that lag does occur *in vivo*. Two predicted rumen NDF digestibilities were then generated:

1) $NDFDP_1 = D(k_1/(k_1+k_2)$

2) $NDFDP_2 = D[e^{-k_2L}-((k_2e^{-k_2L})/(k_1+k_2))]$

Correlations (r) of $NDFDP_1$ and $NDFDP_2$ with *in vivo* NDF digestibility were .75 and .34, respectively. *In vivo* NDF digestibility was more closely related to D (r = .89), which is the 96-hour *in vitro* NDF digestibility, than to $NDFDP_1$, $NDFDP_2$, or any of the other model parameters.

CHAPTER I
INTRODUCTION

A local radio personality in Gainesville, who provides fine music for a very few appreciative listeners, has been known to refer to his program as "Gainesville's best kept secret." The role of forages in providing sustenance to the human population is perhaps one of this country's best kept secrets.

This ignorance is bewildering in light of the scope of forage production in the United States alone (Hodgson, 1976). More land is devoted to forage production than all other crops combined. The dollar value of forages in terms of their contribution to human food of animal origin exceeds the value of any other crop. In 1974 forages supplied 60 percent of all the feed units fed to all classes of livestock, and 82 percent of the units fed to beef cattle. Some 20 to 25 percent of the food supply of the average American is based on forage. And the lack of impact of forages is not limited to the general public. In spite of their massive contribution to food production, forages receive only 4 percent of the funds devoted to agricultural research in the United States.

As energy becomes increasingly expensive, the role of forages in animal production will increase. They represent one of the least energy intensive methods of fulfilling the human requirement for protein. With increasing energy costs, cereal grain production will be diverted to human use or to the production of alcohol as fuel. Beef cattle producers, raising and marketing the least efficient domestic species in converting high quality feed to protein, will come under increasing economic pressure to reduce feed costs. Forages grown on land that is not amenable to the

production of cereal grains, in combination with ruminants that are capable of converting cellulose to human food, will of necessity play an even larger role in the nation's agriculture.

Many factors are involved in the lack of attention to forages. One is that as less people are employed in the agricultural production sector of the economy, fewer people have any knowledge of how food is brought to their table. An additional factor is the nature of forage production itself. It is more fragmented than grain production, and a relatively small fraction (20 percent) is marketed on a cash basis (Rohweder, et al., 1976). Unlike grains, which have a large export market, little forage finds its way abroad. Finally, for animal nutritionists, forage as a feedstuff presents challenges that are more demanding than other feedstuffs. A classification of corn grain into four categories defines it fairly precisely in terms of expected animal production. No such classification of forages is possible. Some 35 species are considered important forage crops, and some 100 species are grown in the United States alone. The ability of these forages to be converted to milk or meat varies with species, plant maturity, soil fertility, rainfall, sunlight, processing and grazing intensity. This diversity is increased many times by the activities of plant breeders seeking improvement in forage quality. Nutritionists are thus presented with a large and heterogeneous assortment of feedstuffs, the evaluation of which in animal trials is economically prohibitive. Laboratory evaluation of this material has identified no single method that is adequate for predicting the feed value of forages, undoubtedly due to their extreme diversity in chemical and physical structure.

The complexities of agricultural production in general, and the quality of forages in particular, have increasingly been studied with tools recently made available by the discipline of systems analysis. The use of systems

analysis and modeling in agricultural research has risen dramatically in the past decade, spawning hundreds of articles and at least one journal devoted entirely to systems research in agriculture. Concommittantly, there has been some resistance to the use of modeling. The blame for this must be shared by those working in this area. Some have shown excessive zeal for the advantages of systems analysis and scorn for traditional scientific thinking. Dillon (1976), for example, suggests that this approach represents a "technological change in our mode of thinking of such magnitude as to imply that we are moving from one socio-technical age to another." Models have been presented with inadequate notation. Rice et al. (1974) presents a diagramatic representation of a model of the grazing system that is virtually unreadable. Nutritionists often do not have a good grasp of the mathematics employed in modeling. These barriers have led to attacks on systems modeling as overly theoretical, when in fact modeling is, at its best, a formal methodology that permists researchers to simplify and order complex relationships.

The advantages of modeling have been described by several authors. Mertens (1977) states that modeling helps to organize information, crystalize thinking, identify new research areas, and test research and hypotheses. Rountree (1977) emphasizes that the whole is greater than the parts and that interactions among the parts precludes studying them without reference to the whole, since it is the interactions themselves that produce the whole's organizational integrity and identity. He also sees systems analysis as a bridge betwen pure mathematics and the empirical sciences. Dillon (1976) states that systems thinking will lead to more efficient and purposeful research. Perhaps Baldwin (1976) put it most succinctly when he wrote that modeling becomes an extremely useful tool when complex interactions between elements that determine output (animal

performance, e.g.) cannot be feasibly evaluated in a quantitative or dynamic fashion by the human mind or traditional research methods.

The purposes of the research described in this dissertation were: 1) to compare ten cultivars of tropical grasses, harvested at different maturities, as to their intake and digestibility by sheep; 2) to evaluate a model of forage cell wall digestion, and to compare *in vitro* estimates of the parameters of the model with *in situ* estimates; and 3) to evaluate an integrated model of ruminal cell wall digestion and intake.

CHAPTER II
REVIEW OF THE LITERATURE

Determinants of Forage Quality

Forage quality has been defined as output per animal when forage availability is not limiting and when animal potential does not vary between treatments (Moore and Mott, 1973). Given no other nutritionally limiting factors, animal output will be determined by net energy intake. Due to the expense of measuring either animal performance or net energy intake, other measurements, highly correlated with animal output, have been used: digestible energy intake, digestible dry matter intake and digestible organic matter intake. The choice of method is dependent on the equipment available, expense and local conditions. At the University of Florida, digestible organic matter intake is the method of choice.

In contrast to forages, highly digestible feeds, such as cereal grains, are evaluated on the basis of their content of digestible, metabolizable or net energy alone. Why then do we require two separate definitions of feed quality? Ruminants consuming highly digestible feeds are able to adjust their intake to provide sufficient net energy to meet their maximum potential for output under 'chemostatic' regulation, the nature of which has been reviewed by Capote (1975). Ruminants consuming most forages are unable to consume sufficient quantitites to meet this potential.

Conrad et al. (1964) noted that as forage quality increases, there is a specific point at which voluntary intake comes under the control of chemostatic mechanisms. Below this point, dry matter intake increases with increasing quality; above it, intake decreases with increasing quality.

Conrad's use of digestibility as the independent variable was probably influenced by the traditional use of digestibility, or total digestible nutrients, to evaluate highly digestible feeds. But this study did recognize the existence of two kinds of intake control. Control of forage intake has been predicated on the assumption that there exists neural sensors in the gastro-intestinal tract that respond to a certain level of fill, causing the cessation of consumption (Campling, 1970). This has been referred to as the 'distension' mechanism.

Fiber, lignin, cellulose, hemicellulose, physical form, tensile strength and many other characteristics have been shown to affect forage quality (Raymond, 1969; Moore and Mott, 1973). A complicating element is that these characteristics often appear to affect forage quality differently in different forages. This has made the accurate prediction of forage quality, from one or more of these, an elusive goal. Success in achieving this goal depends on the understanding of the intermediate mechanisms that link input to output:

Forage chemistry and structure
↓
Intermediate mechanisms
↓
Digestible organic matter intake

Evidence has accumulated that six interrelated factors determine digestible organic matter intake when control is by distension:

1) Fill
2) Potentially digestible and indigestible pool sizes
3) Initial particle size distribution
4) Rate of particle size reduction
5) Rate of passage
6) Rate of digestion

Collectively, control of digestion and passage by these factors determine

forage quality. If quantifiable for a given forage-animal combination, and their relation to each other understood, measurement of these factors will enhance our ability to evaluate forage quality.

Fill. The structure of the gastro-intestinal tract alone suggests that under certain conditions, it will limit feed intake. It is not infinitely elastic nor is it an open tube through which digesta can flow at any rate (Church, 1976). With respect to fill, in effect three compartments exist in the ruminant digestive tract: the reticulo-rumen (rumen), the abomasum and the cecum-colon (large intestine). Exit from these compartments is limited. The omasum, a spherical structure filled with muscular laminae, lies between the rumen and the abomasum, and the reticulo-omasal orifice restricts exit from the rumen. A sphincter, the pylorus, is located at the junction of the abomasum and the small intestine. The anal sphincter restricts movement from the large intestine. Thus, even in the absence of neural sensors to fill, which have yet to be identified (Church, 1979), the physical structure of the digestive tract will act as a limit to intake.

Evidence has accumulated that the rumen is the structure that is limiting in terms of fill. Sawdust of a large particle size introduced into the rumen decreased voluntary intake by 15 percent (Weston, 1966), while particles of polyvinyl chloride, smaller than sawdust, decreased intake by only 7 to 9 percent. The introduction of polypropylene fibers 15 cm long into the rumen via stomach tube depressed intake for 60-65 days (Welch, 1965). Thirty cm long fibers were found unaltered in the rumen some 28 days after administration, while feeding ground fibers had no effect on intake. The introduction of water-filled bladders into the rumen of cattle receiving ad libitum quantities of hay reduced intake (Campling and Balch, 1961). Weston (1966) introduced feed into the rumens of fistulated sheep receiving hay and straw diets. Voluntary intake declined by a quantity equal to 90-110 percent of that introduced. The introduction of sawdust at a rate of 17 percent of

the voluntary feed consumption during a control period, reduced voluntary feed intake by 15 percent.

That the other organs do not materially contribute to intake restriction has been shown (Grovum and Phillips, 1978; Grovum and Williams, 1973b). The rate constant for passage of ^{144}Pr, a heavy metal solid phase marker, was shown to be some twenty times larger from the abomasum than from the rumen. The rate constant for the large intestine was of a similar magnitude to that of the rumen. However, artificially increasing the fill of the large intestine had little effect on intake. Utilizing abomasally cannulated animals maintained on alfalfa (Medicago sativa) hay, a solution of methylcellulose, a bulk laxative, was injected into the abomasum to determine if the intestines are capable of limiting intake. Infusion of 2.95 kg per day had no effect on voluntary intake, although wet fecal output increased from 2.4 to 4.6 kg per day. Only with extremely high levels of infusate (5.4 kg/day) did voluntary intake significantly decline, and it was hypothesized that this was a mechanism to protect the intestines from damage. Furthermore, intestinal transit time was not altered by this treatment.

The component of rumen digesta that constitutes rumen fill has not been as yet identified with certainty. Most workers have reported no relationship between fill (expressed as total digesta, dry matter or organic matter) and voluntary intake (Ulyatt et al., 1967; Thornton and Minson, 1972; Ingalls, et al. 1966). This is an indication that ruminants eat to a constant fill. In the latter study, cell wall constituents constituted the least variable expression of fill. This is in agreement with Van Soest's (1975) "hotel theory," in which plant cell walls, rather than dry matter, constitute the essential fill qualities. Under this

assumption, digestion of cell contents has no effect in reducing fill, just as removal of furniture from a hotel does not reduce the space that the building occupies. Only when cell walls are digested or move to the lower part of the intestinal tract is fill reduced.

Variation in fill can occur in three ways: man induced changes, animal to animal variation and within animal variation. Since within animal variation has only been observed indirectly, and this may be due to rate of passage changes rather than alterations of fill, it will be discussed in a later section. Within a species of animal, the size of the rumen will vary with the size of animal, and thus fill will vary accordingly. Although intake is often expressed as a function of metabolic weight, the volume of different containers will vary directly with the weight of their contents. Capote (1975), utilizing four successive digestibility trials with a group of 24 wethers fed pelleted forages, found that cell wall intake between animals varied least when expressed as a function of body weight to the .96 power. Ivins (1959) and MacLusky (1955) reported that the intake by cattle of forage was closely related to body weight. Other workers have found a wide variation in the relationship of forage intake to body weight (Colburn and Evans, 1968; Karue et al., 1971). Some of the forages used in these studies were high quality temperate forages, where chemostatic regulation rather than fill may be actively limiting intake. An additional source of variation is that even if fill is, theoretically, a function of rumen cell wall content, which in turn is a function of rumen volume, animal-to-animal variation in rumen volume may not be perfectly explained by body weight variation.

Man may also reduce fill by reducing animal intake. In theory this should increase digestibility, since a reduction in fill reduces rate of passage to the abomasum. This has been born out by many researchers.

Blaxter et al. (1956) varied the intake of long, medium-ground and fine-ground hay fed to sheep from 600 to 1500 g daily. Digestibility of dry matter declined, on the average, seven percentage points. Rate of passage, as measured by the appearance of stained particles in the feces, increased with increasing feed intake. Campling et al. (1961) fed hay at 4.5 and 6.8 kg per day, and ad libitum, and observed a decline in digestibility of four percentage points at the higher intake level. Grovum and Hecker (1973) observed increased quantities of digesta in the digestive tract and decreased retention times as sheep intake of alfalfa was increased from 400 to 1200 g per day.

Potentially digestible and indigestible pools. Rates of digestion of cell wall in the rumen are dependent on the nature of the pools upon which they act. If forage cell wall is uniform in chemical and physical structure, then the rate of digestion of cell walls will be quantified by a single rate constant. In the introduction to this dissertation the diversity of forage chemistry and structure was emphasized. There is considerable evidence that forage cell wall is not of a uniform nature.

Blaxter et al. (1956) suggested that there may be a maximum limit of forage dry matter digestibility that was less than complete digestion. Using stains as markers to measure retention time, they altered retention times by varying particle size and the amount of hay fed to sheep. A plot of digestibility against mean time in the digestive tract suggested the exponential equation, digestibility = $D(1-e^{-k_1 t})$, where D is the potentially digestible dry matter (estimated at 81 percent of dry matter for the hay fed in these trials), k_1 is the rate constant for digestion and t is time. This equation is known as the first order reaction curve. This paper was ahead of its time and recognition of the importance of the concept of potentially digestible and indigestible pools did not occur until much later. Lucas

(1958) realized that there was a need for a rigorous definition of the chemical components of feeds with respect to their nutritional value. He proposed the basic outline of the concept of a "nutritive entity," and later expanded on this concept (Lucas, 1962). A nutritive entity was defined as a "nutritionally ideal chemical fraction with invariant digestibility properties;" i.e., it will have a constant true digestibility. The Lucas definition did not take account of retention time; a nutritive entity will vary in its true digestibility, depending on the length of time it spends in the digestive tract. The constant should be the potential digestibility of the nutritive entity; it remains the same, regardless of retention time. Additionally, given no other limiting factors, and a uniform rumen environment, the digestion rate constant of a nutritive entity should be constant. This latter concept is of course an idealized one; nutrient deficiencies (e.g. nitrogen) have been shown to affect rates of digestion, and a decrease in cell wall digestibility with increasing levels of concentrate in the diet is a commonly observed phenomenon. Lucas placed these effects under the umbrella term, "associative effects."

In 1969 Wilkins defined the potential digestibility of cellulose as the "maximum digestibility attainable when the conditions and duration of digestion are not limiting factors." Cellulose digestibility in vitro did not increase with an incubation period of greater than five days, and the use of two incubations of six days each did not result in a different digestibility than 12 days continuous fermentation. Using four grasses at several stages of growth, the potential digestibilities varied from 54 to 88 percent, with an observed decrease in the size of the potentially digestible pool of an individual cultivar with the increasing maturity of that plant. Similar values were found when samples were suspended in nylon

bags (20u) for equivalent duration. A subsequent study found potential cellulose digestibility to be correlated with the percent of schlerenchyma, vascular tissue and lignified tissue in five forage species (Wilkins, 1972). Leaf blade was found to have the highest potential cellulose digestibility followed by the inflorescence, sheath and stem in oats (Avena sativa) and tall fescue (Festuca arundinacea).

The concept of an indigestible fraction is supported by the work of Akin and coworkers who have examined the fate of different forage microstructures when subject to in vitro digesiton. An initial study (Akin et al., 1973) demonstrated histologically that the lignified tissues of Coastal bermudagrass (Cynodon dactylon) appeared to be completely intact after 72 hours of in vitro digestion. However, the lignified tissue of a temperate species, tall fescue, showed some signs of digestion of the lignified portion. This was indicative of an indigestible pool, but one that varied in chemical composition among forage species. A more extensive study of the leaf tissue of one bahiagrass (Paspalum notatum) and two bermudagrasses (Akin et al., 1974) showed that after 72 hours, lignified vascular tissue was undegraded, small vascular bundles, mesophyll and outer bundle sheaths were completely degraded, while epidermis was at various stages of digestion. Histological studies of sections after six and twelve hours of digestion suggested different rates of digestion, or at least different lag times, of different potentially digestible pools. A difference between tropical and temperate species, utilizing 10 different grasses, was also observed (Akin and Burdick, 1975). After 72 hours of digestion the schlerenchyma and lignified vascular tissue of tropical species were undegraded, while some temperate species showed a degree of degradation of the lignified vascular bundles.

The work of Akin and associates suggests a structural or anatomical classification of the potentially digestible pools and the indigestible pool,

at least in tropical species. Minson (1976) has taken a chemical approach to these pools. According to his classification, the potentially digestible pool contains the readily soluble carbohydrates and hydrolyzable polysaccharides (primarily cellulose and hemicellulose), and the indigestible pool consists of lignin, silica, cuticle, and the polysaccharides that are fully protected by lignin. Later, however, Minson had difficulty in quantifying these fractions by in vitro digestion (Goto and Minson, 1977). Using long term fermentation, with centrifugation, decantation and reinoculation at four days, dry matter digestion was found to not be complete at five days, as Wilkins had observed. Reinoculation resulted in a 10.2 percent increase in dry matter digestibility and up to a 28 percent increase in lignin solubility. A similar response was observed when sheep feces were fermented. These authors suggested that 21 days was the minimum incubation time to distiguish between the two pools. Dehority and Johnson (1961) demonstrated a wide variation in the asymptotic peak of in vitro cellulose digestion of mature timothy (Phleum pratense) as particle size was varied with ball milling. Material milled for 6, 24 and 72 hours appeared to have maximum cellulose digestibilities of 58, 65 and 75 percent, respectively. In another phase of this experiment, samples of timothy, ground through a 40 mesh screen, were subjected to digestion for 48 hours. One half of the samples were then ball milled for 72 hours. All samples were reinoculated and cellulose digestion determined at 6, 12, 24, 30 and 48 hours. The samples that were not subject to ball milling showed an insignificant amount of additional digestion, while the ball milled sample experienced loss of 67 percent of the cellulose it contained at the initiation of the second fermentation. The authors suggest that lignin acts as a physical, rather than chemical, barrier which is disrupted with ball milling. Minson (1976) supports this viewpoint.

Inherent difficulties exist in the verification and quantification of an indigestible cell wall pool. Outside of the closed environment of the rumen, lignin and lignocellulose are digestible by microorganisms. Under normal rumen conditions microorganisms which are capable of digesting lignocellulose or of solubilizing lignin may be: 1) not present, due to the nature of the rumen environment; 2) suppressed by the activity of other microorganisms that utilize the more readily available sources of energy, as cellulose digesters are suppressed in grain-fed ruminants; 3) the same organisms which digest non-lignified cellulose, but do not attack lignocellulose when the unlignified material is available as a substrate. Under long term in vitro digestion, with reinoculation, the readily available carbohydrates have been digested, decantation has removed many toxic products, and organisms capable of solubilizing lignin may proliferate. Thus, the transference of pool size derived in the essentially static conditions of the test tube to the dynamic conditions of the rumen may lead to erroneous conclusions. A second difficulty is that the size of the indigestible pool may be a variable rather than a constant pool. If the hypothesized physical barrier that lignin presents can be broken down by ball milling, then to some extent this must also occur with mastication and rumination. Thus, the effect of the lignin barrier in vivo may vary with the duration and intensity of mastication, rumination and the initial particle size, leading to variations in the size of the indigestible pool.

Initial particle size distribution. If the omasum functions as a barrier to the passage of solids out of the rumen, then particle size will affect the rate at which solids move to the abomasum. Alternatively, particle size may contribute to changes in effective rumen fill, for as particle size decreases, density increases. An increase in fill due to

particle size would also lead to increased rate of passage. There is ample documentation that decreases in particle size of forage diets result in increased passage, with a concomittant decline in digestibility.

In Blaxter's classic study of rates of passage in sheep (Blaxter et al., 1956) three forms of a single hay were fed: finely ground and cubed, medium-ground and cubed, and long. Intakes were held constant at three levels: 600, 1200 and 1500 g per day. Rate of passage increased as particle size decreased, irrespective of feeding level. Campling and Freer (1966) found that the intake of ground, pelleted oat straw was 26 percent greater than that of long straw. Grinding of previously chopped alfalfa and wheaten hays resulted in a 50 percent increase in voluntary feed consumption in sheep (Weston, 1967; Weston and Hogan, 1967). A similar response to the pelleting of ryegrass was observed by Greenhalgh and Reid (1973). Intake in cattle, however, was increased by only 11 percent. Others have observed similar results with pelleting or grinding of forages (Osbourn et al., 1976; Osuji et al., 1975). In view of the mass of data with regard to the effect of particle size on intake, it is unfortunate that in none of these studies were there any quantitative measures of initial particle size distribution.

Alwash and Thomas (1974) fed hay diets at two levels of intake. The hays were of four different particle size distributions with a mean particle size of .64, .54, .44 and .20 mm, having been ground through a hammermill fitted with screens of 12.7, 4.75, 3.06 and 1.00 mm, respectively. Differences in retention time among particle sizes were similar at both levels of feeding. However, a doubling of the mean particle size did not result in a doubling of the mean retention time, which suggests that rumination and mastication will increase with increases in particle size (Pearce and Moir, 1964). Additionally, mean particle size is probably an

inadequate index to use in defining a particle distribution. A hypothetical example will illustrate this. If the omasum permits particles of 1.0 mm or less to pass through it and all particles are 1.0 mm in size, they will have a mean particle size of 1.0 mm and all will pass to the abomasum, independent of any particle size reduction. However, given a particle distribution in which one half of the particles are 1.5 mm and the other half are .5 mm, the mean particle size will also be 1.0, but only one half of these particles are capable of passing to the lower tract unaided by mastication. Waldo et al. (1971) suggest that the use of a log normal distribution might be a better method for describing particle distributions.

Rate of particle size reduction. The rate of particle size reduction as well as the initial distribution will affect rate of passage and other components of nutritive value. Of all factors involved in the determination of nutritive value, it is particle size reduction that is least understood. Measures of the variation in rate of particle size reduction have been, for the most part, indirect.

Utilizing six varieties of temperate forages, Chenost (1966) measured the electrical energy necessary to grind forage samples through a 1 mm screen fitted to a laboratory mill. The correlation between the "fibrousness" index and dry matter intake was a .90. However, correlation with digestibility was also high, and intake and digestibility were closely correlated. Troelson and Bigsby (1964) determined a "particle size index" based on the particle size distribution after 10 minutes of artificial mastication. Using four temperate forages cut at different stages of maturity, for a total of 14 different hay samples, he observed that voluntary feed intake was highly correlated ($r = .94$) with particle size index. Omasal digesta from alfalfa hay had a greater relative particle

size than those from the grass hays at comparable maturity stages (Troelson and Campbell, 1968). They suggested that this may be due to the differences in particle shape between the species. Pearce (1967) measured the change in particle distribution in the rumens of sheep fed a roughage ration once daily. More particle reduction occured during the evening hours when rumination was more active. Pearce suggested that this was indicative that rumination, rather than microbial activity, is the major factor responsible for particle size reduction. This concept might be developed into an extension of Van Soest's "hotel theory." If, in terms of space occupying characteristics, the disappearance of cell contents is analogous to the removal of furniture from the interior rooms of a hotel, cell wall digestion may be like the removal of the hotel's windows. The structure continues to occupy the same space, which can only be reduced when the outer walls (cell walls) are demolished by a wrecking ball (rumination).

Rate of passage. The shape of cumulative fecal excretion curves over time, irrespective of type of marker, has been shown to be sigmoidal. Some early researchers (Balch, 1950) have attributed this behavior to lack of instantaneous mixing upon administration of the marker substance, and have ignored the first 5 percent of marker excretion for subsequent calculations. However, both Blaxter et al. (1956) and Brandt and Thacker (1958) independently realized that a sequential, two-compartment model would fit the resulting curves. If the amount of marker administered is normalized to 1, then the cumulative excretion of marker is equal to:

$$1-(1/(k_{21}-k_{11}))\ (k_{21}e^{-k_{22}(t-L)}-k_{22}e^{-k_{21}(t-L)})$$

In this model K_{21} and K_{22} are rate constants for the two compartments, L is a time delay and t is time after marker administration. The model assumes simple first order disappearance of marker from any one compartment.

Dual compartmental models will also resolve into sigmoidal curves, but they would be difficult to relate to the actual physical structure of the digestive tract.

An Australian group has intensively studied the behavior of various sections of the gastrointestinal tract, utilizing a variety of markers and abomasal and ruminal cannulation (Grovum and Williams, 1973a, b, c, 1977; Grovum and Hecker, 1973; Grovum and Phillips, 1973, 1978). Elimination of a particle phase marker, ^{144}Pr, and a liquid phase marker, ^{51}Cr-EDTA, in the rumen, abomasum and cecum-proximal colon, were well described in each compartment by a first order kinetic model with sheep maintained on an alfalfa hay diet. Passage rate constants for ^{144}Pr through the three compartments were .020, .38 and .043 hr^{-1} respectively, and for the ^{51}Cr-EDTA wer .031, .80 and .042 hr^{-1}. This indicated that, as had been previously theorized, the rumen was the limiting compartment with respect to passage, and that at least in the rumen and abomasum, water was capable of passing independently of and at a higher rate than the particulate phase. Ellis ahd Huston (1968) found similar differences between the two phases. When excretion patterns were fitted to the two comparment model, a close fit was observed for both phases (Grovum and Williams, 1973c). A subsequent physical and computer simulation study utilizing reservoirs and flow lines, illustrated that the two compartment model behaved as did the in vivo results, although the rate constants were larger than those in sheep (Grovum and Phillips, 1973). Ulyatt et al. (1976) found no abomasal particles larger than 1 mm in sheep fed alfalfa hay. This suggests that solid phase passage rate estimates apply only to particles less than some critical size.

Rate of digestion. Although Blaxter et al. (1956) had observed that there may be an upper limit of digestibility that was less than complete digestion,

prior to Wilkin's (1969) publication on potentially digestible cellulose, rational methods of describing digestion curves were absent from the literature. As late as 1968, Van Soest reported that the rate constant for digestion of alfalfa and orchard grass (Dactyles glomesata) varied with the duration of digestion, increasing during the first 12 hours and then decreasing at a decreasing rate during the period after 12 hours. The change in the rate constant after 12 hours occurred because cell wall or cellulose was being used as the digestible pool, when only a portion of either quantity was capable of being digested.

Since that time several studies, using multiple sampling times, have determined rate constants for forages, both in vitro and in situ. Gill et al. (1969) determined in vitro cellulose disappearance at six times, using 48 hours digestibility as the end point of digestion. The rate constants for alfalfa varied from .078 to .102 hr^{-1}. Smith et al. (1972) determined rate constants for cell wall digestion on 112 samples representing 15 species of temperate grass and legume hays, using 72 hours as an end point. These values had a range of .040 to .309 hr^{-1}. Digestion rate constants were most highly correlated with the cell contents fraction ($r = .72$), while the 72 hour digestibility was highly correlated with the lignin fraction ($r = .88$), suggesting that lignin is associated with pool size rather than rate of digestion. Separate correlations for grasses and legumes improved the relationships involving the rate constants, but not those involving 72 hour digestibility.

A lag time at the initiation of digestion has been observed in many of these studies. The zero-time intercept of the semilog plots is almost invariably above that of the measured zero-time fraction. This has also been observed in in situ studies (Van Hellen and Ellis, 1977). Mertens (1973) attempted to accommodate this lack of fit with the addition of

various mathematical description of lag time to the basic kinetic model. A discrete lag time was adequate in describing this phenomenon, although observations indicate that digestion during the lag period is increasing, rather than not yet functioning.

Several researchers have noted a decline in forage intake when crude protein levels are below approximately 7 percent (Milford and Minson, 1965; Blaxter and Wilson, 1963). Since protein deprivation affects both microbial and host-animal metabolism, intake changes may be due to several factors. However, there is evidence that rate of digestion may be adversely affected. Houser (1970) observed that the in vitro rate of digestion of low-protein digitgrass hay (4.2 percent crude protein), was increased by the addition of urea, while digestion at 72 hours was unaffected.

Within Animal Variation in the Determinants of Forage Quality

Under the principles thus far discussed, variation in digestible organic matter intake may occur from three general sources: 1) animal to animal variation, most of which will be removed by expressing intake per unit weight, and the remainder will constitute natural variation; 2) man-induced variation which can be controlled; 3) chemical and structural forage factors which, given adequate understanding of their effect on the parameters that determine forage quality, should be estimable. There is also evidence that under certain physiological and environmental conditions, animals can overcome distensive control.

Several experiments with dairy cattle have shown variation between lactating and non-lactating cows. Hutton (1963) utilized six sets of identical twin Jersey crossbred cows, each twin set comprised of one lactating and one non-lactating member. The diets consisted of fresh herbage cut once daily. Intake differences between twins increased over

the 36 weeks post-calving period with the average difference being a 50 percent increase in favor of the lactating twin. Differences in digestibility between twins were minimal (.7 percent), indicating that alterations in fill, rather than the passage rate constant, were occurring. This was confirmed in a second study by Tulloh (1966a) with lactating and non-lactating twins. The water-filled capacity of the rumen was 29 percent greater in lactating cattle. No significant differences between twins was observed in the internal circumference of the duodenum, small intestine or ileum. A 39 percent increase in the weight of digesta in the rumen was observed in the lactating animals. This study also is suggestive of the difficulties in expecting weight differences to account completely for intake differences---the dry cows were heavier than the lactating cows. Similar results were reported in a later study (Tulloh, 1966b).

Rate of passage alteration in the digestive tract of pregnant and non-pregnant Merino ewes were determined by Graham and Williams (1962). Passage in ewes that were 104 days pregnant was higher than that of ewes that were 39 days pregnant. The mechanism responsible may have been an increase in rumination, since the ewes were limit-fed. Exposure of shorn sheep to cold has been shown to reduce the digestibility, rumen turnover time and rumen fill (Kennedy and Milligan, 1978). Differences between shorn sheep and unshorn sheep in intake varied with plant species (Minson and Ternouth, 1971). Shorn sheep had greater intake of digitgrass, similar intakes of Setaria and reduced intakes of alfalfa when compared to unshorn sheep. No consistent differences in digestibility were observed.

Integrated Models of Rumen Digesta Disappearance

The six major factors identified in a previous section as determinants of the quality of a forage have all been identified, directly or

indirectly, as being capable of producing alterations in the digestible organic matter intake of a forage. Variations in forage structure and chemistry will affect one, several, or more likely, all of these parameters. It is not sufficient, for example, to state that the grinding of a forage results in an increase in voluntary intake, a decrease in digestibility and an overall increase in digestible organic matter intake. These six factors do not operate independently; they interact with each other. Only by understanding these interrelationships can the ultimate quantitative effect on forage quality be understood. This effect will vary from forage to forage and may, in a particular species, produce unexpected results (Van Soest, 1975).

Reducing the intial particle size of a forage would have the following effects based on our present knowledge of the way in which these parameters behave, and on intuition based on theoretical principles:

1) an increase in the digestion rate constant as surface area increased.
2) an increase in effective fill if greater packing occurred.
3) an increase in the potentially digestible pool if such grinding results in a rupture of ligno-cellulose bonds.
4) a decrease in the rate of particle size reduction with a decrease in rumination.
5) a decrease in the rate constant for passage, if rumination is at least partially responsible for passage.

The net effect of these changes on the disappearance of digesta would be the following:

1) an increase or a decrease in the rate of passage depending on whether the net effect of the decreased initial particle

size distribution and the increased effective fill is greater than the effect of decreased rate constants for particle size reduction and passage.

2) if passage rate is increased, an increase or a decrease in the extent of digestion depending on whether the effect of increased rate of passage in decreasing the retention time is greater than the effect of increased digestion rate constant.

3) if passage rate is decreased, an increase in the extent of digestion.

Although the vast majority of studies show an increase in intake and a decrease in digestibility with grinding, it is evident that without a theoretical and quantitative understanding of these dynamic relationships, precise predictions of such changes are not possible. Similarly, efforts to predict intake from digestibility are doomed to failure, since they are not causative factors, but the end result of these processes.

Any given system contains within it component subsystems and is itself part of a larger system. Therefore, selection of the level at which to explore a system will depend on the specific goals of the researcher. With respect to forages, one can attempt to model at the level of: 1) the entire grazing ruminant system, including the agronomic subsystems (climate, soil and herbage), animal digestive function and animal metabolism (Rice et al., 1974); 2) the animal only, including both distensive and chemostatic controls (Forbes, 1977); or 3) a component of the diet subjected to only one type of control (Waldo et al., 1972). Once this level is selected the degree of detail or complexity necessary to fulfill objectives must be determined. Finally, the determination must

be made as to whether there is sufficient data available to validate the model. At this station the major concern is ultimately with the evaluation of forage quality. Therefore, this discussion will be limited to models which offer the potential of predicting the quality of a given forage to ruminant animals.

The foundation paper in this field published in 1956 by Blaxter and his associates (Blaxter et al., 1956). Its importance was fourfold: 1) it provided a kinetic explanation for the results of marker studies; 2) it introduced the concept of potential digestibility; 3) it attempted to integrate digestion and passage in one model; 4) it introduced the concepts of fill and distension. Unfortunately, it took more than a decade for other researchers to interpret, apply and extend this model. The two-compartment model for passage has already been discussed. By varying the form and amount of hay fed, they were able to change retention time. As retention time increased, so did digestibility, in a manner suggesting the exponential relation, digestibility = $D(1-e^{-k_1 t})$. Potential digestibility, the rate of constant for digestion, the passage constants (k_{21} and k_{22}) and a time delay (L) were then integrated into a single model to predict in vivo digestibility:

$$\text{Digestibility} = D-D(k_{21}K_{22}/(k_{21}-k_{22}))(e^{-k_{22}L}/(k_1+k_{22})-e^{-k_{21}L}/(k_1+k_{21}))$$

One assumption of the model is that one rate constant for digestion is valid for the entire digestive tract. Using in vivo values to estimate the digestion rate constant, Blaxter noted that an increase in initial particle size resulted in a higher digestion rate constant, and that an increase in intake lowered the digestion rate constant. It is unlikely that either would be valid.

In 1972 a simpler model to describe cellulose disappearance from the rumen was proposed (Waldo et al., 1972), and has since been applied to cell wall disappearance (Mertens, 1973) and organic matter disappearance (Golding, 1976). Its use with respect to intake assumes that the rumen is the limiting part of the gastrointestinal tract. It recognized that previous attempts to resolve disappearance curves into exponential functions failed because they used the total fraction rather than the potentially digestible fraction as the digestion pool. The basic hypothesis of the model is of two rumen pools, a potentially digestible pool which can disappear by passage and digestion, and an indigestible pool which can only escape the rumen by passage. Its simplifying aspects were the assumption of the rumen as the key organ in limiting intake and that an adequate description would be achieved by assuming steady state conditions. The concentration of labeled cellulose (F) in the rumen at 5 hours post-administration is given as:

$$F = De^{-(k_1+k_2)t} + Ue^{-k_2t}$$

Under steady state conditions, labeled cellulose fill (F) per unit intake (I) is:

$$F/I = D/(k_1+k_2)+U/k_2$$

where D = potentially digestible pool, U = indigestible pool, k_1 = digestion rate constant and k_2 = passage rate constant.

The application of this model illustrates how modeling can aid in resolving questions raised by experimental results and force the integration of concepts. Although it is now recognized that digestion and intake are functions of the same dynamic processes, for many years they were treated entirely separately. Various experimental methods have been used to generate prediction equations of either or both of these in vivo parameters. In the process, researchers observed that the coefficients

of variation for intake (for animals receiving the same forage) averaged 10-20 percent, and were much higher than those for digestibility, which averaged 5-10 percent. An example, using the Waldo model, aids in explaining these differences. Two equations, derived from this model, define intake and digestibility under steady state conditions:

$$\text{Intake} = F/(D/(k_1+k_2)+U/k_2)$$

$$\text{Digestibility} = D(k_1/(k_1+k_2))$$

The same variables occur in both equations, with the exception that fill appears only in the intake equation (U can be expressed as 1-D). Thus, for example, a 25 percent change in F results in a 25 percent change in intake and no change in digestibility. Fill contributes an additional source of variation to intake that is not present for digestibility, and the addition of a function of body weight to the equation will not entirely remove this source of variation unless this function of body weight is perfectly correlated with fill. There is an additional explanation for the difference in observed variation between intake and digestibility. First, we must assume that the digestion rate constant does not vary among animals receiving the same forage, which is reasonable since rumen environments are relatively constant and microbes tend to adapt to their diets. Then let us examine what happens when two animals of the same size and fill consume the same forage, but the passage constant, k_2, is 25 percent greater in one animal, perhaps due to differences in rumination, size of the reticulo-omasal orifice, or chewing duration and intensity. The parameters and expected intake and digestiblity are given below.

	F	D	U	k_1	k_2	CI	CD	DCI
Animal 1	500	.60	.40	.04	.020	401	40	160
Animal 2	500	.60	.40	.04	.025	476	37	176
percent change	0	0	0	0	+25	+19	-7.5	+10

F = cellulose fill, g; D = potentially digestible fraction of total cellulose; U = indigestible fraction of total cellulose; k_1 = cellulose digestion rate constant, hr^{-1}; k_2 = cellulose passage rate constant, hr^{-1}; CI = cellulose intake, g/day; CD = cellulose digestibility, percent; DCI = digestible cellulose intake, g/day.

This example shows that a 25 percent increase in the passage rate constant results in a 19 percent increase in cellulose intake, only a 7.5 percent decrease in digestibility and a 10 percent increase in digestible cellulose intake. It provides an explanation, in a simplified, quantifiable way, for these variations, although under actual in vivo conditions these relationships are certainly more complex.

The publication of the Waldo model stirred great interest due to its potential for the prediction of forage quality. It provided a simple, integrated scheme, and if utilized for broader fractions of forage, and a method found to estimate the parameters, than the difficulties in predicting intake might be solved. Golding (1976) applied this model to rumen organic matter disappearance, using 31 tropical grasses. In vivo lignin intake was used to estimate the passage rate constant. The rate constant for digestion was estimated via in vitro digestion, and potentially digestible and indigestible pools were estimated as steady-state rumen concentrations, with the use of equations employing the digestion rate constant and a predicted organic matter digestibility based on 72 hour in vitro digestion. Although steady-state conditions were assumed, these values were entered into the time dependent equation and the time required for organic matter fill to reach 37 percent of estimated steady-state

fill was used to predict digestible organic matter intake. The correlation achieved was quite high ($r = .96$). However, several difficulties with the interpretation of the model existed in this study: 1) the basis for the pool estimates was that the retention time of digestible organic matter is $1/(k_1+k_2)$, but since this fraction leaves the rumen only by digestion its retention time is $1/k_1$, which cannot be used to estimate these two pools; 2) in the model, intake, rather than digestible intake, is a function of retention time, and it is intake that should be used to validate the model; and 3) the passage rate constant was estimated by a value containing intake, i.e. lignin intake, and digestibility was estimated by using a prediction equation based on *in vivo* values of the same forages, and these equations were different for different species of grass.

Mertens (1973) applied the Waldo equation to cell walls, assuming that cell walls constitute rumen fill. He was aware that previous researchers had shown a lag phase in measurements of rates of digestion and attempted to account for lag by various alterations in the digestion rate component of the model (although lag may be an artifact of the methods used to measure rates of digestion). These included the introduction of a discrete lag phase, a sequential two-compartment digestion model, analogous to that proposed by Blaxter *et al.* (1956) for passage, an incremented lag phase and a model containing two potentially digestible pools. A model with a discrete lag phase was adequate in fitting the data, although he recognized that the rejection of other models may have been due to an inadequate number of sampling times. To test the ability of the model to predict cell wall and dry matter intake, he estimated the potentially digestible and indigestible pools, digestion rate constant

and lag time with in vitro rate studies, and estimated the rate constant for passage by an equation based on cell wall intake. A total of 187 temperate and tropical grasses and legumes were used. He found a correlation of .81 with dry matter intake. As with Golding's estimate of the passage constant, Merten's was also derived from intake.

Both authors recognized certain trends and concepts in their research. First, that the independent estimation of the passage constant was necessary if the model was ever to be used in a predictive manner. Golding suggested the use of grinding energy. Second, the importance of this passage constant was seen by both, recognizing that a given percent change in this value resulted in a much greater change in forage quality than did an equivalent change in the digestion rate constant. Third, Mertens felt that the description of particle size degradation and passage by only one rate constant was too simplified, and that the kinetics involved were more complex.

An attempt to rectify this neglect of particle size has recently been presented (Mertens and Ely, 1979). The new model of fiber kinetics employs three rumen particle size pools, two potentially digestible pools (recognizing the microanatomical work of Akin), and two lower tract digestion pools. Its basic assumptions are that particle size is reduced at differential rates that are dependent on the particle size pool, that particles in the large size pool are incapable of passing out of the rumen, and that particles from the small and medium size pools pass at different rates through the omasum. Due to the complexity of the model, evaluation was necessary by simulation techniques, and the results agreed fairly well with those in the literature.

The models thus far discussed might be classified as structural models in that they attempt to understand forage quality in terms of the structural

components of the diet: cell walls and associated microanatomical structures. A group of researchers in California have for several years been actively synthesizing a chemical model of the determinants of feed quality (Ulyatt et al., 1976; Reichl and Baldwin, 1976; Vera et al., 1977; Baldwin et al., 1977). Their objectives were threefold: 1) development of a model based on currently accepted and defensible concepts; 2) identification of specific aspects of ruminant digestion where current concepts are inadequate; 3) development of a model which could be used to test hypotheses regarding factors that affect feed quality.

The model presently consists of a central subunit to account of microbial activity and growth, a summary computation subunit, and 12 chemical subunits consisting of soluble carbohydrate, organic acids, starch, pectin, hemicellulose, cellulose, lipids, soluble proteins, insoluble proteins, nonprotein nitrogen, lignin and ash. Particle size is the only physical attribute of the forage contained in the model, and only as a binary factor (it is either greater than or less than the maximum size capable of passage). The effects of chewing are associated with forage input (leafiness and tensile strength), and rumination is in the model but only as an externally altered factor. It does not respond to other factors in the model.

The model is described by over 800 equations, and thus by necessity it was evaluated by simulation. Their conclusion was that the model performed generally well but was weak in three major areas: 1) existing analytical methods are inadequate in describing pectin and organic acids in legumes; 2) the effects of rumination and chewing are inadequately accounted for; 3) rates of passage of soluble and insoluble material from low

quality feeds are poorly described.

The author of this dissertation felt that the Waldo model presented a compromise between empirical methods of predicting forage quality and more complex models that may better describe the ruminant digestive process but may be useless for prediction due to our inability to measure all pool sizes and rate constants. Furthermore, it was felt that this model had not been adequately validated. Due to the many abbreviations and symbols used in this text, a table of definitions is provided as an aid to the reader (table 1).

TABLE 1. ABBREVIATIONS AND SYMBOLS USED IN TEXT, TABLES AND FIGURES

Item	Definition
Animal and chemical data	
ADF	Acid detergent fiber, % dry matter
BW	Body weight, kg
$BW^{.75}$	Metabolic weight, kg
CELL	Cellulose, % dry matter
CP	Crude protein, % dry matter
DNDF	Digestible ash-free neutral detergent fiber, % dry matter
DNDFI	Digestible ash-free neutral detergent fiber intake, g/day
DM	Dry matter
DOM	Digestible organic matter, % dry matter
DOMI	Digestible organic matter intake, g/day
HEMI	Hemicellulose, % dry matter
LIG	Permanganate lignin, % dry matter
NDF	Ash-free neutral detergent fiber, % dry matter
NDFD	Neutral detergent fiber digestibility, %
NDFI	Ash-free neutral detergent fiber intake, g/day
OM	Organic matter, % dry matter
OMD	Organic matter digestibility, %
OMI	Organic matter intake, g/day
SIL	Silica, % dry matter
suffix '1'	Per body weight
suffix '75'	Per metabolic weight
Model parameters	
D	Potentially digestible pool
F	Fill
k_1	Digestion rate constant
k_2	Passage rate constant
L	Lag time
t	Time
U	Indigestible pool

Table 1 - continued

Item	Definition
Statistics	
CV	Coefficient of variation, %
ns	Not significant
r	Correlation coefficient
r^2	Coefficient of determination
R^2	Multiple coefficient of determination
*	P<.05
**	P<.01
***	P<.001

CHAPTER III
DIGESTIBILITY AND INTAKE OF CULTIVARS OF BERMUDAGRASS, DIGITGRASS AND BAHIAGRASS FED TO SHEEP -- EXPERIMENT 1

Introduction

Bermudagrass (Cynodon dactylon), digitgrass (Digitaria decumbens) and bahiagrass (Paspalum notatum) are major pasture grasses in the southeastern portion of the United States. Plant breeders have endeavored to develop new cultivars that combine the characteristics of high yield, disease resistance, cold tolerance, improved propagation and improved animal performance. Several recently developed cultivars have shown promise in one or more of these characteristics. The objective of this study was to determine the quality of several previously released cultivars of these grasses at different stages of maturity, in terms of their intake and digestibility when fed to sheep, and to provide a wide range of forage samples for the evaluation of laboratory methods used to predict forage quality.

Materials and Methods

Grasses were grown during the summer and fall of 1975, on three areas of the Agronomy Farm, "Green Acres," near Gainesville, Florida on upland sandy soil. Three digitgrasses, 'Transvala', 'Slenderstem' and X50-1 were grown in area A. Three digitgrasses, X124-4, X215-3 and X45-2, and three bahiagrasses, 'Argentine', 'Paraguay' and 'Pensacola', were grown in area C. Coastcross-1 bermudagrass was grown in area D. Each area was divided into two replications and cultivars were allotted randomly within each replication. The plots were fertilized with 560 kg/ha of 12-12-12 in May. Prior to staging(initial mowing and removal

of mowed material), all plots were fertilized with 74 kg/ha of ammonium nitrate (33 percent N), and this application was repeated after the two-week regrowth was harvested. Area D received an application of 0-10-20 at a rate of 560 kg/ha plus micronutrients at this time. The harvesting schedule is shown in table 2. After staging, the two-week old regrowths from areas A and C were harvested from the entire plot, and subsequent four, six and eight-week cuttings were second regrowths. In area D, only the six-week cutting was a second regrowth. On September 2, at the time of the four week harvest of area C, an infestation of striped grass loopers (Mocis latipes) was recorded. Damage was particularly severe in area C, and leaf blade disappearance was noted, especially among the digitgrasses. Applications of an insecticide (Lanate) were made on September 3 and 19 in all areas, and October 10 in area D. At each harvest the grasses were cut with a flail harvester, artificially dried and stored in open weave bags.

Thirty-six sheep, averaging 44.5 kg liveweight, were allotted randomly to hays in each of six periods. Hays from one field replication were fed during the first three periods, and from the other replication during the second three periods. Each period consisted of a fourteen-day preliminary period during which sheep were acclimated to metabolism crates and hays, followed by a seven-day collection period when the amount of hay offered, refused (orts) and wasted (dropped on the floor), and the amount of feces excreted were measured. Hays were fed ad libitum, adjusting the hay offered to provide approximately 200 g of orts daily. Water, calcium phosphate and trace-mineralized salt were available at all times. At the beginning of each collection period the anticipated amount of hay needed for the collection period was emptied, mixed, sampled and rebagged. Feces were collected in canvas bags. The waste, orts and 20 percent of the

TABLE 2. HAY HARVESTING SCHEDULE

Item	A	C	D
Date of harvest			
July 25		Staged	
August 4-7		2_1	
August 6-8	Staged		
August 13	2_1		
August 25			Staged
September 2-5		4_2	
September 9			2_1
September 12	4_2		
September 16-19		6_2	
September 23			4_1
September 26	6_2		
September 30-October 3		8_2	
October 10	8_2		
October 21			$(\begin{smallmatrix}6_2\\8_1\end{smallmatrix})$

[a]Numbers refer to ages (weeks) at harvest; subscripts refer to cutting following staging.

feces were dried at 50 C for a minimum of two days. Samples were pooled by animal, hays and orts ground through a hammermill and all samples ground in a Wiley mill to pass through a 4 mm screen. Approximately 200 g were reground through a one mm screen for subsequent analysis.

Dry matter was determined at 105 C for 24 hours and organic matter at 500 C for a minimum of six hours for the determination of organic matter intake (OMI), digestibility (OMD) and digestible organic matter intake (DOMI). Neutral detergent fiber was determined by a modification of the technique of Goering and Van Soest (1970), in which the decahydronapthalene and sodium sulfite were omitted, and residues were filtered through glass wool in porcelain gooch crucibles. Neutral detergent fiber digestibility (NDFD), intake (NDFI) and digestible neutral detergent fiber intake (DNDFI) were calculated.

Least squares analysis and correlations (Snedecor and Cochran, 1967), and multiple comparisons (Duncan, 1955) were performed utilizing SAS (SAS Institute, 1976). TYPE-3 sums of squares were used for the analysis of variance tests of significance.

Results and Discussion

Determination of intake expressions. Prior to the final statistical analysis, the logarithm of the intake variables in g/day was regressed upon the logarithm of body weight (BW) plus all the discrete variables in the model, in order to determine the power of BW appropriate for the expression of intake. Due to the differences in areas in staging time, insect damage, fertilization and regrowths (second or first), the following equation was employed:

$$\text{Log intake} = B_0 + B_1 x \log BW + B_2 \times \text{area} + B_3 \times \text{rep (area)} + B_4 \times \text{regrowth} + B_5 \times \text{area} \times \text{regrowth} + B_6 \times \text{regrowth} \times \text{rep (area)} + B_7 \times$$

cultivar (area) + B_8 x regrowth x cultivar (area) + B_9 x period (rep) + error

The coefficient B_1 is the least squares estimate of the exponent of BW. Estimates of this exponent were .92, 1.04, .93 and 1.02 for OMI, DOMI, NDFI and DNDFI, respectively.

The proper expression of intake for roughage diets has been a matter of some controversy, and this has been reviewed by Capote (1975). The results reported here agree with those of Capote, in that greater variation is removed by expressions that relate intake to BW than to $BW^{.75}$. There exists no uniform method of reporting intake in the literature; some researchers prefer to report it as per body weight and others per metabolic weight.

Models of forage intake (Mertens, 1973; Baldwin et al., 1977) assumes that fill of the rumen and rates of material disappearance from the rumen limit intake. Since rumen volume is theoretically related to weight, intake of the same roughage by different size animals of the same class would be expected to vary directly with the weight of the animals. The data presented here support that conclusion. Since digestibility is assumed to be independent of animal weight within a species, DOMI should also be related to animal weight, since DOMI is the product of OMD and OMI.

In utilizing various expressions of intake, it is important to keep in mind the purpose to which these expressions will be put. In evaluating various forages, using animal performance at ad libitum intakes as a criterion, the manner in which livestock regulate intake is irrelevant. What is essential to know is what animal performance, in terms of wool production, milk production, work or growth, can be expected with these diets. Basal metabolic rate is well established as a function of metabolic weight

(Kleiber, 1932; Brody and Procter, 1932). Methods of estimating net energy values using comparative slaughter techniques use metabolic weight as a reference base (Garrett, 1971). The National Research Council uses metabolic weight as its reference base in computing the net energy requirement for both maintance and gain in beef cattle (NRC, 1976). Therefore, $DOMI/BW^{.75}$ (DOMI75) is the appropriate parameter to use when comparing the value of several forages, under the assumption that animal variation in the utilization of digestible energy of a given feedstuff is best removed by metabolic weight.

Alternatively, if the purpose of an experiment is to either validate models of ruminant intake, or to predict intake with laboratory techniques, the correct expression for intake data is on a weight basis, assuming fill is a limiting factor. All suggested models of rumen digesta disappearance that incorporate fill (when the diet consists of low quality feeds fed ad libitum) are resolvable into expressions of OMI/BW (OMI1) and OMD, or NDFI/BW (NDFI1) and NDFD, if estimates of pools and rate constants can be obtained. Validation of the model with animal data should be accomplished using body weight as the reference base. If such models are proven reasonably accurate and useful as tools to evaluate forage quality, then these quantitites should be converted to DOMI75 for comparative purposes.

Cultivar comparisons. OMD, OMI1, DOMI75, NDFD, and NDFI1 were statistically analyzed using equation (1) with the 'log BW' term removed. Analyses of variance are presented in the appendix (tables 15 and 16). Significant main effects included area, regrowth (except for NDFI1), cultivar with area, and period with replication for all expressions of intake. The greater variation accounted for by the area x regrowth interaction than by the cultivar (area) x regrowth interaction, could

be due to any of the factors involved in area differences, including insect damage and harvest date. The period (rep) effect for intake measurements can be attributed to to environmental changes over the course of the digestion trial. Across all regrowths, the digitgrasses in area C were superior in OMD ($P<.01$) to the bahiagrasses.

Least square means of intake and digestibility by cultivar, replication and regrowth are presented in appendix table 17. In light of the significant regrowth x cultivar (area) effect, multiple comparisons among cultivars were made only within each area-regrowth combination (tables 3-6). Within area A, the four-week Transvalva was superior in OMD (68.8) and NDFD (71.1) to the other two cultivars. No other significant differences in digestibility were observed in this area. Intakes of Slenderstem were the highest of the six-week regrowths, with a DOMI75 of 41.2 versus 32.3 for the Transvala, and 31.5 for X50-1. At eight weeks the ranking of these cultivars was reversed, with X50-1 registering its highest DOMI75 over all regrowths, and this was superior to the other two varieties. In area C, two-week old X124-4 had a higher DOMI75 than most other varieties (50.2) and was higher in DOMI75 at four weeks than two of the bahiagrasses. No other significant differences in DOMI75 were observed after four weeks. At six weeks, cultivar X124-4 had the highest OMD, and across all regrowths in this area, this cultivar appeared to best maintain its quality.

Regrowth effects within each area are illustrated in figures 1-9. In area C, OMD declined rapidly from the two-week regrowth to the four-week regrowth in all cultivars. Part of this decline may have been due to insect damage because, with the exception of X215-3, all cultivars in area C recovered at six weeks, recording higher digestibilities than were

TABLE 3. LEAST SQUARES MEANS OF INTAKE AND DIGESTIBILITY OF TWO-WEEK REGROWTHS

Area (date of harvest)	Grass	Cultivar	Parameter (see table 1) OMD	OMI1	DOMI75	NDFD	NDFI1
Area C (August 4-7)	Digit	X124-4	70.4^{ab}	27.4^{a}	50.2^{a}	74.7^{a}	19.4
		X215-3	67.1^{bc}	24.2^{ab}	42.1^{bc}	73.5^{ab}	18.4
		X46-2	72.3^{a}	26.2^{ab}	49.3^{ab}	77.3^{a}	19.1
	Bahia	Argentine	63.9^{cd}	22.9^{b}	38.8^{c}	67.9^{c}	18.0
		Paraguay	64.6^{c}	23.6^{b}	39.8^{c}	69.1^{bc}	18.5
		Pensacola	64.9^{c}	24.2^{ab}	41.3^{c}	69.0^{bc}	19.2
Area A (August 6-8)	Digit	Slenderstem	66.7	24.8	52.7	72.0	18.4
		Transvala	68.0	24.6	43.0	72.2	18.5
Area D (August 25)	Bermuda	Coastcross-1	59.7	21.3	33.6	60.9	16.0

^{abc}Column means within area with different superscripts are different ($P<.05$).

TABLE 4. LEAST SQUARES MEANS OF INTAKE AND DIGESTIBILITY OF FOUR-WEEK REGROWTH

Area (date of harvest)	Grass	Cultivar	Parameter (see table 1) OMD	OMI1	DOMI75	NDFD	NDFI1
Area C (September 2-5)	Digit	X124-4	65.2[a]	21.4	38.7[a]	70.2[a]	17.1
		X215-3	58.9[bc]	20.4	30.9[ab]	64.6[b]	16.8
		X46-2	58.3[bc]	22.2	33.7[ab]	63.2[b]	18.2
	Bahia	Argentine	55.0[c]	23.1	33.7[ab]	57.7[c]	19.2
		Paraguay	54.5[c]	23.3	33.1[b]	57.3[c]	19.3
		Pensacola	58.0[b]	20.3	30.9[b]	60.8[bc]	16.8
Area A (September 12)	Digit	Slenderstem	61.8[a]	24.2	38.9	63.8[a]	18.2
		Transvala	68.8[b]	24.5	44.2	71.1[b]	18.4
		X50-1	61.6[a]	24.4	39.1	64.6[a]	18.0
Area D (September 23)	Bermuda	Coastcross-1	58.1	17.3	26.4	61.1	14.2

[abc]Column means within area with different superscripts are different (P<.05).

TABLE 5. LEAST SQUARES MEANS OF INTAKE AND DIGESTIBILITY OF SIX-WEEK REGROWTHS

Area (date of harvest)	Grass	Cultivar	Parameter (see table 1) OMD	OMI1	DOMI75	NDFD	NDFI1
Area C (September 16-19)	Digit	X124-4	68.5[a]	21.5	38.4	73.1[a]	17.3[ab]
		X215-3	58.7[bc]	20.1	31.0	63.5[bc]	16.3[b]
		X46-2	61.8[b]	21.6	34.5	65.6[b]	17.2[ab]
	Bahia	Argentine	55.4[c]	23.7	34.5	58.0[c]	19.6[a]
		Paraguay	57.0[bc]	21.4	32.3	59.9[c]	17.8[ab]
		Pensacola	59.5[bc]	22.5	35.5	62.2[bc]	18.7[ab]
Area A (September 26)	Digit	Slenderstem	59.6	26.7[a]	41.2[a]	60.3	20.4[a]
		Transvala	61.3	20.3[b]	32.3[b]	61.6	15.3[b]
		X50-1	60.3	20.1[b]	31.5[b]	62.0	14.8[b]
Area D (October 21)	Bermuda	Coastcross-1	59.4	20.0	30.7	61.1	16.6

[abc]Column means within area with different superscripts are different ($P<.05$).

TABLE 6. LEAST SQUARES MEANS OF INTAKE AND DIGESTIBILITY OF EIGHT-WEEK REGROWTHS

Area (date of harvest)	Grass	Cultivar	Parameter (see table 1) OMD	OMI1	DOMI75	NDFD	NDFI1
Area C (September 30-October 3)	Digit	X124-4	60.6[a]	19.7	30.9	64.3[a]	15.7
		X215-3	60.2[a]	19.7	30.9	64.3[a]	16.0
		X46-2	59.2[ab]	22.1	33.7	61.2[ab]	17.4
	Bahia	Argentine	60.1[a]	22.2	34.4	62.9[ab]	18.4
		Paraguay	54.1[b]	21.9	31.1	57.5[b]	18.2
		Pensacola	57.1[ab]	20.2	29.6	61.0[ab]	17.0
Area A (October 10)	Digit	Slenderstem	58.7	27.0[a]	37.0[a]	58.9	18.6
		Transvala	57.8	21.4[b]	32.8[a]	57.6	16.0
		X50-1	61.7	25.3[a]	41.3[b]	62.0	18.0
Area D (October 21)	Bermuda	Coastcross-1	55.2	21.5	31.2	55.4	17.8

[ab]Column means within area with different superscripts are different (P<.05).

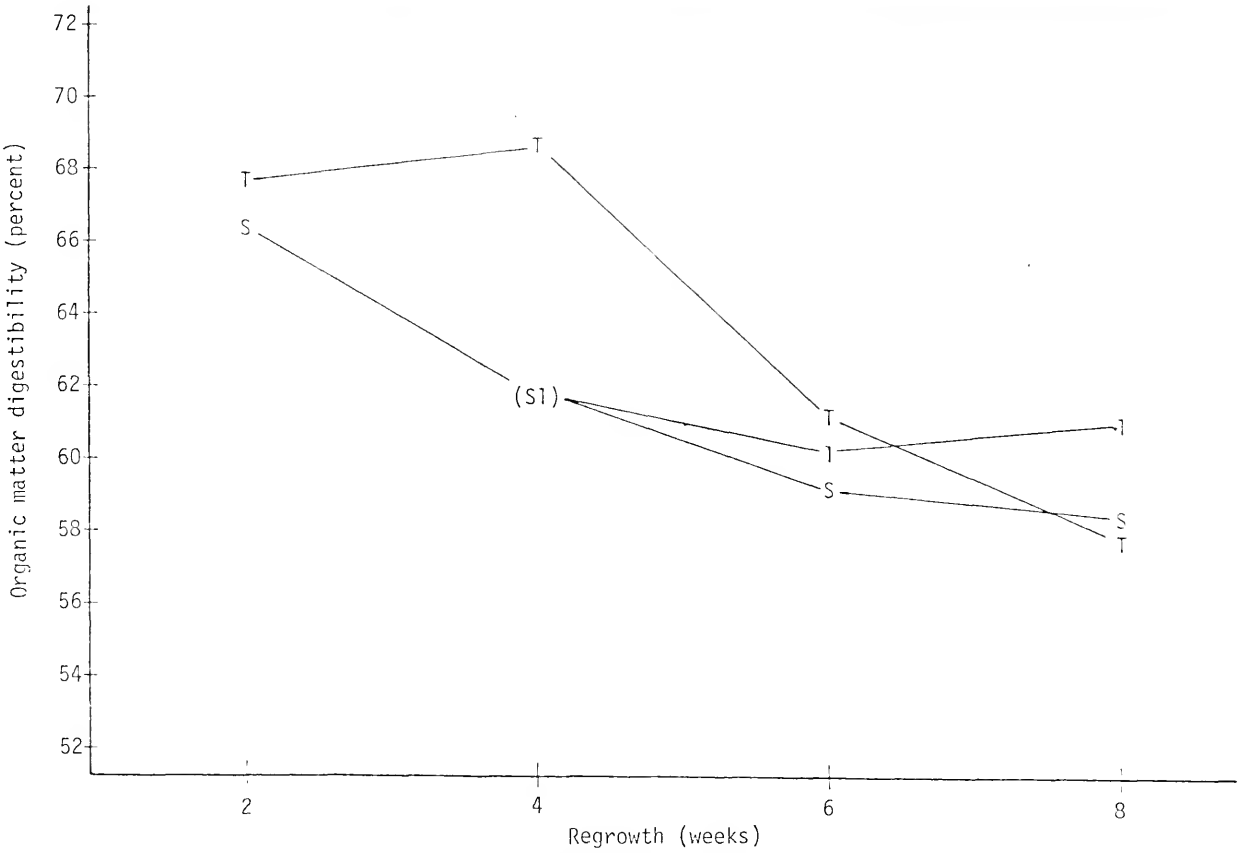

Figure 1. Organic matter digestibility (OMD) of digitgrass from area A versus regrowth. T=Transvala; S=Slenderstem; 1=X50-1.

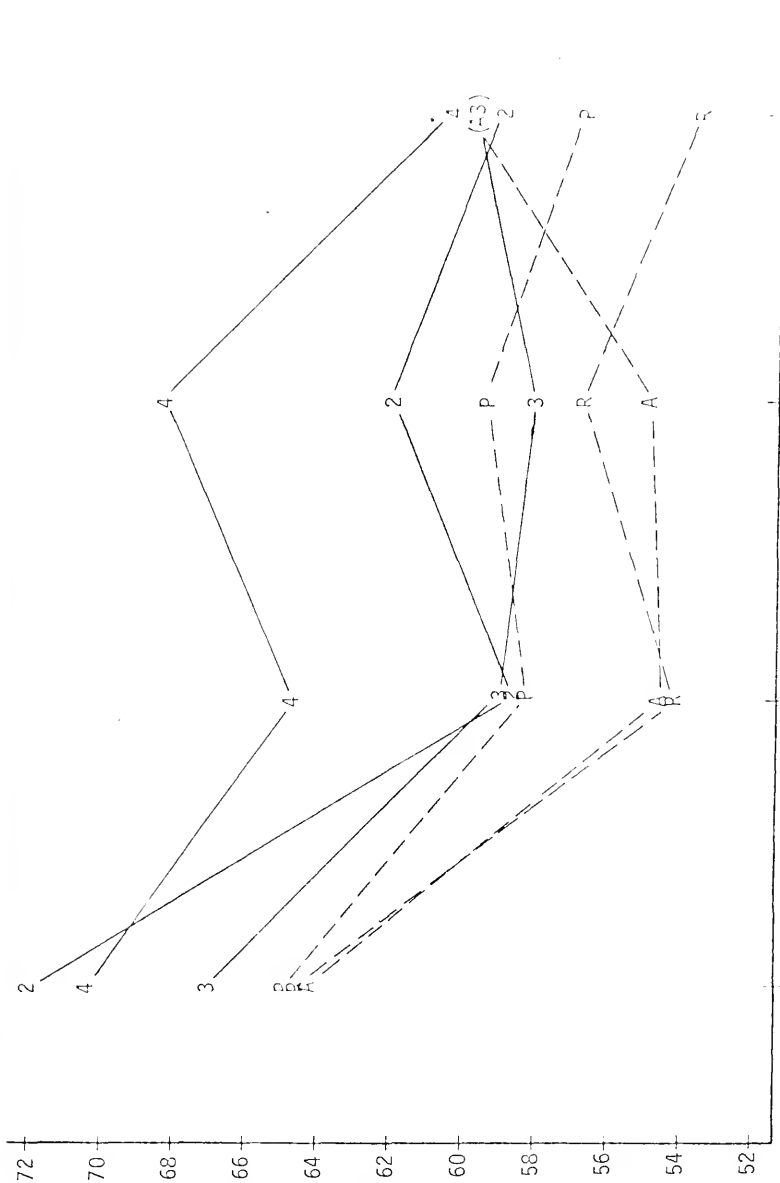

Figure 2. Organic matter digestibility (OMD) of digitgrasses (solid lines) and bahiagrasses (broken lines) from area C versus regrowth. 2=X46-2; 3=X215-3; 4=X124-4; A=Argentine; R=Paraguay; P=Pensacola.

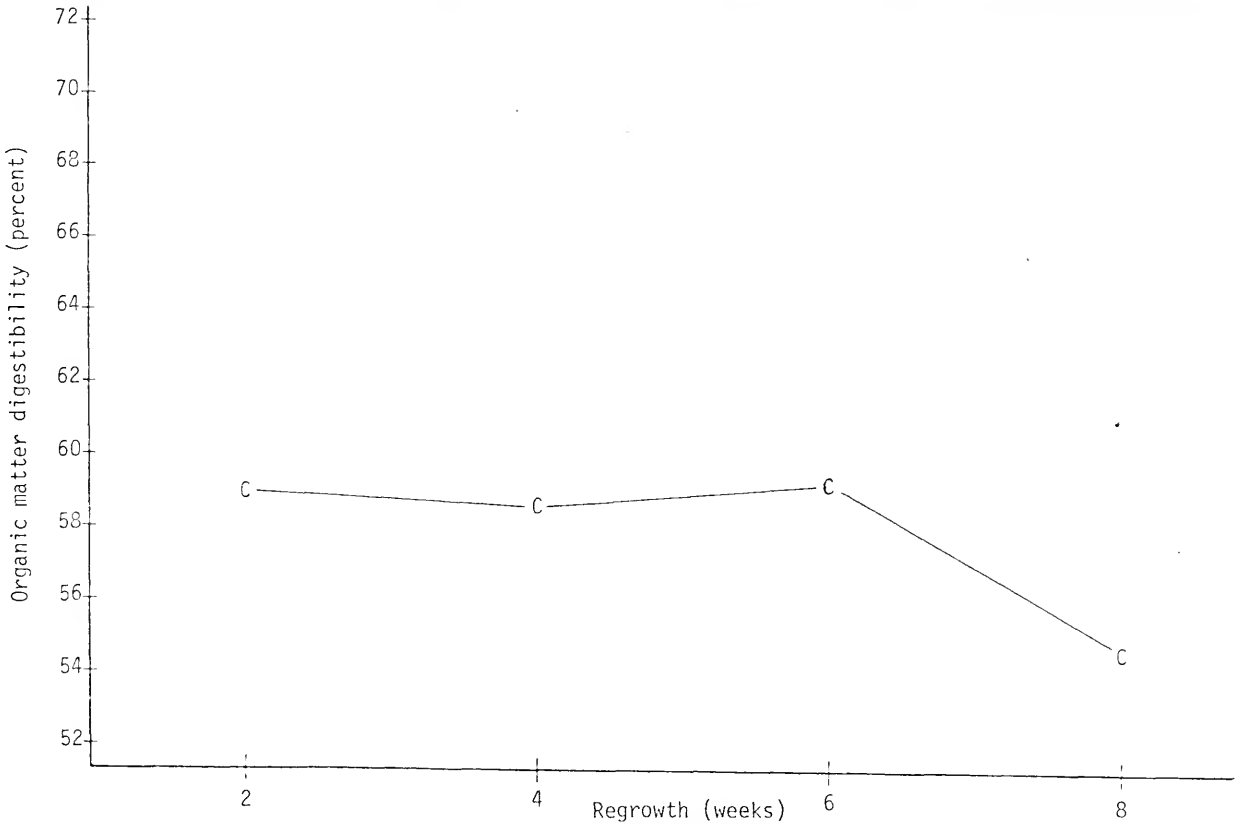

Figure 3. Organic matter digestibility (OMD) of Coastcross-1 bermudagrass versus regrowth.

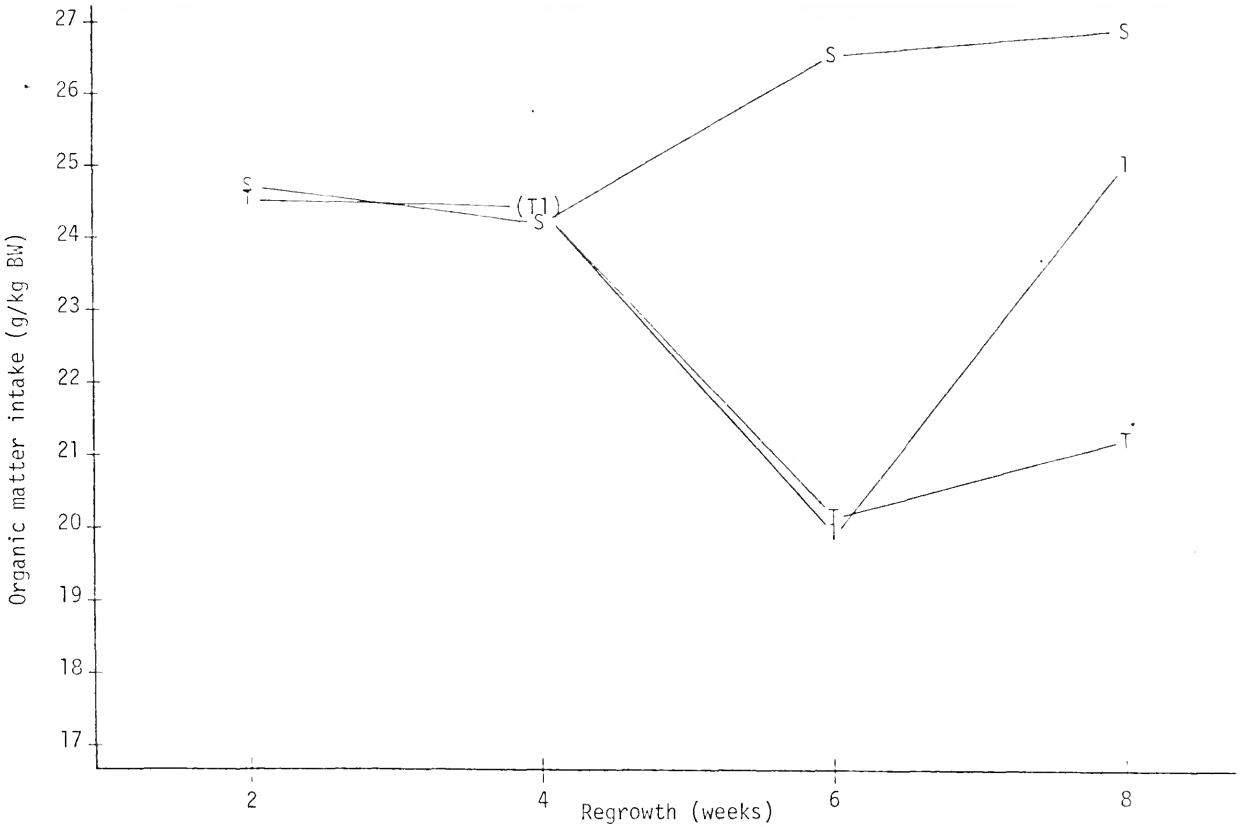

Figure 4. Organic matter intake (OMI1) of digitgrasses from area A versus regrowth. T=Transvala; S=Slenderstem; 1=X50-1.

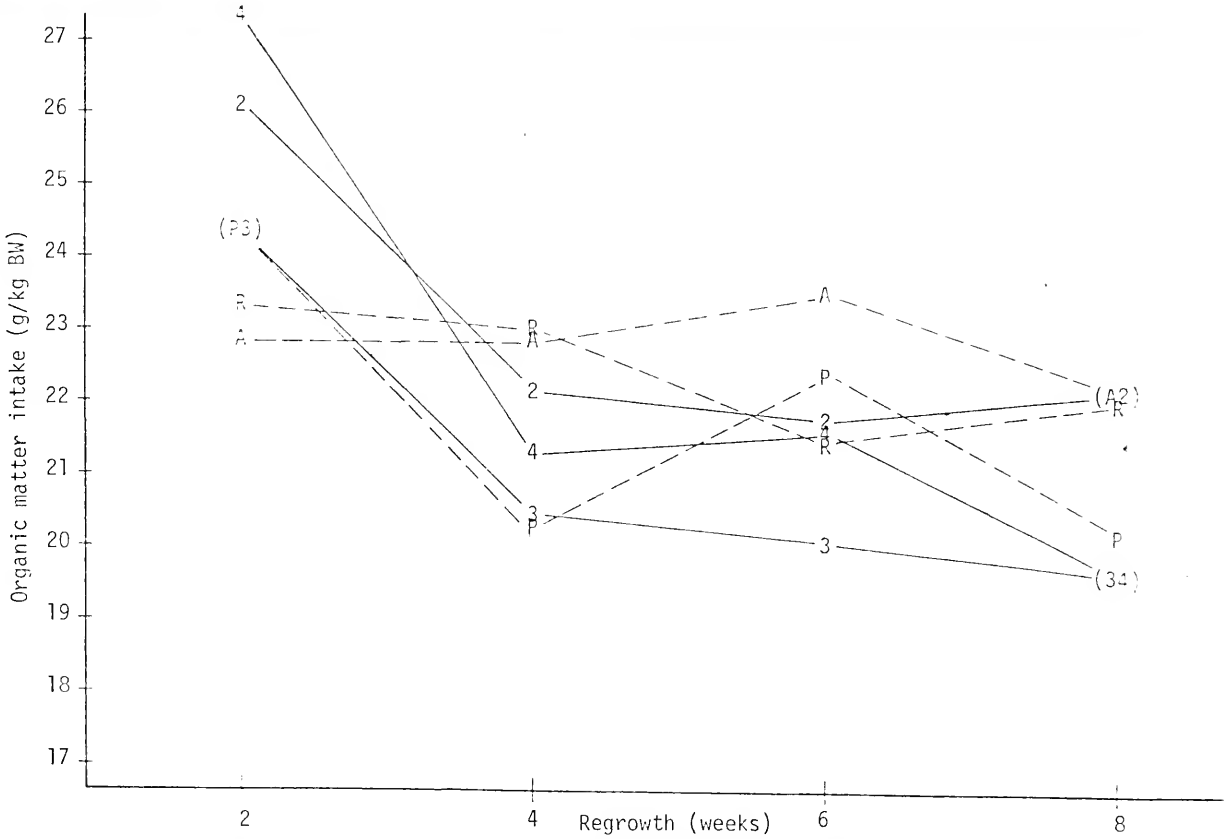

Figure 5. Organic matter intake (OMI1) of digitgrasses (solid lines) and bahiagrasses (broken lines) from area C versus regrowth. 2=X46-2; 3=X215-3; 4=X124-4; A=Argentine; R=Paraguay; P=Pensacola.

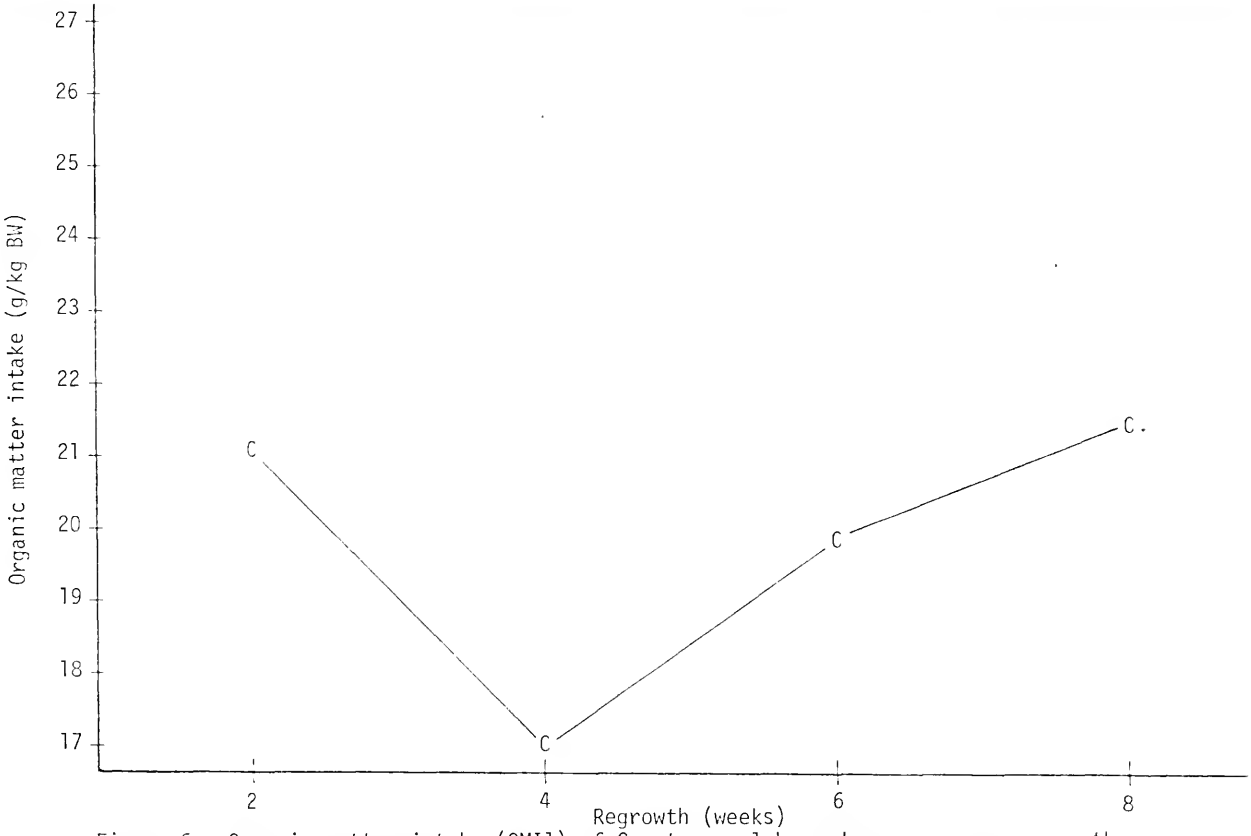

Figure 6. Organic matter intake (OMI1) of Coastcross-1 bermudagrass versus regrowth.

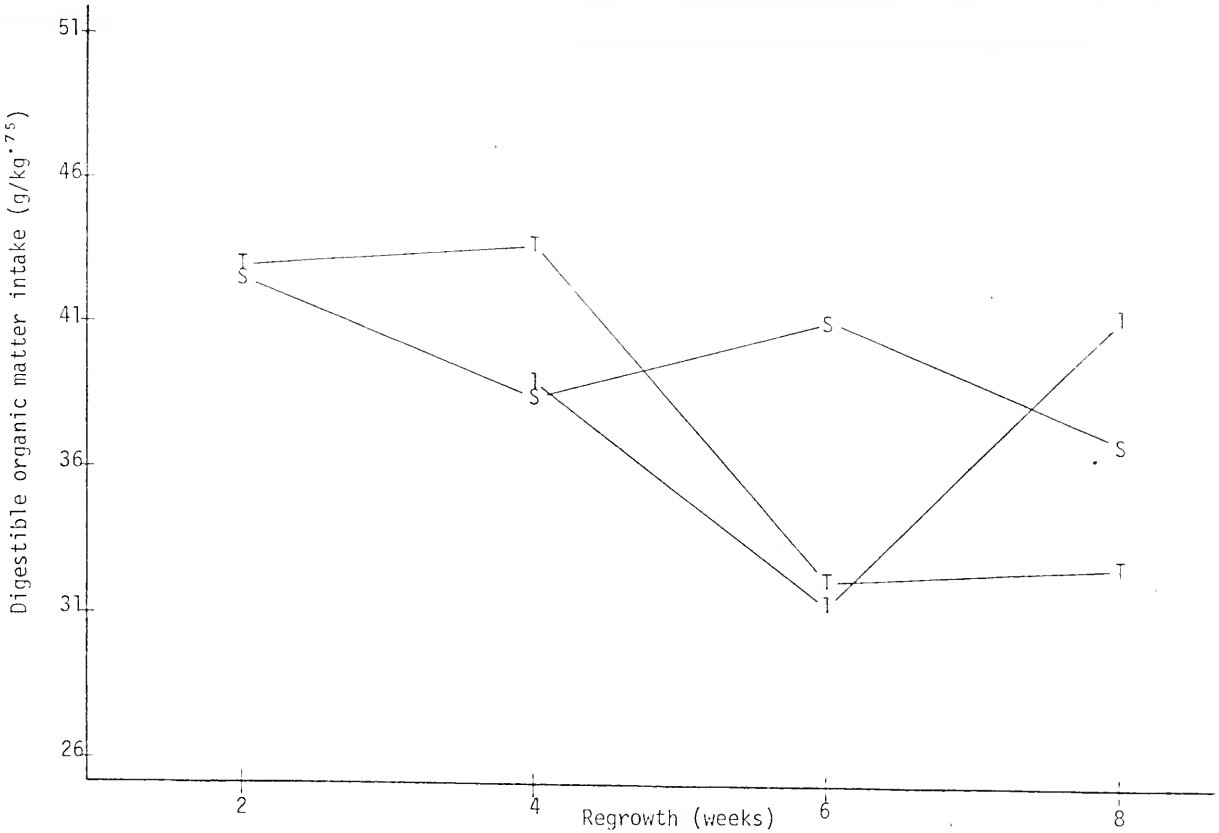

Figure 7. Digestible organic matter intake (DOMI75) of digitgrasses from area A versus regrowth. T=Transvala; S=Slenderstem; 1=X50-1

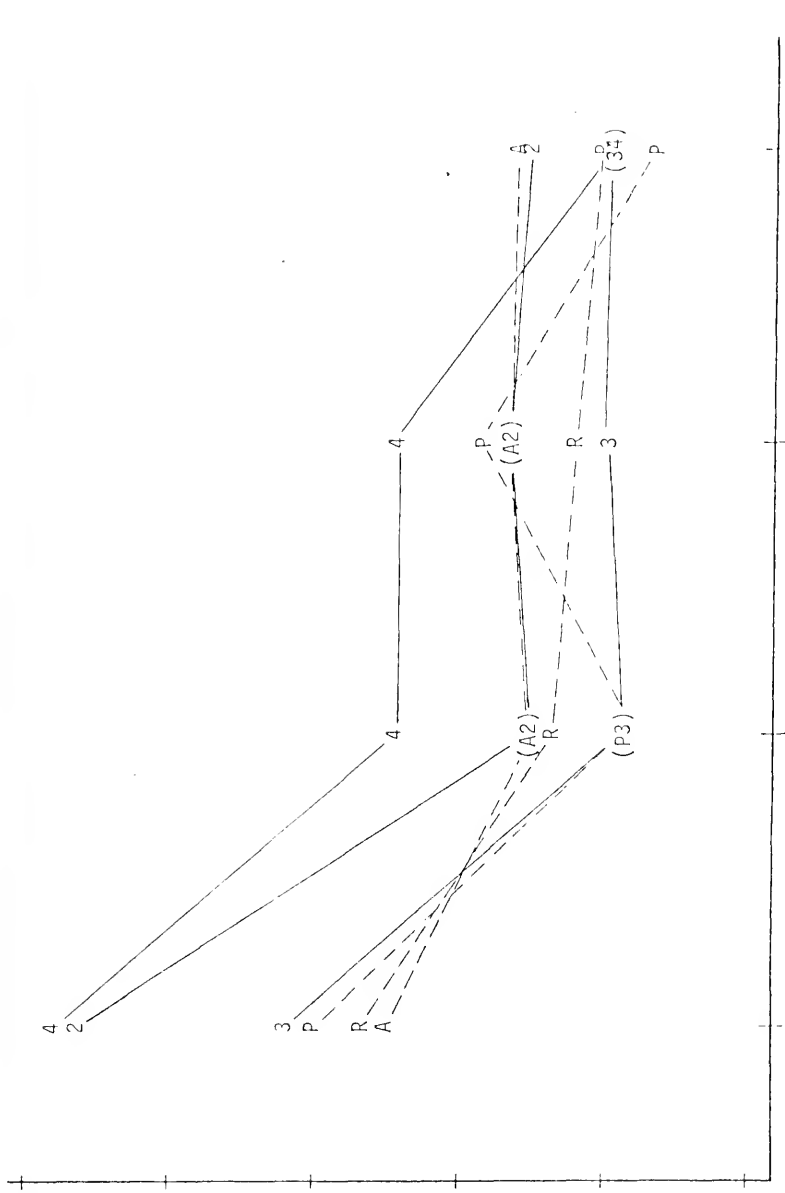

Figure 8. Digestible organic matter intake (DOMI75) of digitgrasses (solid lines) and bahiagrasses (broken lines) from area C versus regrowth. 2=X46-2; 3=X215-3; 4=X124-4; A=Argentine; R=Paraguay; P=Pensacola.

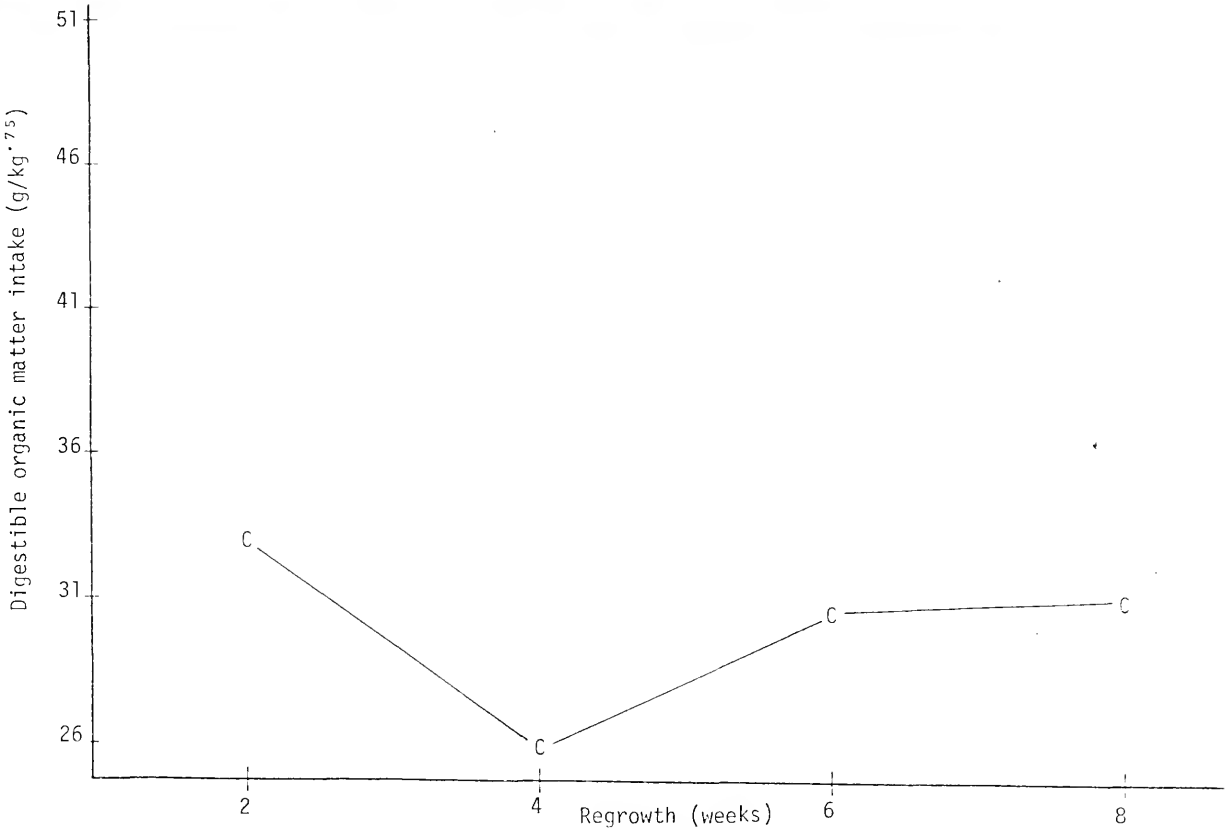

Figure 9. Digestible organic matter intake (DOMI75) of Coastcross-1 bermudagrass versus regrowth.

observed at four weeks. Most cultivars then showed a decline in OMD at eight weeks. In area A there were no drastic shifts in OMD over regrowths, with a gradual decline evident. OMD of the Coastcross-1 was generally constant. OMI1 of the digitgrasses and the Pensacola declined rapidly from the two-week regrowth to the four-week regrowth in Area C, while the OMI1 of the Argentine and Paspalum remained constant. Changes in OMI1 from four to eight weeks were more moderate. In area A, intake of cultivars Transvala and X50-1 declined sharply from four to six weeks of regrowth and then increased at eight weeks, while Slenderstem showed increases in OMI1 over these regrowths. OMI1 of Coastcross-1 in area D declined from two to four weeks and then increased.

Ventura et al. (1975) observed OMD and DOMI75 in Pangola digitgrass at two weeks regrowth to be 69.7 and 47.8, respectively. The average for the two-week digitgrasses in this study were comparable at 68.9 and 45.5; superior cultivars were X124-4 and X46-2 with OMD's of 70.4 and 72.3, and DOMI75's of 50.2 and 49.3, respectively. Minson (1972) found the OMD and DOMI75 of Pangola to be 68.9 and 33.8, respectively, for four-week old material. At this stage of regrowth, digitgrasses in area C were inferior in OMD and about equivalent in DOMI75 to the values Minson observed, while the digitgrasses in area A were superior in DOMI75. Again, insect damage was more severe in area C than in area A, and digitgrasses were damaged more than bahiagrasses.

Moore et al. (1970) determined OMD and DOMI75 on four-week old regrowths of Pensacola bahiagrass to be 61.6 and 28.8, respectively. The four-week Pensacola in this study had an OMD of 58.0 and DOMI75 of 30.9. Four-week regrowths of Coastal bermudagrass studied by Grieve and Osbourn (1965) had dry matter digestibility of 64.8 and digestible dry matter intake per

metabolic weight of 42.4 compared to OMD of 58.1 and DOMI75 of 26.4 for the four-week Coastcross-1 in this experiment.

Interrelationships among animal measurements. The relationship between various measures of intake and digestibility were examined with a correlation procedure (table 7). NDFD was highly correlated with OMD ($r = .96$), and DNDFI1 with DOMI1 ($r = .95$). NDFI1 was also highly correlated with OMI1 ($r = .94$). These correlations suggest the importance of cell wall intake and digestibility in determining forage quality. Intake and digestibility as separate functions were not well related, the highest being that between OMD and OMI1 ($r = .47$). This is in agreement with other studies that have used several forage species. Minson (1972) demonstrated that while within-species correlations between intake and digestibility may often be has high as .96, correlations across several species will be lower. In the research presented here, however, within-genus and within-species correlations were also low. The average correlation between OMI1 and OMD, within genera was .42, and within species, .48.

TABLE 7. CORRELATION MATRIX OF INTAKE AND DIGESTIBILITY

Item[a]	OMD	DOM	OMI1	DOMI75	NDFD	DNDF	NDFI1
DOM	.96						
OMI1	.48	.49					
DOMI75	.77	.75	.92				
NDFD	.96	.90	.41	.70			
DNDF	.71	.73	.24	.48	.81		
NDFI1	.36	.38	.94	.82	.33	.35	
DNDFI1	.73	.72	.88	.95	.74	.65	.88

[a]Explanation of abbreviations in table 1.

Summary

Six cultivars of digitgrass (Digitaria decumbens), three cultivars of bahiagrass (Paspalum notatum) and one cultivar of bermudagrass (Cynodon dactylon) were harvested at different stages of regrowth (two to eight weeks), artificially dried, and fed ad libitum to sheep in a digestibility and intake trial. Animal variation in organic matter intake (OMI) and neutral detergent fiber intake (NDFI) was best removed by powers of body weight that were closer to 1.0 than to .75. Organic matter digestibility (OMD) varied from 57.8 to 72.3, 54.1 to 64.9, and 55.2 to 59.7 percent for the three genera, respectively. Digitgrasses grown in the same area as the bahiagrasses were superior in OMD ($P<.01$), with a mean digestibility of 63.4 versus 58.7 percent. Digestible organic matter intake per metabolic weight ($g/kg^{.75}$) for the three genera varied from 30.9 to 50.2, 29.6 to 41.3, and 26.4 to 33.6, respectively. No consistent regrowth effects, either within genera or within species, were noted. This may have been because there was severe insect damage to some of the grasses between the two and four-week cuttings. The experimental digitgrass X124-4 and 'Slenderstem' digitgrass maintained higher digestible organic matter intakes across all regrowths. The overall correlation between organic matter intake and digestibility of these grasses was .47, which was not improved when considered within genera ($r = .42$) or within species ($r = .48$). Digestibilities of organic matter and neutral detergent fiber were closely related ($r = .96$) as were OMI and NDFI ($r = .94$).

CHAPTER IV
ESTIMATION OF CELL WALL DIGESTION RATES AND POTENTIALLY DIGESTIBLE AND INDIGESTIBLE CELL WALL IN FORAGE AND FECES BY *IN VITRO* AND *IN SITU* DIGESTION-- EXPERIMENT 2

Introduction

Several recent efforts to improve the prediction of forage quality have involved the development of models that describe rumen digesta disappearance (Waldo *et al.*, 1972; Mertens, 1973; Baldwin *et al.*, 1977). These models presuppose the existence of a potentially digestible fraction, capable of complete digestion given sufficient fermentation time, and an indigestible fraction which undergoes no digestion, regardless of the duration of fermentation. The concept of these fractions originates from the research of Blaxter *et al.* (1956) and Wilkins (1969), both of whom showed a ceiling of digestion that was below 100%, the latter author employing long term *in vitro* digestions. Goto and Minson (1977) had difficulty in quantifying these fractions *in vitro*. Reinoculation of forage samples after four days incubation resulted in a 10.2% increase in dry matter digestibility and up to a 28% increase in lignin solubility. These authors suggested that 21 days was the minmum incubation time. *In vitro* derived estimates of lag times, rate constants and digestible and indigestible pools have been employed to estimate the corresponding *in vivo* values (Mertens, 1973; Golding, 1976). Such use assumes that *in vitro* values are approximately equal to, or highly correlated, with *in vivo* values.

The purpose of this experiment was threefold: (1) to determine the relationship of *in vitro* estimates of lag times, rate constants and potentially digestible and indigestible fractions to estimates obtained *in situ*;

(2) to determine in which of these parameters two hays, of the same species but differing in quality, differed; and (3) to determine if the indigestible fraction is fully recoverable in the feces.

Materials and Methods

Digestion and intake trial. Two bermudagrass (Cynodon dactylon) hays, one designated high quality (HQ) and the other low quality (LQ), were fed to mature wethers. The wethers were subjected to a fourteen day preliminary period during which they were acclimated to metabolism crates and hays, followed by a seven day collection period when the amounts of hay offered, refused (orts) and wasted, and the amount of feces excreted were measured. The hays were fed ad libitum, adjusting the hay offered to provide approximately 200 g of orts daily. Water, calcium phosphate and trace mineralized salt were available at all times. Feces were collected in canvas bags. The waste, orts and 20 percent of the feces were dried at 50 C for a minimum of two days. Samples were pooled by animal, ground to pass through a 4 mm screen and approximately 200 g ground through a 1 mm screen for subsequent analysis.

Rate study. The HQ and LQ hays were fed to two fistulated steers in a switchback design. Samples of hay, orts and feces from the above digestion trial were composited by hay, across animals, using amounts proportional to that obtained from individual animals. Samples of both hays were incubated in vitro and in situ, two replications per cell. Orts and feces from sheep fed the LQ hay (LQ orts and LQ feces) were incubated only when the steers were consuming LQ hay. Similarly, orts and feces from sheep fed the HQ hay (HQ orts and HQ feces) were incubated only when the steers were consuming the HQ hay. Residues were recovered after 2, 4, 6, 10, 15, 21, 27, 33, 39, 45, 51, 57, 69, 84, 100 and 129 hours of incubation, both in vitro and in situ.

Acropor (A-5000)[1] copolymer material, with a porosity of 5 microns, was used in the in situ study. Bags, 12.5 cm long with a diameter of 2.5 cm, were fabricated by sealing the seam and one end with epoxy glue. One half gram of sample was placed in each bag, and the open end sealed with epoxy. Four of these bags, consisting of samples of both hays, and the orts and feces representing the hay which the steer was consuming, were placed in nylon utility net bags[2], 17 x 16 cm, with a mesh size of .3 cm. The addition of three marbles (1.5 cm diameter) to each bag gave them the approximate density of water. Sixteen of these bags were attached to a doubled nylon monofilament (36.3 kg test) and placed in the rumen of each steer. To insert the bags in the rumen it was necessary to remove some digesta, but it was replaced when the bags were in place. At each recovery time, one net bag (with Acropore bags) was removed, washed with cold running water, placed in ice and taken to the laboratory. Acropor bags were removed from the net bags, thoroughly washed with cold running water, and refrigerated until analysis, which was carried out within 40 hours of removal from the rumen.

Within one week after the last in situ sample was removed, rumen fluid was collected for the in vitro phase of the study. The fluid was strained through cheesecloth and glass wool, and combined with a buffer solution (McDougall's Saliva), one part rumen fluid to four parts buffer. Samples of hay, orts and feces, .5 g each, were innoculated in 100 ml polyethylene centrifuge tubes with 50 ml of inoculum. The tubes were flushed with CO_2, capped with rubber stoppers fitted with Bunsen valves, and incubated at

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[1]Gelman Instrument Company, Ann Arbor, MI

[2]Sterling Marine Products, Montclair, NJ

39 C, swirling the tubes three times daily. At each recovery, centrifuge tubes were placed in an ice water bath and refrigerated until analysis which was carried out within 40 hours.

Chemical analyses. Dry matter was determined at 105 C for 24 hours and organic matter at 500 C for a minimum of six hours. Protein was determined by Kjeldahl (AOAC, 1970). Acropor bags were opened at both ends and the residue washed into 600 ml Berzelius beakers with 100 ml of neutral detergent fiber solution. Centrifuge tubes were emptied into 600 ml Berzelius beakers and rinsed with 100 ml neutral detergent solution into the beakers. Ash-free neutral detergent fiber (NDF) as a percent of dry matter was determined by a modification of the technique of Goering and Van Soest (1970) in which the decahydronapthalene and sodium sulfite were omitted, and residues were filtered through glass wool in porcelain gooch crucibles.

Statistical analysis and estimation of model parameters. Initial analysis of NDF residue, as a function of time and the discrete variables in the statistical model, was accomplished by least squares analysis (Snedecor and Cochran, 1967), using the GLM procedure of SAS (SAS Institute, 1976). TYPE-1 sums of squares were used for polynomial effects and TYPE-3 sums of squares for all other effects. NDF residue was then fitted, one run at a time,to three models, utilizing the NLIN procedure of SAS:

$$\text{NDF residue} = De^{-k_1(t-L)} + U \quad (\text{for } t>L) \qquad (1)$$

$$= D + U \quad (\text{for } t \leq L)$$

$$\text{NDF residue} = D_1e^{-k_{11}(t-L)} + D_2e^{-k_{12}(t-L)} + U \quad (\text{for } t>L) \qquad (2)$$

$$= D_1 + D_2 + U \quad (\text{for } t \leq L)$$

$$\text{NDF residue} = (-D/(k_{13}-k_{14})(k_{14}e^{-k_{13}t} - k_{13}e^{-k_{14}t}) + U \qquad (3)$$

D, D_1 and D_2 are potentially digestible pools, expressed as percent of NDF.

U is the indigestible pool, t is time (hr), L is lag time (hr) and e is the base of the natural logarithm. Rate constants for digestion (hr^{-1}), are k_1, k_{11}, k_{12}, k_{13}, and k_{14}. Model (1) is a lag time model, model (2) assumes two separate potentially digestible pools (a fast and a slow digesting pool, D_1 or D_2), each with its own rate of digestion (k_{11} or k_{12}), and model (3) is a sequential model that assumes NDF disappearance occurs in two phases, disappearing at k_{13} and k_{14}, respectively. Estimates of model parameters were analyzed by least squares analysis using the original statistical model, but with time omitted.

Results and Discussion

Digestion and intake trial. Results of the digestion trial are presented in table 8 and analysis of variance in appendix table 18. The HQ hay had a higher OMD and DOMI75 than did the LQ hay ($P<.001$), and a higher OMI1 ($P<.01$). It was also lower in NDF and higher in CP.

Comparison of hays and methods of digestion. Analysis of variance of NDF residue as a function of time is presented in appendix table 19. A quartic time effect was present ($P<.01$). Method of digestion (*in vitro* vs. *in situ*) as a main effect was not significant, but all interactions with method were ($P<.001$). These interactions are illustrated in figure 10. With increasing time of digestion the difference in NDF residue between the HQ and LQ hays widened, from a 6% difference at zero time to an 11% difference at 129 hours. A larger difference between the two methods was observed in the LQ hay than in the HQ hay. The method x time interaction was evidenced by the *in vitro* method yielding less NDF residue than the *in situ* method prior to 10 hours. After 10 hours, greater digestion was observed with the *in situ* method, and this difference increased with increasing duration of digestion. Wheeler *et al.* (1979) observed greater digestion at 72 hours *in situ* than *in vitro*. The higher rate of *in vitro*

digestion during the early hours of fermentation might be due to the more immediate contact of sample and inoculum that occurs in the centrifuge tube, compared to Acropor bags where the small porosity might restrict liquid flow into the bags. It was also possible that the dilution of the NDF solution with 50 ml inoculum during analysis of the *in vitro* samples might affect NDF values, although intuitively this should increase rather than decrease NDF. Mertens (personal communication) suggested that

TABLE 8. IN VIVO AND CHEMICAL CHARACTERISTICS OF HQ AND LQ HAYS[a]

Hay	OMD[a]	OMI1	DOMI75	NDF	CP
HQ	54.3	24.7	36.7	73.2	10.5
LQ	44.3	22.2	25.4	78.1	8.3

[a]Explanation of abbreviations in table 1.

the initial rapid rate of *in vitro* fermentation may be due to analytical technique. An experiment to examine this is described at the end of this chapter.

Differences in hays and method observed in the initial analysis of variance are not readily resolvable into biologically meaningful comparisons. The conversion of the data to expressions of model parameters affords a better method of comparison and provides estimates that can be introduced into models of rumen digesta disappearance. When NDF residue was fitted to models (2) and (3), estimates of the rate constant for the fast digesting pool (k_{11}), and the initial rate constant (k_{12}), respectively, nearly always gave confidence intervals that included zero. For several runs the NLIN procedure was unable to solve the equations. Therefore, these two models were discarded.

Estimates of the rate parameters (for model 1) are given in table 9 and selected contrasts in table 10. For the hays only, which were in a cross-

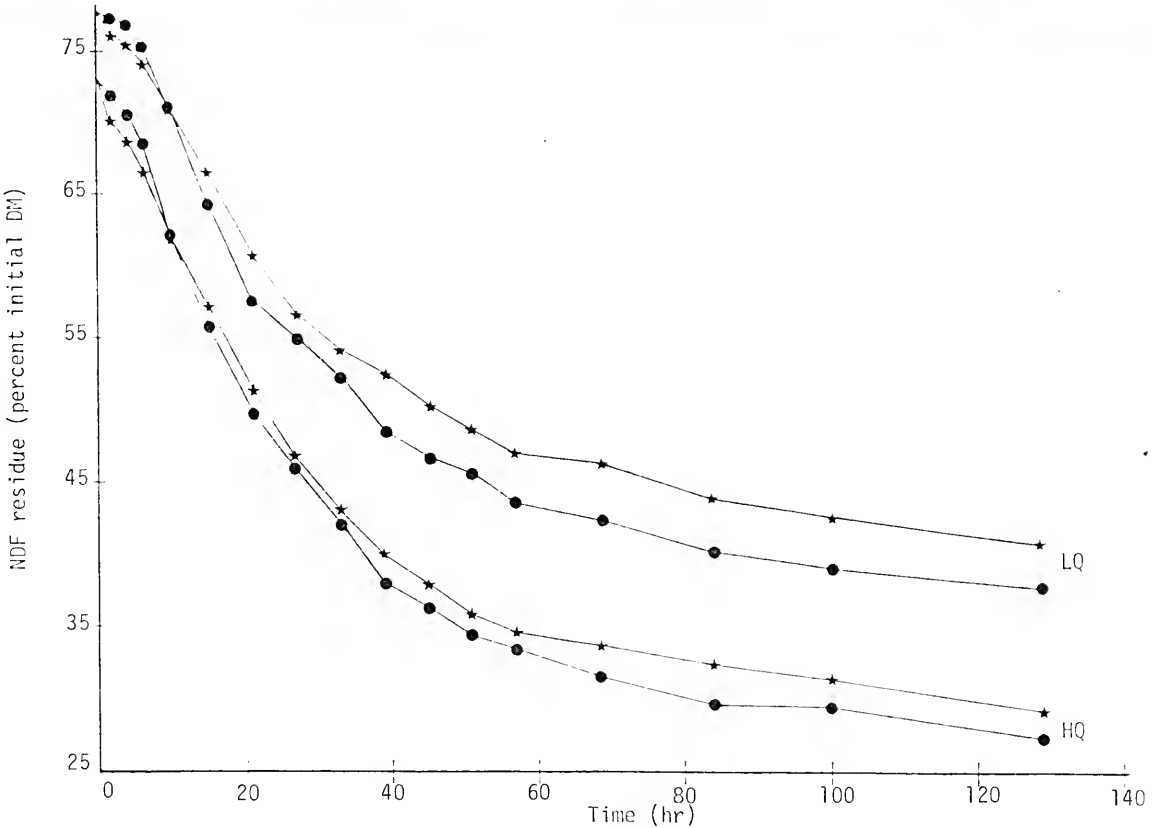


Figure 10. In vitro (stars) and in situ (circles) disappearance of NDF of HQ and LQ hays.

TABLE 9. RATE PARAMETERS OF HAY, ORTS AND FECES[a]

Item	In situ					In vitro				
	D (%DM)	D (%NDF)	U (%DM)	k_1	L	D (%DM)	D (%NDF)	U (%DM)	k_1	L
Hays										
LQ	38.8	50.2	38.5	.0413	5.1	34.6	45.6	41.3	.0344	5.4
HQ	43.8	61.3	27.6	.0409	4.5	39.6	57.0	29.9	.0387	4.7
Orts										
LQ	38.5	50.2	38.2	.0360	5.6	32.6	43.9	41.6	.0370	5.2
HQ	40.1	57.4	29.8	.0403	3.8	37.7	54.6	31.3	.0370	4.7
Feces										
LQ	17.5	22.8	59.0	.0179	9.6	11.5	15.5	62.6	.0229	17.3
HQ	23.6	34.1	46.3	.0170	7.3	15.3	22.1	53.8	.0309	16.5

[a]Explanation of abbreviations in table 1.

TABLE 10. SELECTED CONTRASTS

Contrast	Parameter[a]				
	D (%DM)	D (%NDF)	U (%DM)	k_1	L
HQ vs. LQ hays	***	***	***	ns	ns
In vitro vs. in situ for hays	***	***	**	ns	ns
HQ hay vs. HQ orts[b]	***	***	**	ns	ns
LQ hay vs. LQ orts	ns	ns	ns	ns	ns
HQ hay vs. HQ feces[b]	***	***	***	***	***
LQ hay and LQ orts vs. LQ feces	***	***	***	***	*

[a]Explanation of abbreviations and symbols in table 1.

[b]Not orthogonal.

over design with steer diet, least squares analysis of variance is provided in table 20. All differences in hays and method occurred in the estimates of the potentially digestible (D) and indigestible (U) cell wall pools, both of which were different for the hays and the two methods. No differences were detectable in the digestion rate constant or the estimates of lag time, the latter being highly variable from run to run (CV = 19.0%). The HQ hay had a higher potentially digestible fraction and a lower indigestible fraction than did the LQ hay, whether expressed as a percent of dry matter or percent of NDF. The in situ method provided higher estimates of the potentially digestible fraction and lower estimates of the indigestible fraction than did the in vitro method. Estimates of NDF residue at 129 hours were always greater than actual values, indicating that simple first order kinetics, using a single digestible pool, may not adequately describe digestion kinetics. This effect was also observed by Mertens (1973) in vitro. Disappearance of NDF from 84 to 129 hours was approximately linear, rather than approaching an asymptote (figure 10).

Comparison of hays, orts and feces. Analysis of variance of NDF residue as a function of time by steer diet is provided in appendix table 21. The analysis of variance for parameter estimates is given in appendix tables 22 and 23. No difference between the LQ hay and LQ orts were observed (tables 9 and 10). Pool estimates of the potentially digestible fraction were higher for the HQ hay than for the HQ orts, even though the HQ orts contained approximately 1% less NDF at zero time (figure 11). Thus it would be incorrect to conclude that no animal selectivity took place just because the cell wall content of hay and orts were approximately equal.

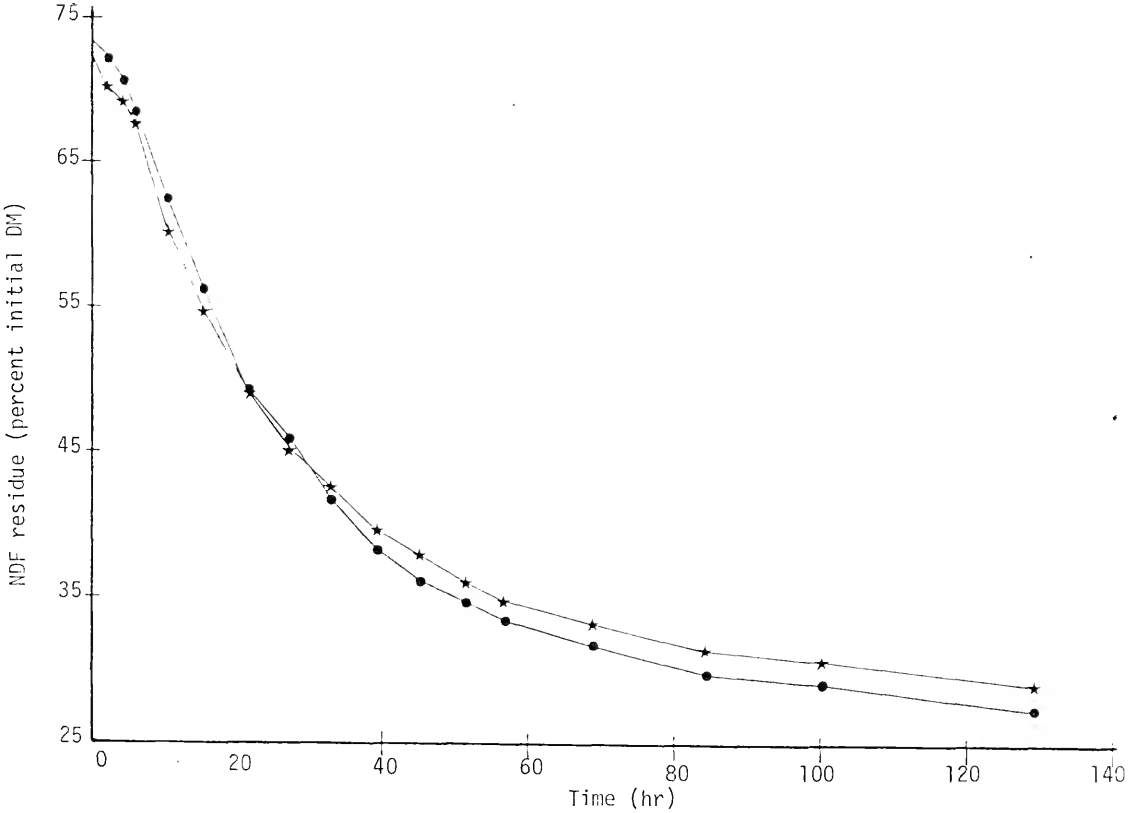


Figure 11. In situ disappearence of NDF of HQ hay (circles) and HQ orts (stars).

Comparison of hay with feces showed significant differences in all parameters (tables 9 and 10). It is expected that fecal material would be higher in indigestible cell wall, and lower in potentially digestible cell wall. However, if the potentially digestible pool is truly of a uniform nature, i.e. a combination of unlignified hemicellulose and cellulose (Minson, 1976), the digestion rate constant should be equivalent with that of the respective hay. Forage microanatomical studies by Akin (Akin and Burdick, 1975; Akin et al., 1974) strongly indicate that more than one potentially digestible pool exist. However, as observed earlier, the resolution of single value data into two rate curves is no easy matter. It demands extensive sampling times and extremely accurate data (Gutfreund, 1972), the latter being difficult when dealing with a chemically heterogeneous substance such as cell walls.

Digestibility of the indigestible fraction. The use of an indigestible fraction makes determination of rate constants tractable. If it satisfies the requirements of an 'ideal' marker (Engelhardt, 1974; Faichney, 1975), it might prove useful in rate of passage studies and in determining digestibility when complete fecal collection is impractical. Berger et al. (1979) used indigestible NDF and ADF as passage markers, determined by 96 hour in vitro digestions followed by NDF and ADF analyses. In this study, application of the estimates of U in hay, orts and feces to the intake and excretion data of the digestion trial resulted in positive digestibilities of U in both hays, irrespective of method of estimation. Using in situ, estimates of U, the digestibilities of U were 18.1 and 14.3 percent for the HQ and LQ hays, respectively; using in vitro estimates, digestibilities were 15.6 and 14.8 percent, respectively.

Effect of method of determination of the NDF content of five hays. An experiment was conducted to determine if analytical factors were the cause of the observed rapid rate of *in vitro* digestion during the first few hours. Five hays were studied, in a 5 x 6 design, with the following treatments:

A. Hay samples placed in centrifuge tubes, the following solutions added, centrifuge tubes immediately iced, and analyzed for NDF:
   1. 50 ml normal inoculum (40 ml buffer + 10 ml rumen fluid)
   2. 40 ml buffer + 10 ml sterilized rumen fluid
   3. 50 ml buffer

B. Hay samples placed in Berzelius beakers, the following solutions added, and analyzed for NDF:
   4. 50 ml buffer
   5. 50 ml deionized water
   6. no solution added (control)

The results of this trial appear in table 11 and analysis of variance in appendix table 24. All samples containing buffer (no.'s 1, 2, 3, 4) showed a drop in NDF compared to the control (no. 6) of approximately 2 percent. The addition of 50 ml deionized water (no. 5) did not have this effect and NDF values were the same as in the control. Evidently, the buffer solution solubilizes, either by itself or in combination with NDF solution, a portion of cell wall that is not solubilized by the NDF solution alone. Therefore, part of the apparent digestion of NDF which took place in the first few hours of *in vitro* digestion (figure 10) was an artifact of the *in vitro* technique.

TABLE 11. EFFECT OF METHOD OF DETERMINATION ON NDF CONTENT OF FIVE HAYS

			Treatment[a]					
			A			B		
Genus	Cultivar	Regrowth	1	2	3	4	5	6
Digit	X46-2	2	63.5	63.7	63.9	64.1	64.9	65.8
Bahia	Argentine	2	72.4	72.4	72.1	72.3	74.9	74.6
Bahia	Paraguay	4	77.8	76.9	76.6	77.0	79.8	79.7
Ditit	Slenderstem	6	68.6	69.3	69.0	67.9	69.8	70.3
Bermuda	Coastcross-1	8	74.2	73.3	74.6	73.9	76.4	76.8
	$\bar{x}$		71.3	71.1	71.2	71.0	73.2[b]	73.4[b]

[a]A=samples initially in centrifuge tubes; B=samples initially in beakers; 1=50 ml normal inoculum; 2=40 ml buffer + 10 ml sterilized rumen fluid; 3=50 ml buffer; 4=50 ml buffer; 5=50 ml deionized water; 6=no solution added (control).

[b]Means in a row with different superscripts are different ($P<.01$).

Summary

Two bermudagrass hays (Cynodon dactylon), one high quality (HQ) and one low quality (LQ), were fed ad libitum to wethers in a digestion and intake trial. Organic matter digestibilities (%) were 54.3 and 44.3, and organic matter intakes per body weight (g/kg) were 25.7 and 22.2, respectively. Samples of hays, orts and feces from this trial were digested in vitro, and in situ (utilizing Acropor bags), in two fistulated steers fed these hays, in a crossover design. Samples were removed at 16 times, ranging from 2 to 129 hours and analyzed for neutral detergent fiber (NDF). NDF residue was fitted to the set of equations: NDF residue = $De^{-k_1(t-L)}+U$ for $t>L$, and NDF residue = D+U for $t\leq L$, where D=potentially digestible cell wall (%), k_1=digestion rate constant (hr^{-1}), t=time (hr), L=lag (hr), and U=indigestible cell wall (%). No differences were detected in k_1 or L, the latter being highly variable between runs. All differences between hays and method of digestion occurred in the estimates of D and U. The HQ hay had a higher fraction of D than did the LQ hay. In situ digestion provided higher estimates of D than did in vitro digestion. Orts from sheep fed the HQ hay had less of fraction D than did the HQ hay, although they were approximately equal in NDF content. Application of the estimates of U in hay, orts and feces to the intake and excretion data of the digestion trial resulted in positive digestibilities of U in both hays, irrespective of method of digestion, with a range of 14.3 to 18.1 percent. In a separate experiment the addition of McDougall's saliva to 100 ml neutral detergent reagent solubilized an additional two percent of forage NDF. Therefore, apparent increased early digestion of NDF in vitro, compared to in situ, was an artifact.

CHAPTER V

EVALUATION OF A MODEL OF RUMEN CELL WALL DISAPPEARANCE-- EXPERIMENT 3

Introduction

Several models of rumen digesta disappearance have been suggested (Baldwin et al., 1977; Mertens and Ely, 1979). Validation of these models has been accomplished by computer simulation, whereby the effect of alteration in the parameters of the model are compared with values found in the literature, since direct estimates of the pool sizes and rate constants have not been possible. Waldo et al. (1972) proposed a model of rumen cellulose disappearance that is of less complexity than the above models. It describes two cellulose fractions, a potentially digestible pool and an indigestible pool, both of which can disappear from the rumen by passage, but only the former is capable of being digested. Mertens (1973) applied this model to rumen cell wall disappearance. He used in vitro estimates of the rate constant for passage by an equation relating cell wall intake to passage. Predicted dry matter intake was correlated with actual dry matter intake ($r = .81$) in a wide variety of 187 forages. Golding (1976) applied the Waldo model to organic matter intake, again using in vitro techniques, and estimated the passage rate constant with an equation relating lignin intake to passage. A correlation of .96 was found between predicted and actual digestible organic matter intake per metabolic weight.

Both Mertens (1973) and Golding (1976) used intake to both predict and validate the Waldo model. Thus, there was an internal correlation that may have lead to spurious conclusions about the validity of this model. The

purpose of this experiment was to evaluate the Waldo model, as applied to cell walls, using a procedure that was independent of the methods used to generate parameter estimates.

Materials and Methods

In vitro rate study. From the 76 hay samples used in experiment 1, 60 were selected (table 12) on the basis of the number of animal observations (>1), coefficient of variation (CV) for organic matter intake (<19) and CV for organic matter digestibility (<10). In each of 5 runs, hay samples were incubated in vitro and NDF residues recovered at 96 hours and at one of the following: 12, 24, 36, 48 or 60 hours. Thus there were five estimates of the 96 hour residue.

Rumen fluid was collected from a fistulated crossbred steer, maintained on bermudagrass hay. One hour prior to obtaining rumen fluid, the steer was fed 600g soybean meal. The rumen fluid was strained through cheesecloth and glass wool, and combined with a buffer solution (McDougall's Saliva), one part rumen fluid to four parts buffer. Samples of hay were inoculated in centrifuge tubes with 50 ml of inoculum. The tubes were flushed with CO_2, capped with rubber stoppers fitted with Bunsen valves, incubated at 39 C, and swirled three times daily. At each recovery, centrifuge tubes were placed in an ice water bath and refrigerated until analysis, which was carried out within 40 hours.

Dry matter was determined at 105 C for 24 hours and organic matter at 500 C for a minimum of six hours. The centrifuge tubes were emptied into 600 ml Berzelius beakers, and rinsed with 100 ml neutral fiber solution into the beakers. Ash-free neutral detergent fiber (NDF), as a percent of initial dry matter, was determined by a modification of the technique of Goering and Van Soest (1970), in which the decahydronapthalene and sodium

sulfite were omitted, and residues were filtered through glass wool in porcelain gooch crucibles.

TABLE 12. IN VIVO AND CHEMICAL CHARACTERISTICS OF HAYS[a]

Genus (# of cultivars)	OMD	OMI1	DOMI75	NDF	CP
Cynodon (1)	54-62	17-24	26-36	68-80	5-14
Digitaria (6)	55-73	19-30	28-57	73-80	6-14
Paspalum (3)	54-67	18-26	26-45	63-76	4-13

[a]Explanation of abbreviations in table 1.

Estimation of digestion rate constant, and potentially digestible and indigestible pools. Examination of the raw data indicated that the first twelve hours of digestion were part of the lag time (L) for many of the samples, and residues collected at this time were not included for parameter estimation. The 96-hour residue was considered the indigestible fraction (U), and ((hay NDF content)-U) considered the potentially digestible fraction (D). Residues at 96-hour were subtracted from the residues at 24, 36, 48 and 60 hours, and subjected to a log transformation for regression prediction of the digestion rate constant (k_1) in hr^{-1} and the zero-time intercept of the regression line (D_i), where time (t) is the dependent variable:

$$\text{NDF residue} = D_i e^{-k_1 t} + U$$

$$(\text{NDF residue}) - U = D_i e^{-k_1 t}$$

$$\ln((\text{NDF residue}) - U) = \ln(D_i) - k_1 t$$

The sum of D_i + U was almost invariably greater than the hay NDF content due to the assumption that digestion is not initiated until the end of the lag time. Lag time was determined as:

$$L = (\ln(D_i) - \ln(D))/k_1$$

Estimation of rumen NDF digestibility. The Waldo model yields two equations, one for intake and one for digestibility. If the digestion rate constant, the potentially digestible pool and the indigestible pool are estimated by the *in vitro* procedure, and a constant rumen fill assumed, these values can be entered into the intake equation, and the equation can be solved for the passage rate constant (k_2).

It is, of course, worthless to re-enter this estimate into the intake equation to evaluate the model since the estimates of intake will be equal to actual intake. However, the parameters can be entered into the equation for rumen NDF digestibility and the resulting estimates for digestibility should, if the model is accurate, yield better estimates for digestibility than do any of the parameters alone.

Two methods for predicting rumen NDF digestibility, depending on how k_2 is estimated, are possible. The first assumes that lag (L) is an artifact of the *in vitro* system and does not occur *in vivo*. In this case cell wall intake (NDFI) is a function of rumen cell wall fill (F), the rate constant for digestion (k_1), the rate constant for passage (k_2), the potentially digestible cell wall as a fraction of total cell wall (D), and the indigestible cell wall as a fraction of total wall (U):

$$NDFI/hr = F/[(D/(k_1+k_2))+(U/k_2)]$$

Dividing both sides by body weight (BW):

$$(NDFI/hr)/BW = (F/BW)/[(D/(k_1+k_2))+(U/k_2)]$$

Assuming F is a constant 16.03 g per kg BW (Mertens, 1973):

$$(NDFI/hr)/BW = 16.03/[(D/(k_1+k_2))+(U/k_2)]$$

Converting intake to a daily basis per kg BW (NDFI1), by multiplying by 24:

$$NDFI1 = 385/[(D/(k_1+k_2))+(U/k_2)]$$

Collecting terms, this then takes the form of the quadratic equation:

$$0 = [385/NDFI1](k_2)^2+[(385k_1/NDFI1)-1](k_2) - Uk_1$$

This equation can be solved for k_2 and entered in the digestibility equation to yield a predicted cell wall digestibility ($NDFDP_1$):

$$NDFDP_1 = D\ (k_1/(k_1+k_2))$$

The second method of estimating NDFD ($NDFDP_2$) is with the assumption that the lag observed in vitro occurs in vivo. If we again assume a constant fill per unit body weight, than daily cell wall intake per kg BW is defined by:

$$NDFI1 - 385/[((D/k_2)(1-e^{-k_2L})) + ((De^{-k_2L})/(k_1+k_2)) + (U/k_2)]$$

This cannot be solved directly for k_2, but a solution can be achieved by Newton's method for approximating the roots of equations (Thomas, 1972). Predicted digestibility $NDFDP_2$ will then be:

$$NDFDP_2 = D[e^{-k_2L}-((k_2e^{-k_2L})/(k_1+k_2))]$$

The two estimates of rumen NDF digestibility should be highly correlated with in vivo NDF digestibility (NDFD), if the model is valid. Regression and correlation (Snedecor and Cochran, 1967) were performed with the computer statistical package, SAS (SAS Institute, 1976).

Results and Discussion

NDF residues (percent of initial dry matter) are presented in appendix table 25 and model parameter estimates in appendix table 26. Model parameter ranges by genera are presented in table 13. Estimates of these parameters fell within the ranges previously found for tropical grasses (Mertens, 1973). The regression equation for estimation of digestion parameters yielded an r^2 of .98 and a CV of 7.4. Digitgrasses had generally lower quantities of indigestible cell wall (U) than the other two genera, when expressed on a dry matter basis. Variation in D, when expressed as a percentage of dry

TABLE 13. MODEL PARAMETER RANGES[a]

Parameter	Genus		
	Cynodon	Digitaria	Paspalum
D (%DM)	42-48	44-57	45-56
D (%NDF)	53-64	62-85	62-77
U (%DM)	25-36	10-28	17-30
k_1	.043-.069	.038-.078	.035-.062
k_{21}[b]	.020-.036	.015-.032	.020-.038
k_{22}[c]	.025-.041	.020-.039	.030-.050
L	5.2-11.2	-1.0-17.4	11.5-19.3

[a]Explanation of abbreviations and symbols in table 1.

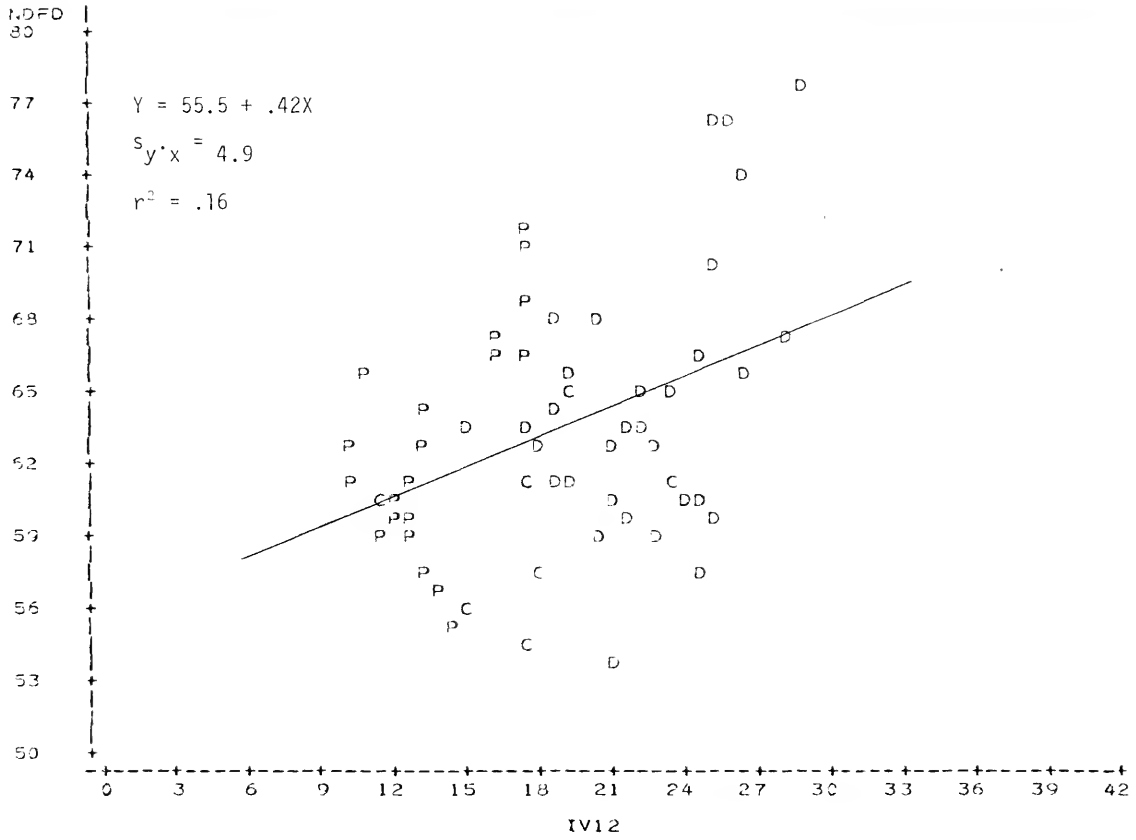
[b]k_{21} derived from model that assumes zero lag (L) in vivo.

[c]k_{22} derived from model that assumes lag (L) in vivo is same as L in vitro.

matter, was less than when expressed as a percentage of NDF, suggesting that the former is a more constant proportion of forage dry matter than U or NDF.

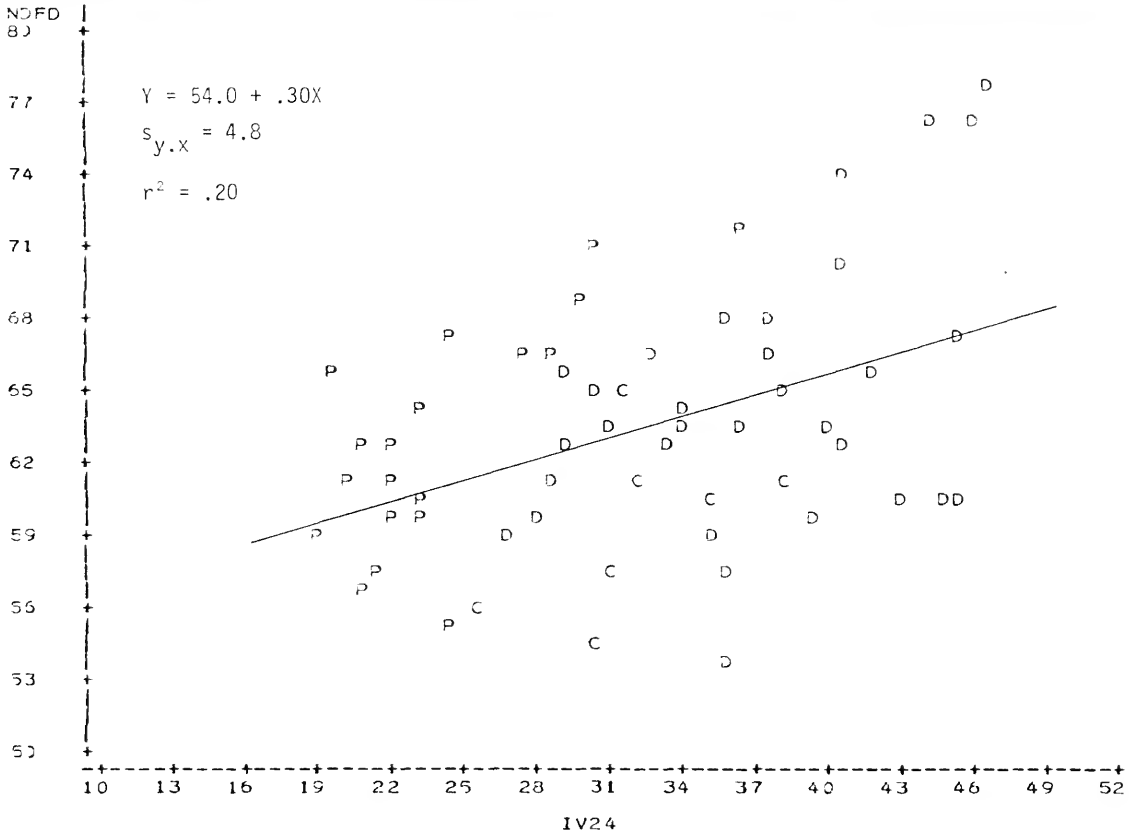
Correlations of predicted NDFD with in vivo NDFD were .75 for $NDFDP_1$ and .34 for $NDFDP_2$. Evidently, the lag contributed random variation to the estimate. Correlation of $NDFDP_1$ was not above what has been reported elsewhere for correlations of in vitro estimates with in vivo digestibilities (Weller,1973; Velasquez, 1974). Since $NDFDP_1$ and $NDFDP_2$ are predictions of rumen cell wall digestibility, the remaining potentially digestible fraction was mathematically subjected to further digestion, simulating digestion and passage in the large intestine, using the same rate constants for digestion and passage that were estimated for the rumen. This improved the correlation with $NDFDP_1$ to .81, but had no effect on the correlation with $NDFDP_2$. It should be observed, however, that as the remaining potentially digestible fraction is subjected to increasing digestion, the limit is its complete digestion, which in this case is the 96-hour in vitro digestibility.

This highest correlation with NDFD was with parameter D, the 96-hour in vitro digestibility. In vitro digestibility at each recovery time was calculated (figures 12 - 17). The coefficient of determination between in vivo and in vitro NDF digestibility increased with increasing fermentation times from .16 at 12 hours to .80 at 96 hours, with a concomitant decline in deviation from the regression lines. A considerable difference existed between the standard 48-hour in vitro digestibility and that at 96 hours, suggesting that 96 hours might provide more accuracy in predicting NDFD in routine forage screening procedures. A possible explanation for this result is that actual retention times of digesta subject to digestion are closer to 96 hours, when the large intestine is included, and that digestion of potentially digestible cell wall approaches completion during



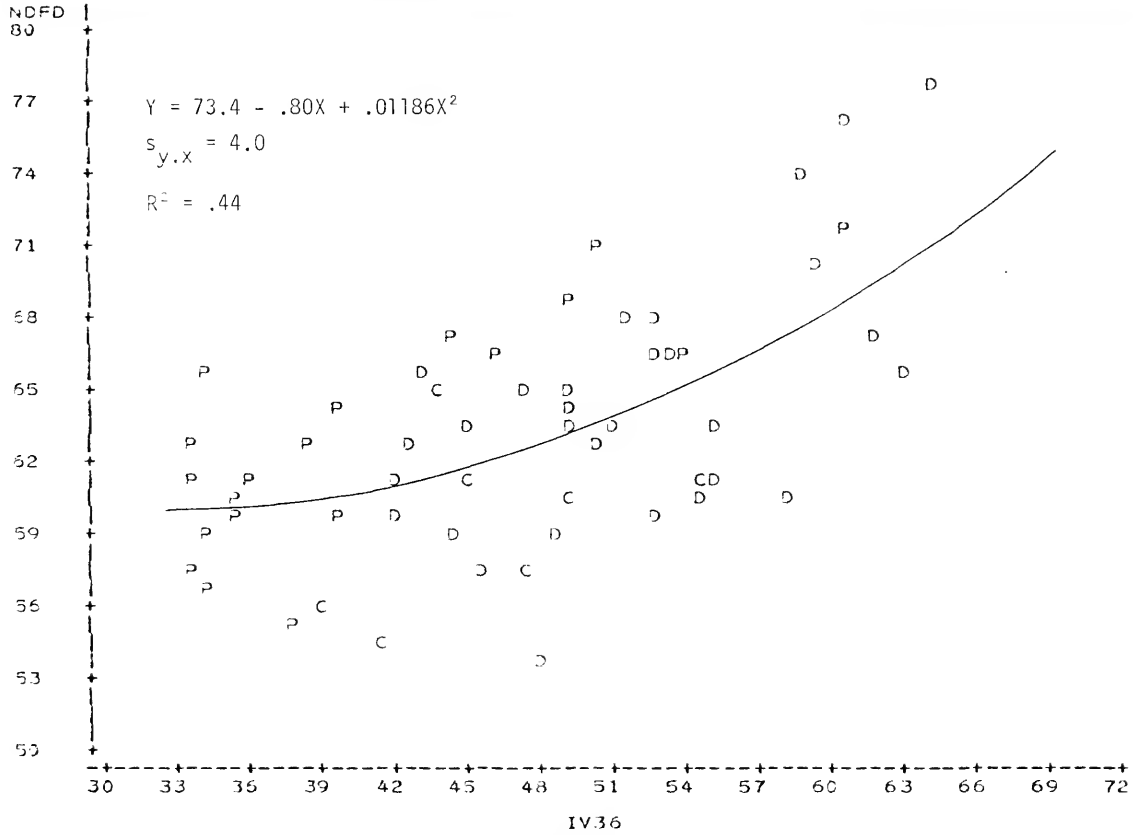
NOTE: 1 OBS HIDDEN

Figure 12. Relationship between in vivo (NDFD) and twelve-hour in vitro (IV12) neutral detergent fiber digestibility (percent). C=Cynodon; D=Digitaria; P=Paspalum.



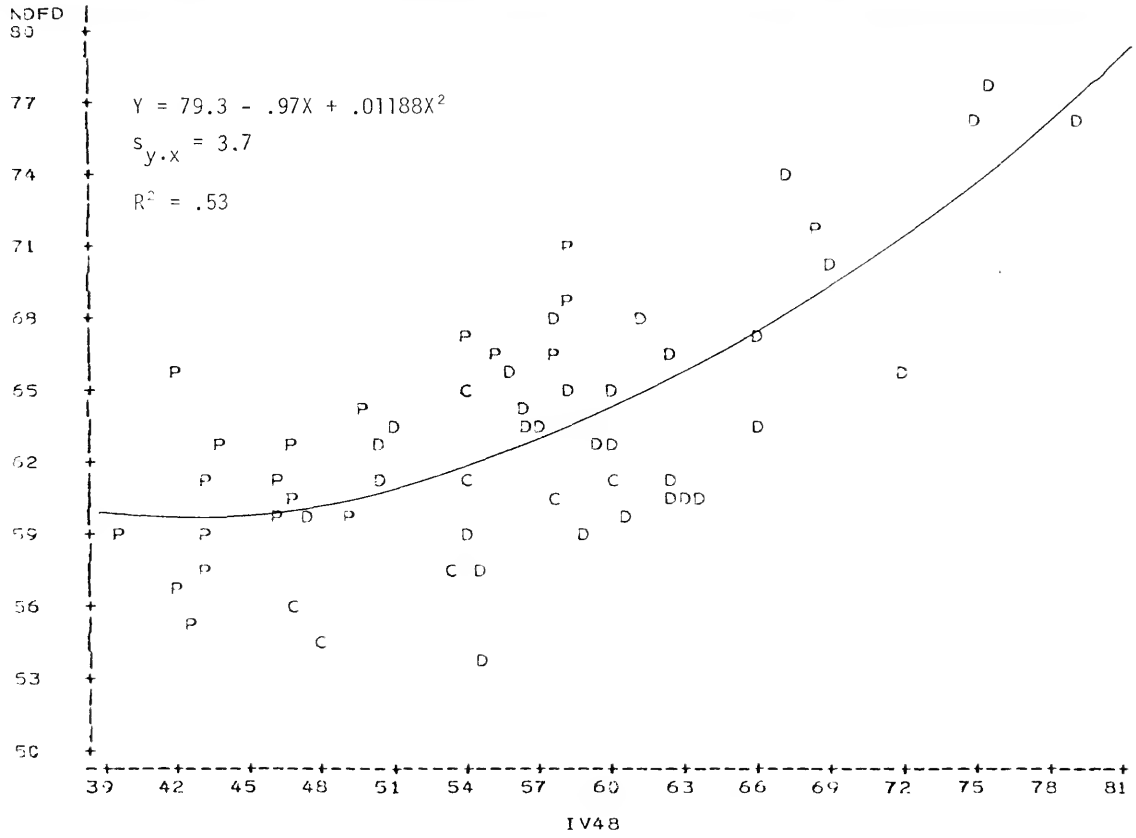
NOTE: 2 OBS HIDDEN

Figure 13. Relationship between in vivo (NDFD) and twenty-four-hour in vitro (IV24) neutral detergent fiber digestibility (percent). C=Cynodon; D=Digitaria; P=Paspalum.



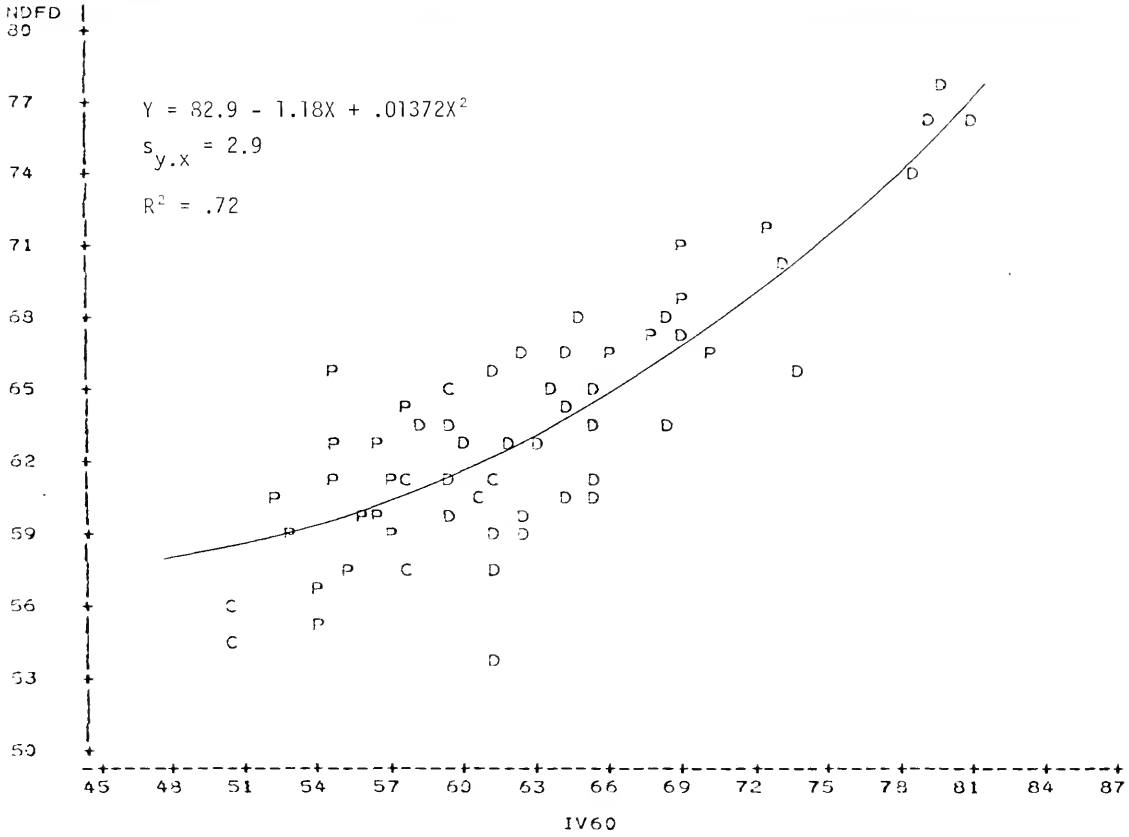
NOTE: 1 OBS HAD MISSING VALUES OR WERE OUT OF RANGE 3 OBS HIDDEN

Figure 14. Relationship between in vivo (NDFD) and thirty-six-hour in vivo (IV12) neutral detergent fiber digestibility (percent). C=Cynodon; D=Digitaria; P=Paspalum.



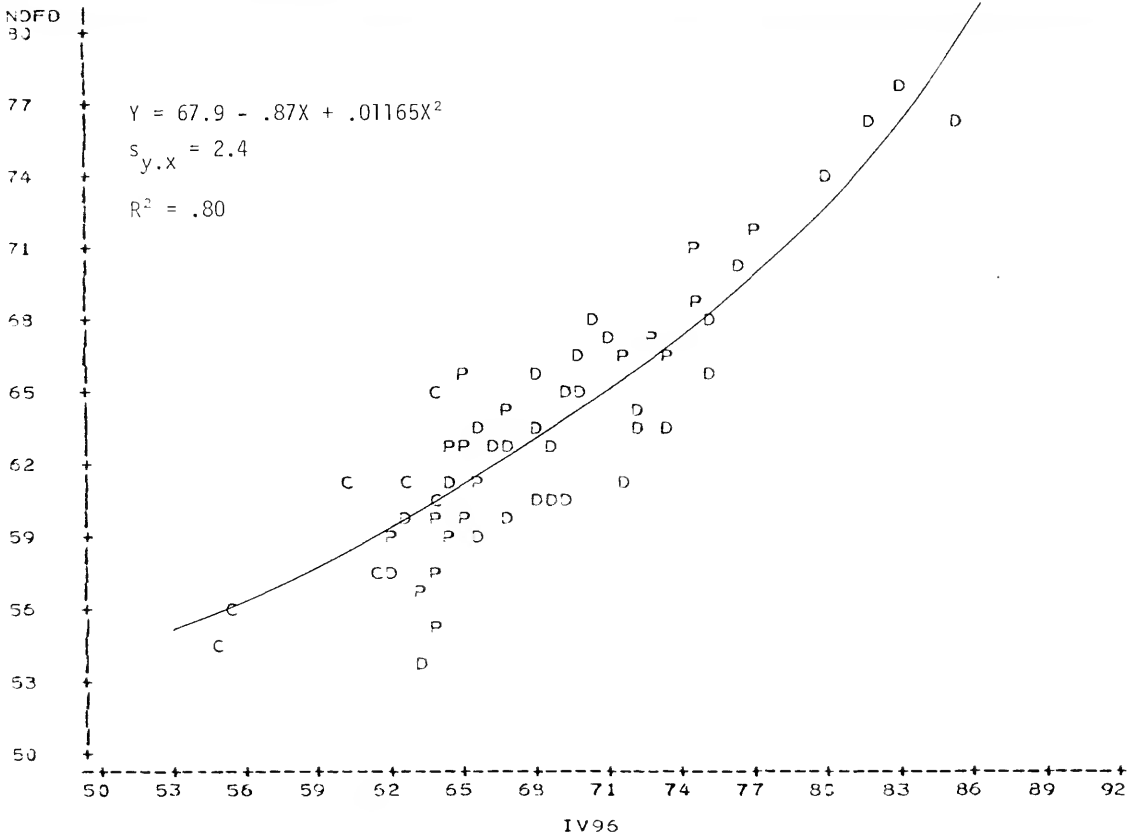
NOTE: 1 OBS HIDDEN

Figure 15. Relationship between in vivo (NDFD) and forty-eight-hour in vitro (IV48) neutral detergent fiber digestibility (percent). C=Cynodon; D=Digitaria; P=Paspalum.



NOTE: 1 OBS HIDDEN

Figure 16. Relationship between in vivo (NDFD) and sixty-hour in vitro (IV60) neutral detergent fiber digestibility (percent). C=Cynodon; D=Digitaria; P=Paspalum.



NOTE: 4 OBS HIDDEN

Figure 17. Relationship between in vivo (NDFD) and ninety-six-hour in vitro (IV96) neutral detergent fiber digestibility (percent). C=Cynodon; D=Digitaria; P=Paspalum.

this time. Correlations of k_1 and L with in vivo measurements were not high (appendix table 27).

Correlations between model parameters and chemical composition (Hartadi, unpublished data) are presented in table 14. Highest correlations were between lignin (as a percent of dry matter or of acid detergent fiber) and the potentially digestible and indigestible pools. This is consistent with the concept that lignin serves as a limit to potential digestibility, rather than an inhibitor of the rate of digestion (Minson, 1976). Lag time was not highly correlated with any chemical component. The correlations of the digestion rate constant with NDF and cellulose (-.71 and -.76) suggest that with increasing amounts of fiber in forages, the character of the potentially digestible fraction changes. This is consistent with the concept that there is more than one potentially digestible pool (Mertens and Ely, 1979), and that a single pool model, while conceptually more desirable than empirical models, is inadequate in describing rumen cell wall disappearance. However, as previously stated, in the absence of the ability to measure rates of digestion on individual pools, the ability to resolve one-component data into two-component models is dependent on strict adherence to first order kinetics. With the degree of biological variation that occurs in the measurement of NDF digestion over time, accurate fitting of a two-component model is not feasible. An additional difficulty in identifying pool sizes and rate constants would occur if rumination tends to free some of the lignin-bound cellulose, thus increasing the potentially digestible fraction.

TABLE 14. CORRELATIONS BETWEEN MODEL PARAMETERS AND CHEMICAL COMPOSITION[a]

Parameter	Chemical component							
	NDF	CP	ADF	CELL	LIG(%ADF)	LIG	HEMI	SIL
D (%DM)	.08	.32	-.07	.27	-.77	-.61	.07	-.24
D (%NDF)	-.56	.56	-.41	.23	-.79	-.79	-.25	.09
U (%DM)	.73	-.60	.48	.37	.71	.77	.34	-.20
k_1	-.71	.53	-.61	-.76	-.23	-.45	-.02	<.01
k_{21}[b]	.70	-.39	.30	.36	.45	.50	.41	-.37
k_{22}[c]	.53	-.18	.03	.18	.03	.06	.55	-.30
L	.34	-.13	-.06	.11	-.27	-.22	.47	-.22

[a]Explanation of abbreviations and symbols in table 1.

[b]k_{21} derived from model that assumes zero lag (L) in vivo.

[c]k_{22} derived from model that assumes lag (L) in vivo is same as L in vitro.

Summary

Sixty hay samples, representing three genera (*Cynodon dactylon*, *Digitaria decumbens* and *Paspalum notatum*) and previously fed to sheep in Experiment 1, were subjected to *in vitro* neutral detergent fiber (NDF) digestion for 12, 34, 36, 48, 60 and 96 hours. NDF residue at 96 hours was assumed to be the indigestible NDF (U), and initial NDF less U was assumed to be the potentially digestible pool (D). The rate constant for digestion (k_1) was determined by the regression equation:

$$\ln((\text{NDF residue})-U) = \ln(D_i)-k_1 t$$

where $\ln(D_i)$ is the zero-time intercept of the regression line and t is time (hr). Lag time (L) was determined as:

$$L = (\ln(D_i)-\ln(D))/k_1$$

The rumen NDF passage rate constant (k_2) was estimated using these parameter estimates, an assumed rumen NDF fill of 16.03 g per kg body weight, *in vivo* NDF intake per kg of body weight (NDFI1) and one or the other of two options: 1) that digestion lag does not occur *in vivo*:

$$0 = [385/\text{NDFI1}](k_2)^2+[(385k_1/\text{NDFI1})-1](k_2)-Uk_1$$

which is in the form of a quadratic equation; or

2) that digestion lag does occur *in vivo*, in which case:

$$\text{NDFI1} = 385/[((D/k_2)(1-e^{-k_2L}))+((De^{-k_2L})/(k_1+k_2))+(U/k_2)]$$

which was solved for k_2 by means of an iterative method for finding the roots of equations. Predicted rumen NDF digestibilities are then defined as:

1) $\text{NDFDP}_1 = D(k_1/(k_1+k_2)$

2) $\text{NDFDP}_2 = D[e^{-k_2L}-((k_2e^{-k_2L})/(k_1+k_2))]$

Correlations (r) of NDFDP_1 and NDFDP_2 with *in vivo* NDF digestibility were

.75 and .34, respectively. In vivo NDF digestibility was more closely related to D (r = .89), which is the 96-hour in vitro NDF digestibility, than to $NDFDP_1$, $NDFDP_2$, or any of the other model paratmeters. The coefficient of determination between in vivo and in vitro NDF digestibility increased with increasing fermentation times, from .16 at 12 hours to .80 at 96 hours.

CHAPTER VI
GENERAL DISCUSSION

The experiments described in chapters IV and V were aimed at validating the Waldo model as applied to rumen cell wall disappearance. An additional goal was to provide information as to future directions in experimentation and modeling in the area of methodology for the prediction of forage quality. Some general observations follow.

Precise identification of the indigestible pool (U) was not possible, as evidenced by the observed positive digestibilities of this fraction of cell wall. To date, lenghty *in situ* or *in vitro* fermentations have been the only method of estimating U. If this fraction exists *in vivo*, a preferable method of quantification would be by chemical techniques. This may be precluded, however, by the possibility that the indigestible pool consists of several compounds which occur in different quantitites in different forages, or that the size of this pool varies with particle size. If no such fraction exists, i.e. there is some digestion of all components of cell wall, models that incorprate this fraction will be faulted and subject to error.

Parameter estimates were different for feces than for hays. This is to be expected in the estimates of pool sizes, but if the potentially digestible pool is truly uniform with respect to its digestion properties, the rate constant for digestion of hay and feces should be approximately the same. Thus, the fraction of feces amenable to further digestion is not of the same chemical and/or physical nature as that measured in the hays.

A study with two bermudagrass hays indicated that there was no difference in the digestion rate constant between hays, or between *in vitro* and *in situ*

digestion. This may have been due to the fact that only two hays, both of the same species, were used. When in vitro derived digestion rate constants were measured on a set of 60 hays, considerable variation occurred in this parameter. Correlations of this parameter with in vivo measurements of intake and digestibility were not high.

No positive relationship between lag time and in vivo measurements was observed, either directly, or indirectly through model solutions. This suggests that in vivo digestion lag does not exist (a possibility, since the addition of media to actively growing microbial cultures results in no changes in microbial growth rate), that in vitro lag is a poor estimate of in vivo lag, or that lag time is not of crucial importance in predicting digestibility or intake.

The potentially digestible pool (equivalent ot the 96-hour in vitro cell wall digestibility) was a better predictor of in vivo cell wall digestibility than a model equation employing this pool, the indigestible pool, and the rate constants for passage and digestion. The estimate of the passage rate constant was dependent on two factors: that the overall model was adequate in explaining cell wall disappearance from the rumen, and that in vitro model parameter estimates were equal to, or closely related to, those that exist in vivo. Therefore, the Waldo model, for the prediction of forage quality with the use of the in vitro technique, is deficient.

The recent use of infrared spectroscopy for the evaulation of feeds has given hope to many that this technique has great potential for increasing the accuracy and decreasing the cost of making predictions of forage quality. This technique is purely empirical, and the same difficulties that occur in using empirical equations to relate chemical analyses to forage quality (the inability to generalize these equations) may occur with the infrared method. A firmer understanding of the factors affecting forage quality

can only serve to aid in its prediction, regardless of the method used.

Mathematical models can only organize and incorporate known relationships, and are dependent on so-called "traditional" research for their further development. It has been stated by this author and others that one of the most important gaps in our knowledge is the role of physical factors in determining forage quality. Although some general principles are understood in this area, there is little quantitative understanding of the nature of the relationship between the animal (rumination and rate of passage), and the praticle size distribution and tensile strength of forages. The rate of particle size reduction in the rumen has not been studied intensively, and no independent estimates of passage constants are possible without greater understanding of these relationships. A second area for future reserach is in the chemical characterization of plant cell walls in relation to animal consumption. Characterization of this fraction by its content of lignin, cellulose and hemicellulose may, in the end, be inadequate for defining cell wall digestibility and intake.

APPENDIX

TABLE 15. ANALYSIS OF VARIANCE OF ORGANIC MATTER INTAKE AND DIGESTIBILITY FOR EXPERIMENT 1[a]

Item	Digestibility and digestible intake			Intake	
	df	OMD	DOMI75	df	OMI1
Sums of squares					
Area	2	183**	622***	2	130***
Rep(area)	2	7	9	2	4
Regrowth	3	551***	692***	3	75*
Area x regrowth	6	273**	434*	6	95*
Regrowth x rep(area)	8	59	360	8	120*
Cultivar(area)	7	1029***	712**	7	115*
Regrowth x cultivar(area)	20	555**	1009*	20	252*
Period(rep)	4	43	713***	4	231***
Error	147	2042	4449	150	986
R^2	-	.66	.64	-	.60
CV	-	6	16	-	12

[a]Explanation of abbreviations and symbols in table 1.

TABLE 16. ANALYSIS OF VARIANCE OF CELL WALL INTAKE AND DIGESTIBILITY FOR EXPERIMENT 1[a].

Item	Digestibility		Intake	
	df	NDFD	df	NDFI1
Sums of squares				
Area	2	298***	2	32*
Rep(area)	2	7	2	2
Regrowth	3	1048***	3	17
Area x regrowth	6	438***	6	60*
Regrowth x rep(area)	8	78	8	71*
Cultivar(area)	7	1416***	7	85**
Regrowth x cultivar(area)	20	622**	20	126
Period(rep)	4	144	4	149***
Error	145	2188	148	615
R^2	-	.73	-	.53
CV	-	6	-	12

[a]Explanation of abbreviations and symbols in table 1.

TABLE 17. LEAST SQUARES MEANS OF INTAKE AND DIGESTIBILITY BY CULTIVAR, REPLICATION (REP.) AND REGROWTH FOR EXPERIMENT 1[a]

Cultivar	Rep.	Regrowth (weeks)	Hay no.	Number of animals	OMD	DOM	OMI1	DOMI1	DOMI75	NDFD	DNDF	NDFI1	DNDFI1
Argentine	1	2	7	3	64.5	60.6	24.1	15.6	41.2	68.9	51.0	18.9	13.1
		4	27	2	56.4	53.5	23.9	13.6	36.0	60.0	47.9	20.1	12.1
		6	48	1	52.8	50.2	23.7	12.5	32.6	55.2	43.7	19.6	10.8
		8	70	4	59.1	55.7	23.8	14.1	36.9	61.6	48.0	19.6	12.1
	2	2	10	3	63.3	59.5	21.7	13.8	36.4	67.0	49.7	17.1	11.5
		4	33	3	53.7	51.1	22.4	12.1	31.4	55.3	43.0	18.3	10.2
		6	54	2	58.1	55.1	23.6	13.8	36.5	60.8	47.6	19.5	12.0
		8	76	3	61.1	57.9	20.5	12.5	32.0	64.3	50.7	17.1	11.0
Coastcross-1	1	2	35	-	-	-	-	-	-	-	-	-	-
		4	57	3	57.9	51.2	17.2	10.0	27.0	61.4	45.0	14.3	8.8
		6	84	2	62.7	57.6	19.5	12.2	32.0	64.8	49.5	16.4	10.6
		8	86	2	56.3	53.3	24.2	13.7	36.9	56.4	44.3	20.0	11.4
	2	2	36	2	59.6	49.7	21.3	12.7	33.6	60.9	38.0	16.0	[illegible]
		4	56	3	58.3	52.2	17.3	10.0	25.7	60.7	45.1	14.1	8.5
		6	85	3	56.1	52.2	20.6	11.6	29.3	57.4	43.3	16.8	9.7
		8	87	3	54.0	50.3	18.7	10.1	25.6	54.4	41.9	15.5	8.5
Paraguay	1	2	5	3	67.1	62.8	25.1	16.9	44.9	71.8	52.5	19.5	14.0
		4	25	3	54.6	51.7	24.1	13.1	34.2	57.5	45.8	20.1	11.6
		6	45	3	60.0	56.8	22.9	13.8	36.8	62.6	49.7	19.1	12.0
		8	68	3	56.6	53.3	23.5	13.3	34.8	59.6	46.7	19.7	11.8
	2	2	11	3	62.2	58.1	22.0	13.7	34.8	66.5	49.2	17.4	11.6
		4	32	2	54.5	51.8	22.5	12.4	32.0	57.0	44.7	18.5	10.6
		6	53	2	53.9	51.2	20.0	10.9	27.7	57.3	44.8	16.5	9.5
		8	75	3	51.6	48.8	20.2	10.7	27.5	55.4	43.2	16.7	9.4
Pensacola	1	2	3	2	66.6	52.8	24.3	16.2	42.3	71.4	54.2	19.5	14.0
		4	24	3	59.6	56.6	22.6	13.4	35.9	62.5	49.5	18.9	11.7
		6	45	2	63.4	60.5	25.6	16.3	43.4	65.6	52.4	21.4	14.0
		8	67	3	57.9	55.4	22.2	12.9	33.2	61.3	49.5	18.7	11.5
	2	2	12	2	63.2	59.5	24.1	15.2	40.3	66.6	49.5	18.9	12.6
		4	31	3	56.5	53.6	17.9	10.1	26.0	59.1	46.5	14.8	8.7
		6	52	3	55.7	53.0	19.3	10.7	27.6	58.8	46.4	16.1	9.4
		8	74	3	56.4	53.7	18.3	10.3	25.9	60.8	48.5	15.3	9.3

Table 17 - continued

Cultivar	Rep.	Regrowth (weeks)	Hay no.	Number of animals	OMD	DOM	OMI1	DOMI1	DOMI75	NDFD	DNDF	NDFI1	DNDFI1
Slenderstem	1	2	16	-	-	-	-	-	-	-	-	-	-
		4	38	2[b]	58.8	55.0	24.3	14.6	37.7	60.6	43.8	18.6	11.6
		6	60	2	60.0	56.9	28.1	17.0	43.8	60.1	43.3	21.4	12.9
		8	78	3	58.7	56.2	23.3	13.7	35.4	60.0	44.5	18.0	10.8
	2	2	20	1	66.7	60.8	24.8	16.5	42.7	72.0	48.1	18.4	13.2
		4	42	3	64.9	60.7	24.1	15.7	40.1	67.0	46.6	17.9	12.0
		6	64	2[c]	59.2	56.5	25.3	15.0	38.6	60.5	42.2	19.5	11.8
		8	82	3	58.7	56.3	25.7	15.0	38.7	57.7	41.7	19.3	11.1
Transvala	1	2	21	1	69.4	64.1	26.5	18.3	47.1	74.4	52.4	20.2	15.0
		4	43	2	70.9	66.4	27.8	20.0	51.3	73.4	52.5	21.1	15.7
		6	65	2	63.3	59.9	21.1	13.4	34.9	63.0	43.9	15.5	9.8
		8	83	2	60.8	58.0	21.8	13.3	36.0	61.4	44.0	16.3	10.1
	2	2	k7	2	66.5	61.3	22.8	15.2	38.9	70.0	47.7	16.8	11.7
		4	40	3	66.8	62.7	21.2	14.5	37.2	68.7	47.8	15.6	11.0
		6	62	2[c]	59.4	56.5	19.6	11.6	29.6	60.2	42.1	15.1	9.3
		8	80	3	54.8	52.4	21.1	11.6	29.6	53.7	38.1	15.7	8.5
X124-4	1	2	8	3	71.1	62.3	30.4	21.6	56.5	76.2	48.1	21.8	16.6
		4	28	2[b]	67.0	61.1	21.6	16.5	42.5	72.4	52.3	17.0	14.0
		6	49	3	67.2	62.9	20.4	13.7	36.1	71.9	54.6	16.5	11.9
		8	71	3	59.7	56.1	19.7	11.8	30.9	63.5	47.6	15.6	9.9
	2	2	13	2	69.7	62.1	24.5	17.1	43.8	73.3	45.9	17.1	12.6
		4	30	3	63.4	58.6	21.3	13.5	34.8	67.9	50.6	17.1	11.6
		6	51	3	69.7	65.3	22.7	15.9	40.7	74.2	56.0	18.2	13.5
		8	73	3	61.4	57.4	19.7	12.1	30.9	65.2	49.0	15.9	10.4
X214-3	1	2	6	2[b]	67.2	60.2	25.4	15.9	44.3	72.8	49.8	18.9	13.2
		4	26	4	58.1	43.3	22.6	13.2	34.1	64.6	49.6	18.9	12.2
		6	47	3	54.8	50.6	18.9	10.6	27.5	60.3	46.3	15.6	9.6
		8	69	3	62.0	58.1	20.7	12.9	33.6	66.0	51.0	17.0	11.2
	2	2	14	2	67.0	59.0	23.0	15.4	40.0	74.2	49.5	17.9	13.3
		4	29	2	59.7	54.6	18.1	10.9	27.6	64.7	48.5	14.8	9.6
		6	50	3	62.6	57.2	21.3	13.3	34.6	66.6	49.0	17.0	11.3
		8	72	3	58.5	54.0	18.7	10.9	28.1	62.6	46.5	14.9	9.3

Table 17 - continued

Cultivar	Rep.	Regrowth (weeks)	Hay no.	Number of animals	OMD	DOM	OMI1	DOMI1	DOMI75	NDFD	DNDF	NDFI1	DNDFI1
X46-2	1	2	2	2	73.1	65.3	28.0	20.5	53.3	78.0	50.6	20.3	15.8
		4	23	3	61.4	56.2	23.4	14.3	37.5	66.6	50.3	19.2	12.8
		6	44	3	63.6	58.8	21.6	13.7	35.9	68.0	51.2	17.4	11.8
		8	66	4	60.2	56.0	24.4	14.8	38.5	63.6	47.7	19.6	12.5
	2	2	9	3	71.6	65.0	24.5	17.6	45.4	76.6	51.2	17.9	13.8
		4	34	3	55.2	51.1	21.1	11.7	29.9	59.8	44.9	17.1	10.3
		6	55	3	60.1	56.3	21.5	12.9	33.1	63.3	47.2	17.0	10.8
		8	77	3	58.2	55.0	19.9	11.5	28.8	58.9	43.0	15.2	8.9
X50-1	1	2	-	-	-	-	-	-	-	-	-	-	-
		4	39	3	62.4	58.9	26.6	16.6	43.2	65.7	45.9	19.6	12.9
		6	61	3	60.1	56.0	20.0	12.0	31.9	62.6	43.4	14.9	9.3
		8	79	2	49.5	56.7	23.2	13.7	36.6	59.0	41.6	17.1	10.1
	2	2	-	-	-	-	-	-	-	-	-	-	-
		4	41	3	60.7	57.2	22.1	13.5	34.9	63.4	44.9	16.4	10.5
		6	63	3	60.5	57.9	20.2	12.2	31.2	61.5	43.1	14.7	9.0
		8	81	2	63.9	60.7	27.4	17.6	46.0	65.1	46.3	20.6	13.5

[a]Explanation of abbreviations in table 1.

[b]One additional animal for OMI1 and NDFI1.

[c]One less animal for NDFD, DNDF, NDFI1 and DNDFI1.

TABLE 18. ANALYSIS OF VARIANCE OF DIGESTIBILITY AND INTAKE OF HQ AND LQ HAYS FOR EXPERIMENT 2[a]

Item	df	Parameter		
		DOMI75	OMI1	OMD
Sums of squares				
Hay	1	349.5***	34.7**	272.3***
Error	9	24.0	25.7	18.7
R^2	-	.94	.57	.94
CV	-	5.3	7.0	3.3

[a]Explanation of abbreviations and symbols in table 1.

TABLE 19. ANALYSIS OF VARIANCE OF NDF RESIDUE OF HQ AND LQ HAY SAMPLES WHEN DIGESTED BY IN VITRO AND IN SITU METHODS FOR EXPERIMENT 2[a]

Item	df	Statistic
Sums of squares		
Method	1	72.7
Period	1	83.6
Diet	1	3.5
Animal	1	59.9
Pooled interactions	3	114.1
Run(method-period-diet-animal)[b]	8	153.1***
Sample	1	363.1***
Sample x method	1	38.5***
Sample x period	1	20.0*
Sample x diet	1	12.7*
Sample x animal	1	2.5
Time	1	4406.5***
$Time^2$	1	548.9***
$Time^3$	1	110.7***
$Time^4$	1	27.3**
Pooled time x method	4	416.3***
Pooled time x sample	4	312.8***
Error	438	1412.9
R^2	-	.99
CV	-	3.5

[a]Explanation of abbreviations and symbols in table 1.

[b]Error term for method, period, diet, animal and pooled interactions.

TABLE 20. ANALYSIS OF VARIANCE OF RATE PARAMETERS OF HQ AND LQ HAY SAMPLES WHEN DIGESTED BY IN VITRO AND IN SITU METHODS FOR EXPERIMENT 2[a]

Item	df	Parameters				
		D(%DM)	D(%NDF)	U(%DM)	k_1(x 10^6)	L
Sums of squares						
Method	1	130.2***	145.1***	46.0**	150.1	.3
Period	1	.3	.9	1.3	240.2*	2.1
Diet	1	1.1	<.1	.6	15.5	6.4
Animal	1	4.0	5.7	1.7	124.6	.3
Pooled interactions	3	26.8*	42.0*	21.4	343.4	23.0
Run(method-period-diet-animal)[b]	8	11.5	27.1*	20.3*	268.3*	38.3**
Sample	1	184.7***	928.9***	908.4***	28.6	3.2
Sample x method	1	<.1	.1	.4	38.2	<.1
Sample x period	1	<.1	.5	1.9	.1	2.5
Sample x diet	1	.1	.6	1.2	.6	<.1
Sample x animal	1	<.1	1.1	2.3	3.1	1.2
Error	10	6.3	8.6	6.7	102.7	8.7
R^2	-	.98	.99	.99	.93	.90
CV	-	2.0	1.7	2.4	8.3	19.0

[a]Explanation of abbreviations and symbols in table 1.

[b]Error term for method, period, diet, animal and pooled interactions.

TABLE 21. ANALYSIS OF VARIANCE OF NDF RESIDUE OF HAYS, ORTS AND FECES FOR EXPERIMENT 2[a]

Item	Animal diet			
	LQ Hay		HQ Hay	
	df	Statistic	df	Statistic
Sums of squares				
Method	1	73.1	1	62.6*
Animal[b]	1	130.0	1	6.0
Method x animal	1	<.1	1	106.1*
Run(method x animal)[c]	4	169.3***	4	27.9**
Sample[d]	3	258.6***	3	573.1***
Sample x method	3	10.4	3	37.7**
Sample x animal	3	77.2***	3	42.4**
Time	1	44518.8***	1	24081.8***
$Time^2$	1	10605.6***	1	6161.6***
$Time^3$	1	1046.9***	1	717.7***
$Time^4$	1	.6	1	.6
Pooled time x method	4	394.3***	4	246.5***
Pooled time x sample	12	7995.0***	12	5079.8***
Error	434	1332.4	421	831.2
R^2	-	.99	-	.99
CV	-	3.1	-	2.6

[a]Explanation of abbreviations and symbols in table 1.

[b]Confounded with period.

[c]Error term for method, animal and animal x method.

[d]HQ and LQ hays, LQ orts and LQ feces when LQ diet fed to animals; HQ and LQ hays, HQ orts and HQ feces when HQ diet fed to animals.

TABLE 22. ANALYSIS OF VARIANCE OF RATE PARAMETERS OF HAYS, LQ ORTS AND LQ FECES WHEN DIGESTED BY IN VITRO AND IN SITU METHODS FOR EXPERIMENT 2[a]

Item	df	Parameters				
		D(%dm)	D(% NDF)	U(% dm)	k_1(x 10^6)	L
Sums of squares						
Method	1	187.2**	218.9**	56.3*	12.2	17.7
Animal[b]	1	1.5	3.3	3.1	119.0	25.8
Method x animal	1	19.3	45.2*	32.9	390.7	2.4
Run(method x animal)[c]	1	12.8	20.7	12.5	333.3	218.5
Sample	3	2875.9***	5831.6***	3665.3***	1696.2***	325.9*
Sample x method	3	2.8	9.0	2.7	176.5	81.0
Sample x animal	3	2.2	3.2	1.6	317.4	30.6
Error	13	27.1	48.9	22.5	661.4	386.0
R^2	-	.99	.99	.99	.83	.67
CV	-	4.5	4.4	3.1	20.6	72.7

[a]Explanation of abbreviations and symbols in table 1.

[b]Confounded with period.

[c]Error term for method, animal and animal x method.

TABLE 23. ANALYSIS OF VARIANCE OF RATE PARAMETERS OF HAYS, HQ ORTS AND HQ FECES WHEN DIGESTED BY IN VITRO AND IN SITU METHODS FOR EXPERIMENT 2[a]

Item	df	Parameters				
		D(% dm)	D(% NDF)	U(% dm)	k_1(x 10^6)	L
Sums of squares						
Method	1	115.7***	186.0***	70.7**	5.6	46.4*
Animal[b]	1	2.5	4.5	.8	1.8	1.1
Method x animal	1	15.6	15.7	3.1	1.5	20.1
Run(method x animal)[c]	4	2.6	8.4	8.0	90.5	21.1
Sample	3	1269.8***	2580.7***	1424.6***	683.3***	172.8***
Sample x method	3	20.6*	54.1**	23.5**	232.4**	52.4*
Sample x animal	3	2.0	7.6	6.4	65.4	8.3
Error	12	17.7	23.2	9.7	111.8	39.8
R^2	-	.99	.99	.99	.89	.93
CV	-	3.5	2.9	2.4	8.5	30.0

[a]Explanation of abbreviations and symbols in table 1.

[b]Confounded with period

[c]Error term for method, animal and method x animal.

TABLE 24. ANALYSIS OF VARIANCE OF METHOD OF DETERMINATION ON NDF CONTENT OF FIVE HAYS FOR EXPERIMENT 2[a]

Item	df	Statistic
Sums of squares		
Treatment	5	30.5***
Hay	4	672.9***
Error	20	5.4
R^2	-	.99
CV	-	.72

[a]Explanation of abbreviations and symbols in table 1.

TABLE 25. IN VITRO CELL WALL RESIDUES AS A PERCENT OF INITIAL DRY MATTER BY CULTIVAR, REPLICATION (REP.) AND REGROWTH FOR EXPERIMENT 3

Cultivar	Rep.	Regrowth (weeks)	Hay no.	Duration of fermentation, hr										
				0	12	24	36	48	60	96^{a}_{12}	96_{24}	96_{36}	96_{48}	96_{60}
Argentine	1	2	7	73.5	60.6	51.7	37.2	30.8	23.0	18.4	18.6	19.5	18.2	18.1
		4	27	79.4	69.3	60.9	51.3	42.9	34.7	29.5	28.8	28.8	28.6	29.1
		8	70	77.4	67.8	61.8	49.7	41.5	33.1	26.9	27.2	27.0	26.6	26.1
	2	2	10	74.6	62.3	56.4	41.4	34.3	23.8	19.7	20.0	20.6	21.7	19.9
		4	33	78.7	67.5	59.5	49.1	45.1	36.1	27.7	28.6	28.5	28.3	29.0
		8	76	77.8	67.3	59.9	46.8	39.1	33.0	26.8	26.1	26.2	25.7	25.1
Coastcross-1	1	4	57	72.4	59.9	49.1	39.6	33.4	30.6	29.2	29.4	29.2	28.3	28.1
		6	84	75.6	61.3	51.5	42.4	35.0	30.7	27.0	27.8	27.7	27.3	26.7
		8	86	79.6	67.5	59.2	48.8	42.3	39.4	36.5	36.3	36.3	34.4	34.8
	2	2	36	67.7	52.0	41.7	30.7	27.2	26.4	26.6	24.9	24.9	25.2	24.7
		4	56	71.6	63.4	46.6	36.4	30.3	28.3	26.0	26.0	26.5	25.7	25.8
		6	85	75.5	61.8	52.1	39.6	35.1	31.8	28.9	30.1	28.9	29.4	28.6
		8	87	76.8	63.4	53.7	45.0	40.2	38.2	34.2	34.5	35.0	35.9	34.2
Paraguay	1	2	5	73.3	60.6	46.7	28.8	23.3	20.2	18.6	16.7	16.9	16.7	16.2
		4	25	79.7	69.2	62.9	52.9	45.4	35.8	28.2	28.6	29.0	30.2	28.2
		6	46	79.0	70.8	61.4	48.7	42.0	34.7	27.7	28.1	28.2	28.6	27.7
		8	68	76.8	67.6	59.7	46.3	39.1	33.9	28.8	26.2	26.6	26.2	26.8
	2	2	11	73.8	62.0	53.7	39.6	33.3	25.1	21.0	20.7	21.7	20.8	20.4
		4	32	78.7	67.9	62.2	51.8	45.5	36.2	29.7	28.1	29.5	29.2	27.7
Pensacola	1	2	3	75.3	62.2	52.2	37.5	31.6	23.1	19.2	19.0	20.2	18.9	18.4
		4	24	79.7	69.4	63.3	53.0	44.8	36.4	26.7	28.4	28.6	28.7	27.9
		6	45	79.3	70.8	63.8	52.2	46.2	36.0	27.4	27.3	28.6	28.8	27.4
		8	67	79.4	71.5	62.1	52.9	45.1	36.0	30.6	29.2	29.9	28.7	28.6
	2	2	12	75.0	61.9	53.5	34.6	31.6	22.3	20.1	20.0	20.1	21.4	19.1
		4	31	78.1	68.4	63.0	51.5	47.2	37.1	29.0	30.5	30.6	29.9	28.8
		6	52	78.5	69.5	63.7	51.8	44.4	33.8	27.5	28.0	27.4	30.3	27.7
		8	74	79.1	69.5	60.7	51.0	42.0	38.0	29.4	28.6	27.6	29.1	29.1
Slenderstem	1	4	38	71.9	54.5	39.3	30.2	26.3	24.9	22.2	22.4	22.5	22.4	22.1
		6	60	70.3	52.5	42.6	33.2	27.8	26.3	24.2	23.5	22.5	22.7	23.3
		8	78	74.0	57.9	53.5	42.9	39.0	30.2	27.4	28.5	27.5	27.9	27.1
	2	4	42	70.4	50.6	38.3	26.7	23.9	22.0	20.0	21.8	19.8	20.4	19.8
		6	64	69.8	52.6	38.4	29.0	25.8	24.9	22.8	22.0	21.6	22.8	21.9
		8	82	72.2	54.5	46.2	39.2	32.9	28.0	26.7	27.0	29.0	27.5	27.0

Table 25 - continued

Cultivar	Rep.	Regrowth (weeks)	Hay no.	Duration of fermentation, hr										
				0	12	24	36	48	60	96^{a}_{12}	96_{24}	96_{36}	96_{48}	96_{60}
Transvala	1	6	65	68.8	56.4	48.8	34.2	27.9	25.5	20.8	21.1	23.1	22.6	21.4
		8	83	71.5	58.3	50.9	41.6	35.6	29.1	23.3	26.3	25.6	26.3	25.2
	2	2	18	69.5	52.2	41.5	28.2	21.4	18.7	16.4	16.5	17.0	17.3	15.6
		6	62	68.9	54.3	39.3	31.4	25.8	24.5	22.3	22.4	22.6	21.8	21.4
		8	80	69.9	55.3	44.7	36.5	31.6	27.3	27.2	25.5	25.9	25.2	24.7
X124-4	1	2	8	63.4	47.4	34.4	25.0	15.7	13.2	11.3	12.0	12.0	12.0	11.3
		8	71	74.5	58.5	49.2	36.7	32.3	25.7	21.1	21.7	20.6	20.1	19.9
	2	4	30	75.4	60.1	48.4	36.7	29.3	23.9	18.9	18.5	19.4	19.2	18.4
		8	73	75.0	58.5	52.0	39.2	31.3	27.3	21.8	24.3	22.6	22.3	21.5
X215-3	1	4	26	77.2	62.7	50.9	39.2	33.8	27.5	21.7	23.0	21.8	20.7	21.2
		8	69	75.9	61.5	53.8	43.2	33.4	29.3	25.7	24.0	25.2	23.8	23.5
	2	2	14	66.4	48.9	39.5	27.2	21.8	14.3	12.8	13.3	13.4	13.7	12.9
		6	50	73.3	60.7	49.4	34.6	30.9	27.6	23.6	22.1	22.4	22.3	21.4
		8	72	74.5	58.7	49.6	42.7	37.1	29.8	23.2	24.3	25.4	26.3	24.[illegible]
X46-2	1	2	2	65.8	46.9	35.2	23.6	16.1	13.3	11.3	12.4	10.6	10.8	10.4
		4	23	76.2	57.5	47.6	35.7	28.7	27.5	23.5	23.2	23.4	22.3	22.3
		6	44	75.8	61.7	47.4	35.9	32.1	26.7	22.2	21.9	22.3	24.1	21.9
		8	66	74.8	63.8	47.7	38.1	32.3	31.2	26.9	25.7	24.9	26.0	26.1
	2	2	9	65.6	48.8	36.7	18.4	13.7	12.4	10.1	9.4	10.0	9.2	9.0
		6	55	74.3	61.3	51.2	41.1	36.3	30.1	23.5	24.0	24.3	24.0	23.4
		8	77	73.8	58.6	53.9	40.9	33.9	28.7	24.4	25.6	26.0	25.7	24.8
X50-1	1	4	39	69.8	51.5	40.7	25.9	19.6	18.4	18.5	16.6	16.7	17.0	16.9
		6	61	68.6	53.0	41.0	34.1	27.3	26.4	23.2	23.3	23.8	23.5	22.6
		8	79	69.7	53.8	45.2	36.0	28.8	26.3	23.7	23.2	24.8	24.2	23.2
	2	4	41	70.6	54.9	42.6	31.8	24.1	22.2	19.0	19.4	19.2	18.6	18.2
		6	63	69.7	56.2	47.4	31.1	26.3	24.0	20.6	19.7	19.8	19.2	20.1
		8	81	71.1	54.4	44.1	36.2	28.3	24.6	21.8	21.6	23.1	21.7	21.2

[a]Determined in same run as samples denoted by subscript.

TABLE 26. MEASURED AND ESTIMATED MODEL PARAMETERS BY CULTIVAR, REPLICATION (REP.) AND REGROWTH FOR EXPERIMENT 3

Cultivar	Rep.	Regrowth (weeks)	Hay no.	Parameter[a] D(% dm)	D(% NDF)	U(% dm)	k_1	k_{21}[b]	k_{22}[c]	L
Argentine	1	2	7	55.0	74.7	18.6	.0510	.0242	.0367	14.8
		4	27	50.5	63.6	28.9	.0452	.0324	.0435	16.6
		8	70	50.6	65.4	26.8	.0432	.0313	.0421	16.7
	2	2	10	54.2	72.6	20.4	.0598	.0203	.0336	19.3
		4	33	50.3	63.9	28.4	.0372	.0309	.0382	12.5
		8	76	51.8	66.6	26.0	.0399	.0267	.0343	13.3
Coastcross-1	1	4	57	43.6	60.2	28.8	.0571	.0207	.0252	10.4
		6	84	48.3	63.9	27.3	.0501	.0248	.0312	10.8
		8	86	43.9	55.2	35.6	.0434	.0359	.0410	8.3
	2	2	36	42.5	61.9	25.3	.0693	.0229	.0281	8.8
		4	56	45.6	63.7	26.0	.0616	.0203	.0259	11.2
		6	85	46.3	61.3	29.2	.0529	.0256	.0309	9.1
		8	87	42.0	52.8	34.8	.0468	.0267	.0289	5.2
Paraguay	1	2	5	46.3	76.7	17.1	.0590	.0225	.0343	11.8
		4	25	50.8	63.8	28.8	.0415	.0339	.0444	16.3
		6	46	51.0	64.5	28.1	.0441	.0310	.0410	15.4
		8	68	49.9	65.0	26.9	.0411	.0326	.0421	14.1
	2	2	11	52.9	71.6	20.9	.0514	.0229	.0340	15.7
		4	32	49.8	63.3	28.8	.0375	.0297	.0377	14.8
Pensacola	1	2	3	56.2	74.6	19.1	.0513	.0250	.0376	14.4
		4	24	51.7	64.8	28.0	.0388	.0315	.0409	15.5
		6	45	51.4	64.8	27.9	.0387	.0381	.0494	16.2
		8	67	49.9	62.8	29.5	.0429	.0308	.0407	16.9
	2	2	12	54.8	73.1	20.1	.0620	.0236	.0379	16.3
		4	31	48.3	61.9	29.8	.0359	.0242	.0299	14.0
		6	52	50.4	64.1	28.2	.0488	.0238	.0334	19.2
		8	74	50.4	63.7	28.7	.0349	.0250	.0301	11.5
Slenderstem	1	4	38	49.6	69.0	22.3	.0529	.0260	.0277	2.1
		6	60	47.0	66.9	23.2	.0507	.0324	.0380	5.8
		8	78	46.3	62.6	27.6	.0546	.0272	.0368	15.3
	2	4	42	50.1	71.1	20.4	.0558	.0231	.0249	2.3
		6	64	47.6	68.3	22.1	.0481	.0274	.0266	-1.0
		8	82	44.8	62.0	27.4	.0782	.0270	.0389	15.7

Table 26 - continued

Cultivar	Rep.	Regrowth (weeks)	Hay no.	Parameter[a] D(% dm	D(% NDF)	U(% dm)	k_1	k_{21}[b]	k_{22}[c]	L
Transvala	1	6	65	47.0	68.3	21.8	.0535	.0198	.0259	11.3
		8	83	46.2	64.6	25.3	.0502	.0234	.0307	13.3
	2	2	18	52.9	76.2	16.5	.0607	.0186	.0267	10.3
		6	62	46.8	67.9	22.1	.0486	.0190	.0198	1.6
		8	80	44.2	63.2	25.7	.0537	.0226	.0276	9.3
X124-4	1	2	8	51.7	81.5	11.7	.0722	.0208	.0391	13.7
		8	71	53.8	72.2	20.7	.0412	.0212	.0260	8.1
	2	4	30	56.5	74.9	18.9	.0469	.0217	.0295	10.6
		8	73	52.5	70.0	22.5	.0440	.0220	.0276	9.3
X215-3	1	4	26	55.6	71.9	21.7	.0398	.0281	.0341	7.6
		8	69	51.5	67.8	24.4	.0461	.0246	.0324	12.4
	2	2	14	53.2	80.1	13.2	.0764	.0161	.0323	17.4
		6	50	51.0	69.5	22.4	.0398	.0254	.0285	4.7
		8	72	49.7	66.8	24.7	.0427	.0215	.0264	9.8
X46-2	1	2	2	54.7	83.2	11.1	.0590	.0206	.0331	9.8
		4	23	53.3	69.9	22.9	.0442	.0287	.0322	4.1
		6	44	53.3	70.4	22.5	.0461	.0244	.0296	7.3
		8	66	48.9	65.4	25.9	.0400	.0321	.0339	2.2
	2	2	9	56.2	85.3	9.7	.0631	.0152	.0253	9.5
		6	55	50.6	68.0	23.8	.0378	.0265	.0313	7.8
		8	77	48.5	65.7	25.3	.0545	.0207	.0280	14.4
X50-1	1	4	39	52.6	75.4	17.1	.0777	.0205	.0336	13.1
		6	61	45.4	66.1	23.3	.0471	.0210	.0224	2.8
		8	79	45.9	65.9	23.8	.0556	.0246	.0317	10.5
	2	4	41	51.8	73.3	18.9	.0503	.0206	.0255	7.2
		6	63	49.8	71.5	19.9	.0512	.0180	.0234	10.0
		8	81	49.2	69.2	21.9	.0527	.0282	.0369	9.9

[a]Explanation of abbreviations and symbols in table 1.

[b]k_{21} derived from model that assumes zero lag *in vivo*.

[c]k_{22} derived from model that assumes lag (L) *in vivo* is same as L *in vitro*.

TABLE 27. CORRELATIONS BETWEEN IN VITRO MEASUREMENTS AND IN VIVO CHARACTERISTICS OF HAYS FOR EXPERIMENT 3[a]

	In vitro NDFD, % at (hr)						Model parameters			
Item	12	24	36	48	60	96	D(%DM)	U(%DM)	k_1	L
NDFD	.39	.44	.63	.69	.83	.89	.72	-.85	.37	.06
NDFI1	.05	.05	.09	.12	.22	.28	.36	-.20	.13	.20
OMD	.51	.53	.70	.76	.86	.86	.58	-.86	.48	-.01
OMI1	.38	.36	.40	.43	.49	.47	.30	-.46	.38	.04
DOMI75	.46	.46	.56	.61	.69	.68	.44	-.67	.47	.05
DNDF	-.22	-.17	.03	.09	.31	.51	.83	-.35	-.16	.31
DOM	.41	.40	.58	.64	.77	.81	.63	-.78	.35	.05

[a]Explanation of abbreviations and symbols in table 1.

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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