COMPARISON OF METHODOLOGIES FOR DETERMINATION OF CONTENT AND DIGESTIBILITY OF HEMICELLULOSE AND CELLULOSE IN TROPICAL GRASS HAYS

BY

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To Allen,

now its your turn...

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Experiments were conducted to: 1) fractionate cell walls of tropical grasses into: holocellulose, cellulose and hemicellulose; 2) determine *in vitro* and *in vivo* digestibility of cell wall components; and 3) study the relationship between these components and quality of forages, as measured by feeding hays to sheep. One cultivar of *Cynodon dactylon* (Coastcross-1), two experimental lines of *Digitaria* species (X46-2 and X124-4) and two cultivars of *Paspalum notatum* (Argentine and Paraguay) cut at four ages (2, 4, 6 and 8 weeks of regrowth) were used in the experiments.

Samples of each hay were analyzed for organic matter (OM), crude protein (CP) and neutral detergent fiber (NDF). NDF fractions (cellulose, hemicellulose and lignin) were determined by two methods: 1) conventional Van Soest detergent analyses

(CELLV, HEMV, PLIG respectively): and 2) classical fractionation using $NaClO_2$ and alkali (CELL, HC, LIG respectively). There were significant differences between fractions obtained by the two methods. HC remained the same or decreased slightly with maturity but HEMV declined. CELL and CELLV showed similar patterns, increasing up to 6 weeks, then decreasing.

Samples were fermented in rumen fluid for 48 hr and digested in acid-pepsin for 48 hr or fermented in rumen fluid only for 48 hr and residual NDF was fractionated as above. In vitro digestibility of NDF and HC was increased by further digestion in acid-pepsin while CELL digestibility showed minimal change. CELL digestibility was generally higher than HC digestibility. Digestion coefficients for each fraction were similar in vivo and in vitro (r^2 = .79 to .92).

NDF, CP and PLIG were the best single chemical analyses for prediction of intake and digestibility, but any in vitro analysis gave better estimates of in vivo digestibility. Two and three variable models improved prediction of organic matter intake (OMI) and digestible OMI.

Hemicellulose obtained by different methods was not the same. Digestion of cell walls in acid-pepsin was due to breakdown of hemicellulose and possibly, dissolution of lignin-hemicellulose bonds. Further studies on chemical bonding, physical degradability and chemical structure of the cell wall appear necessary before being able to predict animal performance from laboratory analyses.

CHAPTER I
INTRODUCTION

The world food shortage is most critical in that portion of the world known as the tropics, which is faced with rising populations and diminishing food supplies. The limited availability of land suitable for arable cropping has focused attention upon the grasslands which occupy fifty percent of the earth's land surface. These lands are able to provide food for people by the use of ruminant animals to convert pasture into meat and milk.

The structural carbohydrates in pastures and forages are an important source of energy to ruminants and, in turn, to people. The amount and proportions of structural carbohydrates in the plant cell wall determine the degree to which the ruminant can utilize this energy. Changes in amounts and types of cell wall constituents are responsible for differences in nutritive value of forages.

The cell wall polysaccharides are comprised of fibers of organized, crystalline cellulose in a matrix of hemicelluloses which are associated physically and chemically with lignin (Jones, 1976). Lignin influences digestibility of forages and has been considered the primary structural inhibitor of quality in tropical grasses (Moore and Mott, 1973). Cellulose and hemicellulose fractions are also responsible for variations in digestibility, since they contain the largest proportion of the indigestible substances in the cell wall (Van Soest, 1975).

The role, structure and composition of hemicellulose(s) *vis a vis* forage quality and nutritive value is not well understood. Hemicellulose is often defined as the resultant of a certain set of operations and it has been convenient to estimate hemicellulose as the difference between acid detergent fiber (ADF) and neutral detergent fiber (NDF). Acid extraction may not, however, remove material that is hemicellulosic in nature (Bailey and Ulyatt, 1970; Morrison, 1980). The entire hemicellulosic fraction has been treated as a unit since no nutritionally meaningful fractionation has been found and since hemicellulose digestibility is so closely related to that of cellulose (Van Soest, 1975). Questions arise, however, as to whether this grouping together of structural carbohydrates is biologically rational, given the complexity of living plants and of ruminant digestion.

The objectives of this research were: 1) to fractionate the cell walls of tropical grasses into three components: holocellulose, cellulose and hemicellulose; 2) to determine the *in vitro* and *in vivo* digestibility of the three components; and 3) to study the relationship between these components and the quality of the forages *in vivo*.

CHAPTER II
LITERATURE REVIEW

Forage Quality

The best measure of the nutritive value of a forage is animal performance. Moore and Mott (1973) have defined forage quality as output per animal, being a function of voluntary intake and digestibility when the forage is fed *ad libitum* as the sole source of feed. Intake and digestibility may be limited by the rate and extent of forage digestion in the rumen, which, in turn, are related to the amount of cell wall constituents (CWC).

Under the best of conditions, all forages would eventually be tested in long-term production trials on pasture (Mott and Moore, 1970) having been preceded by a few years of agronomic and laboratory evaluations. It is often not possible for researchers to conduct pasture studies. Instead, trials with penned or caged animals are used to measure voluntary intake and digestibility. Even these animal trials may be beyond the means of some researchers who have to rely exclusively on laboratory evaluation of forages. Fortunately, enough data has been accumulated to show high correlations between some laboratory methods and *in vivo* performance, e.g., the two stage *in vitro* rumen digestion procedure (Tilley and Terry, 1963), and digestibility. Unfortunately, a laboratory procedure that predicts voluntary intake with acceptable accuracy has not yet been developed.

Thus, there is a continuum ranging from what would be the ideal way to measure forage quality (animal response) to a vast array of ways to predict forage quality in the laboratory which may or may not be acceptable under all conditions.

Digestibility

A number of laboratory methods to estimate *in vivo* digestibility have been devised. However, if only one method is to be used to predict digestibility over a wide range of forages, then the method to choose is the two-stage *in vitro* digestion procedure (Oh et al., 1966; Marten and Barnes, 1980).

Digestibility is very important in determining the quality of a forage and is more a function of plant factors than of animal factors. It is now accepted that as a plant matures, its digestibility decreases due to increasing lignification and changes in plant morphology. The cell contents (CC) of forages are considered to be completely digestible while the CWC show variable digestibilities. Riewe and Lippke (1970) found that tropical species accumulate dry matter more rapidly which can be directly associated with an increase in CWC. While temperate and tropical grasses have similar amounts of CWC at early stages of growth, the amount of CWC and its composition changes more rapidly with maturation in tropical species. This increase in CWC may or may not decrease digestibility in tropical grasses more than such an increase would in temperate grasses. Minson and McLeod (1970) showed temperate species to be 12.8 percentage units higher in IVDMD than tropical species; however, Russo

(1976) did not find differences of such magnitude despite higher CWC. Tessema (1972) noted that the overall rate of decline in digestibility was more rapid with tropical grasses than with temperate grasses, that hemicellulose fluctuated considerably in the tropical grasses and that tropical grasses had higher concentrations of lignin. Moore and Mott (1973) have suggested that the apparent lower quality of tropical grasses may be due to lower protein values, which will depress intake, and higher fiber values, which will depress digestibility.

With respect to ruminant nutrition, the detergent analysis system of Van Soest (1967) which separates forages into CC and CWC is useful for forage quality analysis. The CC are soluble and almost totally available to the ruminant while the CWC are completely insoluble, with their digestibility depending on microbial action. The CWC appear to have the most influence on the digestibility and intake of forages. The higher CWC in tropical grasses places increased emphasis on digestibility, especially with respect to its influence on voluntary intake.

Intake

Intake is controlled by two mechanisms: distention and metabolic. In forage-fed ruminants, the distention mechanism exerts the major effect on intake. Many other factors, of course, affect voluntary intake and have been reviewed by Capote (1975) and Golding (1973).

Crampton et al. (1960), Conrad (1966) and others have proposed the following concepts relating to the distention theory in ruminants: (1) probably a specific degree of rumen load reduction is the major determinant of hunger; (2) the rates of forage cellulose and hemicellulose degradation are directly related to the rate at which the rumen load is reduced; and (3) the time it takes for the rumen load to be reduced (when hunger recurs) is characteristic of the specific forage involved. Most researchers now agree that the rumen is where the distention control over forage is exerted. Distention mechanism is determined by (1) rumen fill and (2) retention time (Weston, 1966; Thornton and Minson, 1972). Van Soest's "hotel theory" (1975) suggests that the plant cell walls, not the dry matter of the forage, are responsible for gut fill and only when the cell walls are degraded or move out of the rumen, is gut fill reduced. Retention time is affected by the rates of digestion and passage (Jones and Bailey, 1974; Waldo et al., 1972).

Relationship Between Digestion and Intake

The detergent systems of Van Soest separate the fibrous portion of a forage into cellulose, hemicellulose and lignin. Other classification systems exist: Minson (1976) separated the CWC into a potentially digestible fraction and an indigestible fraction, while work by Abrams (1980) suggests there may be at least two types of digestible cell walls. How these cell walls are utilized by an animal is determined primarily by their rate of passage through the digestive tract.

Chemical composition and structure of the cell walls are probably the most important factors influencing rates of digestion and passage (Akin et al., 1974; Van Soest, 1965). The chemical bonds holding the cell walls together as well as the structure of each cell wall component (lignin, cellulose and hemicellulose) needs to be elucidated. Lignin, for example, may provide a physical barrier to the breakdown of the cell walls (Dekker, 1976). The rate of breakdown is regulated by the organizational structure of the cell walls (Akin et al., 1974; de la Torre, 1974).

Intake and digestibility can be seen then, to be linked together with respect to forage quality. Some alternative definitions of forage quality use the combination of intake and digestibility, such as voluntary intake of digestible energy (Heaney, 1970). The goal is to predict digestible nutrient intake, i.e., animal performance, without conducting long-term pasture production trials. Few attempts have been successful, especially with tropical grasses. As mentioned earlier, laboratory methods currently available can predict forage digestibility but not voluntary intake. Characterization of the chemical structure of the plant cell wall, especially in tropical grasses, would help our understanding of the factors affecting voluntary intake.

The Structure of Plant Cell Walls

The plant cell wall is of two types, a thin primary wall and a thicker secondary wall. The primary wall is laid down by undifferentiated cells that are still growing. The primary wall is transformed

into a secondary wall after the cell has stopped growing. The primary cell walls of many higher plants appear to have very similar structures (Talmadge et al., 1973) but this is not true of secondary walls.

Every living cell, whether plant or animal, is surrounded by a complex membrane made up of lipids and proteins called the plasmalemma. In almost all plant cells, the plasmalemma forms a cell wall which is rigid or semi-rigid and is chemically distinct in that the structurally important components are polysaccharides (Preston, 1974). Cellulose, hemicellulose, pectic polysaccharide, structural protein and lignin have been identified as the major components of the plant cell wall. Some of these components, e.g. lignin, are not polysaccharides, nevertheless, the essential and sudden change at the plasmalemma surface is from a basically lipo-protein to a basically polysaccharide formation (Preston, 1974).

Usually only one of these polysaccharides is crystalline and cellulose predominates. In primary cell walls, the crystalline cellulose fibrils form a core surrounded by a non-crystalline region consisting of other cellulose molecules, non-cellulosic polysaccharides and glycoprotein (Talmadge et al., 1973). In secondary walls, the cellulose fibrils are aggregated into ropelike structures called microfibrils.

The non-cellulosic polysaccharides making up the bulk of the primary cell wall have been defined by their presence in chemical fractions. The two major non-cellulosic fractions are the pectic polysaccharides obtained by extracting cell walls with boiling

water, EDTA, or dilute acid (Aspinall, 1970) and the hemicelluloses solubilized by the subsequent extraction of the same walls with alkali (Whistler and Richards, 1970). In the secondary wall, also, it appears that the extractable non-cellulosic wall compounds surrounding the microfibrils are hemicellulosic (Preston, 1974). But even further, some of the hemicelluloses cannot be removed without degradation of the microfibrils and are therefore integrally mixed with them (Preston, 1974). It has been suggested (Bauer et al., 1973) that the structural function of the hemicellulosic polysaccharides is to interconnect the cellulose fibrils and the pectic polysaccharides of the plant cell wall, and that this function is based on the ability of the hemicellulosic polysaccharides to bind non-covalently to cellulose and to bind covalently, through glycosidic bonds at their reducing ends, to the pectic polysaccharides.

Hemicellulose

Hemicelluloses are often implicitly or explicitly defined as the cell wall and intercellular polysaccharides that can be extracted by alkali from higher land plant tissues that are, or were, lignified (Wilkie, 1979). Hemicellulose is often extended to include certain carbohydrates found in cereal endosperm. Jermyn (1955) wrote "it cannot be too strongly emphasized that 'cellulose' and 'hemicellulose' are normally determined as the resultants of certain sets of operations, rather than as chemically defined species." (p.197) Hemicellulose has, thus, variable definitions depending on the methods or procedures used to isolate it. A more liberal definition of hemicellulose

refers to all of the types of polysaccharides (found in plants) other than celluloses, starches and fructans (Wilkie, 1979).

It is necessary to point out that the majority of literature on hemicellulose and its relationship to forage quality and ruminant nutrition deals with the Van Soest hemicellulose obtained by subtracting ADF from NDF. Work as early as 1970 (Bailey and Ulyatt) points out the problems inherent in this definition of hemicellulose. Discussion of methodology differences follows. To avoid confusion, Van Soest hemicellulose will be distinguished from alkali-soluble non-endospermic hemicellulose (as isolated by classical methodology).

Hemicellulose: Methodology

Hemicellulose was first isolated by Schultze in 1891 (Wilkie, 1979) using dilute alkali to extract polysaccharides from plant tissues. Work on hemicellulose, thereafter, although becoming more sophisticated, continued to use alkali to separate the compound from plant tissue. The continued search for a simpler, more rapid procedure led, however, to a second extraction scheme using acid hydrolysis, either on delignified plant tissue (Routley and Sullivan, 1958) or on whole plant tissue (Van Soest, 1965). Alkali extraction and acid hydrolysis are two considerably different methods of arriving at a presumed similar result. Closer examination of these two general methodologies appears warranted.

Alkali Extraction

In grasses, some of the hemicellulose is covalently bonded to lignin (Morrison, 1974). Earlier, the relationship of lignin and

hemicellulose was not well understood and hemicelluloses were extracted from lignified tissues of grasses by direct treatment with alkali (Wilkie, 1979). However, it has now become common practice to delignify the plant tissue with sodium chlorite and acetic acid according to the procedure first outlined by Whistler et al. in 1948. The delignified plant tissue, termed holocellulose, is primarily composed of hemicellulose and cellulose. The holocellulose is then treated with dilute alkali under nitrogen to extract the hemicelluloses.

It may be possible to extract some of the hemicellulose from holocellulose with hot or cold water. The alkali solutions, either sodium or potassium hydroxide, may range in concentration from 4 to 24% w/v. Dilute alkali extracts only part of the hemicellulose. If exhaustive extractions are required, the concentrations of alkali can be increased from a low concentration to a higher concentration to extract more of the hemicellulose (Wilkie, 1979). Nevertheless, even with this exhaustive extraction, not all of the hemicellulose will be accounted for (Gaillard, 1958).

With filtration or centrifugation, a residue and a supernatant are obtained; the supernatant containing the hemicellulosic materials. With some diligence, as many as three fractions can be derived from the supernatant. Acidification of the supernatant with acetic acid at 0-2 C will precipitate the A fraction (Gordon and Gaillard, 1976) which is composed primarily of long chain xylans with small amounts of arabinose and uronic acid (Bailey, 1973). The polysaccharides remaining in solution can be precipitated by ethanol (Gordon and

Gaillard, 1976) or recovered by dialysis and freeze-drying (Bailey, 1973) to obtain the B fraction. The B fraction contains smaller molecular weight xylans with many side chains and more complex molecules made up of galactose, glucose and rhamnose (Bailey, 1973). These are sometimes further broken down into linear B and branched B fractions based on their separation by iodine precipitation (Gaillard, 1965). The ethanol in this supernatant can be evaporated and a C fraction recovered by dialysis for 5-7 days, followed by freeze-drying. The C fraction has not been well studied but is composed primarily of lignin, arabinose, galactose, glucose and xylose (Gordon and Gaillard, 1976). In addition to iodine precipitation, individual mono- or polysaccharides may be isolated from these fractions by copper precipitation, gel filtration or quaternary ammonium salt precipitation (Bailey, 1973). The sugars thus isolated can then be identified by any number of methods ranging from the colorimetric (Dubois et al., 1956) to chromatographic (paper, thin-layer, gas or high performance liquid).

It becomes apparent, then, that examination of hemicellulose and its components can very rapidly become a major undertaking, particularly when it is desired to elucidate exact amount or structures of components. Work along these lines thus falls on a few hardy souls upon whom the rest of the researchers become dependent for answers. It is also apparent that such procedures have little value in a situation where a large number of samples need to be analyzed.

Acid Hydrolysis

A fair approximation of the hemicellulosic material in a plant can be obtained by treating depectinated plant tissue with boiling N acid for 2 - 3 hours (Bailey, 1973), hot water (Routley and Sullivan, 1958) or cold 72% sulfuric acid overnight followed by 2 hours boiling (Sullivan, 1966). The sugars in the hydrolysate may then be identified.

With the advent of Van Soest's detergent analysis scheme, it became an easy matter to isolate and work with the cell wall and its constituents. The use of neutral detergent on plant tissue leaves a residue which is essentially nitrogen-free and is composed of lignin and the structural polysaccharides, cellulose and hemicellulose. The use of acid detergent on a forage was originally intended as a preparatory procedure for lignin (Van Soest, 1975) but has commonly been used to obtain cellulose and lignin values by using the acid detergent lignin (ADL) procedure (Van Soest and Wine, 1968) and to obtain a hemicellulose value by subtracting ADF from NDF.

The Van Soest procedures are widely used because they are rapid, simple and not extremely expensive. The isolation of the cell walls by neutral detergent is a chemically, as well as structurally, rational procedure and is a convenient starting point for further work on cell wall constituents. Daughtry (1976) for example, combined the use of neutral detergent for cell wall preparation with boiling in 1 N H_2SO_4 to obtain what was termed a hemicellulose hydrolysate.

It is with the other Van Soest procedures, ADF and ADL, that problems arise. ADF minus lignin and silica, for example, is a close

estimate of cellulose (Van Soest and Wine, 1968) but has been found to be contaminated with hemicellulosic residues (Bailey and Ulyatt, 1970; Morrison, 1980). ADL, on the other hand, gives lignin values quite different from lignin values obtained on the same plant sample by other methods. Lignin, like hemicellulose, is difficult to define and becomes defined by the procedure used to isolate it. Many, and sometimes imaginative, adaptations have been made on the Van Soest procedures in an attempt to get fractions that behave in a uniform manner. However, since the cell wall fractions are not uniform, it is unlikely that a simple evaluation procedure will isolate a recognizably uniform entity in all forages at all ages under all environmental conditions.

Recalling that hemicellulose becomes defined by the procedure used to isolate it, these two different approaches-- solubility in alkali and hydrolysis in acid -- can be seen to, in effect, act as a check on each other. The former, by elucidating the chemistry and structure of hemicellulose piece by piece, acts as a baseline against which the results of the more rapid, latter methodology can be checked for accuracy. It is with a very critical eye that information on "hemicellulose" should be examined and compared because, as has been seen, these hemicelluloses may or may not be representative of the hemicelluloses present in plants.

Hemicellulose: Structure

Hemicellulose was originally thought to be a precursor of cellulose, hence the name hemi - cellulose. While the reasoning has been

proven wrong, the name persists. Cellulose is a linear polymer of β-1-4 linked D-glucose units; repeating unit 4-O-β-D-glucopyranosyl-D-glucopyranose, cellobiose (Bailey, 1973). Hemicellulose is composed of two major groups of polysaccharides: pentosans and non-cellulosic hexosans.

Bailey (1973) reviewed the literature on chemical composition of hemicellulose from which the following information is taken. The pentosans are primarily based on xylose. They appear to be linear chains of β-1-4 linked D-xylopyranose units; repeating unit 4-O-β-D-xylanopyranosyl-D xylopyranose, xylobiose. To these chains are attached L-arabinose, D-glucuronic acid or its 4-O-methyl ether, D-galactose and possibly D-glucose. These sugars are usually single side chains but may be connecting points for further branching, particularly via L-arabinose. Here also, is where the linkages with lignin polymers occur via ferulic acid, other phenolic acids or their esters (Wilkie, 1979; Morrison, 1980).

The hexosans in forage grasses are primarily mannans, either glucomannans or galactoglucomannans. These are linear chains of β-1-4 linked D-glucose and D-mannose. The glucomannans are usually free of other sugars, the galactoglucomannans have α-linked galactose side chains. There has also been some evidence (Fraser and Wilkie, 1971; Wilkie and Woo, 1976) of the presence of linear chains of β-linked D-glucose units which are not cellulosic. The glucans have both β-1-3 and β-1-4 linkages. The ratio of the 1-3 to 1-4 linkages in the glucans in any one tissue falls as the plant matures (Buchala et al., 1972). A more thorough discussion of the structural

features in hemicelluloses can be found in a review chapter by Wilkie (1979).

Albersheim and coworkers (Bauer et al., 1973; Keegstra et al., 1973; Talmadge et al., 1973) working with dicotyledonous plants have developed a cell wall model. The cellulose fibers are linked together by other polysaccharides, in particular, hemicellulosic xyloglucans which completely cover the cellulose fibrils (Albersheim, 1978). Their preliminary work on grasses suggests that a model of the cell wall for monocotyledonous plants will be similar to that for dicots. The dominant hemicellulosic polysaccharide in primary cell walls of monocots is an arabinoxylan rather than a xyloglucan (Albersheim, 1978). A modified cell wall model (Bailey et al., 1976) proposes that much of the hemicellulose and all of the pectin is not linked to other CWC and only some of the hemicellulose is bonded to glycoprotein and cellulose. They also point out that cellulose is not the only crystalline structure and that hemicellulosic xylan is capable of forming a crystalline structure.

Hemicellulose in Plants

Hemicelluloses comprise from 10 - 30% of the total dry matter in temperate and tropical grasses (Bailey, 1973) and from 30 - 60% of the cell wall in tropical grasses (Hartadi, 1980; Bailey and Connor, 1973; Russo et al., 1981). While it is difficult to compare hemicellulose values reported by various researchers due to differences in definitions and procedures, it appears that grasses contain more hemicellulose than legumes (Bailey, 1973). Further, the

hemicellulose in grasses is different structurally from legumes. Gaillard (1965) reported that the hemicellulose A fraction of grasses was primarily an arabinoxylan while in legumes it was a xylan with some uronic acids. Legumes seem to have more uronic acids than grasses and some additional sugars such as rhamnose which are not present in grasses (Gaillard, 1965; Collings and Yokoyama, 1979). Lignin has long been noted to be higher in legumes while seemingly having less effect on the digestibility of legumes than it does on grasses. Gordon and Gaillard (1976) suggest that this difference could be due to the different types of hemicellulose present in grasses and legumes and the subsequent differences in lignin-carbohydrate bonds.

The hemicellulose content of a plant changes with growth and maturity. The most marked change in hemicellulose composition in a grass is in the growth period before changing from a vegetative to a floral morphology (Bailey and Connor, 1973). It is expected that hemicellulose content will increase with maturation. Bailey (1973) reported on several studies with temperate grasses where the increase in hemicellulose with age was associated with in increase in stem tissue. For tropical grasses, however, seasonal changes did not appear so great, the hemicellulose content remaining about the same in spite of increasing cell wall content and increasing proportion of stems.

The greatest change in hemicellulose content occurs during growth. The leaves of speargrass (Heteropogon contortus) at vegetative, early seed and dormant stages had 23, 34 and 36% hemicellulose

(of the total dry matter); the stem in early-seed and dormant stages had 37 and 42% hemicellulose (Blake and Richards, 1971). Reid and Wilkie (1969a) found that in older, mature plants, there was little apparent change in the hemicellulose of either leaves or stems. Mature stems of rye, barley, orchardgrass, oats, ryegrass, timothy and wheat had values of 23 - 28% for hemicellulose (Wilkie, 1979) while mature leaves of oats, barley, wheat, kikuyugrass and pampas-grass had values of 21 - 37% for hemicellulose.

Monosaccharide analysis (Buchala and Wilkie, 1973) of the hemicellulose fraction found that with increasing maturity, there was an increase in amount of D-xylose in all tissues (stems and leaves), L-arabinose and D-glucose decreased and D-galactose remained constant or varied slightly.

The hemicellulose changes with growth may be linked to formation of the secondary wall. When a plant cell wall stops elongating, it produces a thicker secondary wall and at this stage, lignin begins to be synthesized into the wall along with continuing synthesis of structural carbohydrates found in the primary wall (Morrison, 1979). It is at this stage that lignin and hemicellulose become linked to each other. With respect to cellulose, its structure does not change during growth. Hemicellulose, on the other hand, does change its structure during growth and with increasing lignification (Morrison, 1979). These changes have sometimes unexpected effects on digestibility, which will be discussed in a later section.

Bailey (1973) reported that in temperate grasses the cellulose content is greater than or equal to the hemicellulose content but,

in tropical grasses, the hemicellulose content is greater than or equal to the cellulose content. A further difference between temperate and tropical grasses is in monosaccharides present in the hemicellulose fraction. Ojima and Isawa (1968) showed that two tropical grasses (Paspalum notatum and Cynodon dactylon) had xylose: arabinose:glucose:galactose ratios of 50:15:30:2 while two temperate grasses (Phleum pratense and Lolium perenne) had ratios of the same sugars of 74:12:11:2.

It is difficult to separate out environmental effects. The possibility exists that the differences in CWC of temperate and tropical grasses may be due to a temperature effect (Cooper and Tainton, 1968). Anatomical and photosynthetic differences do exist. Wilson and Ford (1971) studied three grasses (Panicum maximum, Setaria sphacelata and Lolium perenne) under a controlled environment and found that the effect of increasing temperature on CWC is not linear but passes through a maximum; this maximum may be due to hemicellulose rather than cellulose differences (Bailey, 1973). Reid and Wilkie (1969b) found little difference in monosaccharide composition of hemicelluloses of oat plants grown indoors or out, in light or in dark. Hemicellulose content and composition are not constant and are apparently influenced by environmental conditions.

Almost every study on hemicellulose contains values for cultivars, species and genera and many attempts have been made to relate hemicellulose values to intraspecific or intrageneric variations. The results can be very confusing and probably of little value. As Wilkie pointed out (1979), "it is not possible to determine invariant,

quantitative values for total hemicelluloses . . . and there are no self-evident parameters upon which to base such quantitative comparisons, particularly at the taxa level." (p. 251) Fraser and Wilkie (1971) re-emphasized the danger of comparative studies, the danger arising when the procedure used in isolating materials for study is either undesirably selective or inadequately reproducible. With so many hemicellulose procedures in use, it appears that comparative studies should be limited to within locations or laboratories.

Hemicellulose Digestion in the Ruminant

Ruminants obtain a great portion of their dietary energy from the digestion of cell wall polysaccharides when they are fed forage diets. The digestibility of the CWC is very crucial in determining the quality of the forage. Intake, too, is dependent on the breakdown of the CWC in the rumen. This digestion involves initially enzymatic hydrolysis by various rumen microorganisms to release sugars. These sugars can then be fermented to volatile fatty acids. It is at the initial stages of hydrolysis of the various cell wall polysaccharides that differences occur and when such properties as degree of lignification (Bailey et al., 1976) or chemical structure of the polysaccharides affect the digestion of the CWC.

In vitro Studies

Hemicellulose and its component monosaccharides can be separated from plant tissue by chemical methods. Earlier *in vitro* work on hemicellulose degradation in the rumen used these isolated fractions to determine digestibility of hemicellulose. Gaillard (1965) found

that legume hemicellulose was more resistant to digestion than grass hemicellulose; of the linear A and B fractions, A was more resistant; and of the B fractions, the branched B fraction was more resistant to digestion than the linear B. Sullivan (1966) also showed that grass and legume hemicelluloses differed in their digestibilities and suggested that it may be due to a difference in the chemical heterogeneity of the xylans. Waite and others (1964) found that during growth in timothy, ryegrass and orchardgrass, the xylan fraction containing the uronic acids became less digestible. Dekker and Richards (1973) found the glycans in hemicellulose to be more digestible than xylan or uronic acid.

Gaillard (1965), Waite et al., (1964) and Bailey (1967) working with temperate grasses, found the pectins, arabinans and galactans to be extensively digested in young plants but incompletely digested in older plants or in hay. They also concluded that xylans are incompletely digested in mature plants and hay.

Other work has shown that rather than the chemical heterogeneity of the hemicellulose being responsible for lower digestion, that the incomplete digestion was due to the presence of lignin. Beveridge and Richards (1975) studying speargrass, showed no significant difference between the hemicellulose composition of fresh samples and of samples digested for 72 hrs in the rumen. In their earlier work (Dekker, Richards and Playne, 1972) the hypothesis had been that branching would cause resistance to digestion but since there were no differences between the original and "resistant" hemicelluloses in the later study, they attributed the poorer digestibility

to lignification. Bailey and MacRae (1970) isolated "digestion-resistant" hemicelluloses which were capable of further digestion after they had been delignified with $NaClO_2$. Dehority et al. (1962) delignified timothy, alfalfa and orchardgrass by ball-milling and found that hemicellulose fermentation increased. The increases became greater with maturity and, again, they offered the theory that lignin provided a physical barrier preventing degradation of the hemicellulose molecules.

In studies using isolated strains or species of rumen hemicellulolytic bacteria, Dehority (1973) found that fermentation of hemicellulose occurred in two stages. The first stage involved one species of bacteria which degraded or depolymerized the hemicellulose but did not utilize it. In the second stage, another type of bacteria which had limited degrading abilities was able to utilize hemicellulose that had been degraded by the first group. Dehority (1973) also found that cellulolytic bacteria could degrade hemicellulose. These synergistic systems may be oversimplifications but the apparent specificity of the rumen bacteria provides another explanation for variabilities in hemicellulose digestion.

Another avenue of <u>in vitro</u> studies is the use of depectinated cell walls and mixtures of mold cellulase and hemicellulase to study the patterns of hemicellulose digestion. With the enzyme systems, hemicellulose hydrolysis was shown to differ between ryegrass varieties and the extent of hydrolysis decreased with maturity (Bailey and Jones, 1971; 1973). Jones and Bailey (1972) found that prolonged drying did not affect the rate or extent of enzymatic hydrolysis.

Studies on isolated hemicelluloses, its components or enzymes from isolated rumen microorganisms give only a limited idea of what is going on in the rumen. More recent research has been on total plant hemicellulose and *in vivo* studies of hemicellulose digestion in the rumen.

In vivo Studies

Beever et al. (1971) studied the sites of carbohydrate digestion by fitting sheep with rumen cannulas and re-entrant cannulas in the proximal duodenum and terminal ileum. Their results showed that 90% of the cellulose and about 70% of the hemicellulose (in ryegrass) was digested before entering the small intestine. Very little hemicellulose digestion occurred in the small intestine itself but approximately 30% of the hemicellulose was digested in the cecum and colon. These values are averages for fresh, dried and ensiled ryegrass. Other experiments with sheep also showed a significant post-ruminal digestion of hemicellulose especially when the forage was ground and pelleted (Thomson et al., 1972).

Van't Klooster and Gaillard (1976) showed that distal to the duodenum in dairy cows hardly any digestion of hemicellulose occurred on diets of hay, grass and/or concentrates. Digestion of cellulose was almost completed before the small intestine.

In a review of several digestion studies, Ulyatt et al. (1975) found that the proportion of hemicellulose digested in the large intestine was higher than that of cellulose. An analysis of these and other results (Bailey et al., 1976) showed that in forages as the hemicellulose increases, the percent digested in the rumen

increases. The differences noted in all of these studies in the contribution of the lower gastrointestinal tract to digestion of hemicellulose may be due to species differences between cattle and sheep, to the type of feed used or to differences in techniques.

Overall, the in vivo studies show that hemicellulose digestion decreases with maturity and increasing lignification and that hemicellulose is less digestible than cellulose. Models for cell wall carbohydrates and their fate in the ruminant animal (Smith et al., 1971; McLeod and Minson, 1974; Abrams, 1980) suggest the existence of at least one digestible cell wall fraction and one indigestible fraction, generally accounted for by the presence or absence of lignin. Morrison (1979) discusses the physical theory vs. the chemical theory. The physical theory proposes that the lignin and hemicellulose form a complex which has a "cage" effect; in young tissue, the complex is not fully developed and digestion is easily accomplished by the rumen microbes but in older tissue, the "bars" of the "cage" are closer together and enzyme access is restricted. The chemical theory suggests that in young tissue, lignification is at a minimum and hemicelluloses are not very complex; with maturity, complexity of the structure increases. Morrison concluded that both theories may be involved; i.e., in young grass, the proportion of side chains is the major factor controlling digestion while with older tissue the lignin content is the dominant factor.

There are still many gaps in our knowledge of hemicellulose in the plant and its fate in the ruminant, especially with respect to the tropical grasses. The literature on hemicellulose digestibility

is often contradictory but there is the possibility that significant hemicellulose digestion occurs after the rumen and that animal performance is related to the digestibility of hemicellulose. Because of the high hemicellulose content of tropical grasses, the acid-soluble hemicellulose from the Van Soest method may not be an acceptable estimate of the hemicellulose in these plants. An alternate method of measuring hemicellulose is by alkali extraction from plant cell walls. The hemicellulose obtained in this manner may be more representative of the hemicellulose in the plant than the hemicellulose obtained by the Van Soest analysis. Alkali soluble hemicellulose could be a major factor influencing forage quality in tropical grasses. More information on the hemicellulose fraction of tropical grasses may lead to a better understanding of the factors influencing quality. This dissertation investigates cell wall content and digestibility in tropical grasses, with special emphasis on hemicellulose.

CHAPTER III
CHEMICAL COMPOSITION OF TROPICAL GRASS HAYS

Introduction

While the present official method of forage analysis is the proximate analysis system first developed in the late 1800's, the Van Soest (1965) system which partitions forages into cell contents (CC) and cell wall constituents (CWC) is now extensively used for evaluation of forages. Use of neutral detergent reagent on a forage leaves a residue which is essentially nitrogen-free and is composed of the structural components of the plant, the CWC (Van Soest, 1965; Van Soest and Wine, 1968). The CC, which were removed by the neutral detergent reagent, contain the sugars, starches, lipids and proteins. The CC are essentially digestible by the ruminant while the CWC have incomplete and differential digestibilities (Van Soest, 1975; Abrams, 1980). The Van Soest system of forage analysis is biologically rational and it has been recommended for grading hays by the American Forage and Grassland Council (AFGC) (Rohweder et al., 1976; Barnes and Marten, 1979).

Nevertheless, in spite of the widespread acceptance and use of the Van Soest system, problems still remain, particularly with respect to tropical and subtropical grasses (Moore and Mott, 1973). Due to physiological and photosynthetic differences and environmental influences, tropical grasses are higher in CWC than temperate

grasses (Cooper and Tainton, 1968; Wilson and Ford, 1971; Moore and Mott, 1973). Prediction equations such as proposed by the AFGC rank the tropical grasses very low due to their high CWC, yet animal performance does not necessarily reflect the predicted low quality of these grasses.

Due to these anomalies, questions arose as to whether the Van Soest method, which had been developed on temperate forages, was suitable for analysis of tropical grasses. While the separation of CC and CWC appears rational (Van Soest, 1975), the further fractionation of the CWC using acid detergent reagent to obtain cellulose, hemicellulose and lignin, warrants further examination. The breakdown of the CWC by the acid detergent reagents appears incongruent with earlier, classical work on hemicellulose, for example, which used alkaline conditions to fractionate the CWC. Resolution of the differences between the Van Soest hemicellulose and hemicellulose obtained via classical methodology may lead to a clearer understanding of the factors affecting forage quality in tropical grasses.

The objective of this study was to compare the cell wall fractions of tropical grass hays obtained by the Van Soest analysis with cell wall fractions obtained by classical methodology, involving an alkali extraction procedure.

Experimental Procedures

One cultivar of *Cynodon dactylon* ('Coastcross-1' bermudagrass), two experimental lines of *Digitaria* species (X124-4 and X46-2) and two cultivars of *Paspalum notatum* ('Paraguay' and 'Argentine'

bahiagrass) grown in an upland sandy soil in north-central Florida during the spring-summer were used in this experiment. At the beginning of the growing season a complete fertilizer was applied to provide 72 kg N, 72 kg P_2O_5 and 72 kg K_2O/ha. Another nitrogen fertilization at a rate of 66 kg N/ha was applied during July. All plots were staged during July and August by mowing and removing the growth; thereafter, two field replications of the 2, 4, 6 and 8 week regrowths were harvested and artificially dried in a portable wagon at approximately 50 C. The dry hays were stored in woven bags and chopped prior to feeding.

Voluntary intake and digestibility were determined simultaneously by feeding the grasses ad libitum to mature wethers. Each hay was fed to at least two (and often three) wethers in separate trials, with no wether receiving the same hay twice. Animals were treated for internal parasites, fed a standard hay and weighed before each trial. The seven day collection period was preceded by a fourteen day preliminary period. Each animal was housed in a wooden pen with slotted floors and had access to water, trace mineralized salt and deflourinated phosphate at all times. Ad libitum feeding was achieved by allowing a feed refusal (orts) of 200-300 g of hay daily. Daily samples were taken of the hay offered during the trial. The orts were removed and weighed daily. Feces were collected in canvas bags, weighed daily and 20% kept for further analyses. The waste (hay dropped through the floor of the cages) was weighed. All samples were dried at 50 C. Each sample was pooled by animal, ground to pass a 4 mm screen in a Wiley mill, mixed and a

representative portion reground through a 1 mm screen. A composite hay sample was made by mixing proportional amounts from each animal fed that specific hay. Animals were weighed at the end of the trial. The composite hay samples were used for the laboratory analyses in this experiment.

The hay samples were analyzed for dry matter (DM) and organic matter (OM) (A.O.A.C., 1975). Neutral detergent fiber (NDF) was determined as outlined by Goering and Van Soest (1970) using sintered glass crucibles for filtration. NDF residues and all subsequent residues were dried at 55 C in a forced air oven overnight and at 55 C in a vacuum oven for 1-3 hrs.

Hartadi (1980) determined NDF (using glass wool for filtration) termed NDF(H), acid detergent fiber (ADF), permanganate lignin (PLIG), hemicellulose (HEMV), cellulose (CELLV) and crude protein (CP) (A.O.A.C., 1975; Goering and Van Soest, 1970).

The NDF residues were delignified with sodium chlorite ($NaClO_2$) according to a procedure adapted from Whistler et al. (1948). An Eberbach heated shaking water bath set at 70 C was fitted with clamps to hold six 500 ml Erlenmeyer flasks and was placed in an explosion proof hood. Rubber tubing, attached to a manifold, was directed above each flask to deliver CO_2 continuously in order to displace ClO_2 formed during the reaction. NDF residues were placed in the flasks and $NaClO_2$ equivalent to the weight of the sample and 100 ml of 1% acetic acid were added to each flask. The weight of NDF residue transferred was determined by weighing the sintered glass crucible before and after the transfer. Flasks were placed in

the water bath and shaken for 30 minutes. Flasks were removed from the bath in order to add an additional .5 gm of $NaClO_2$. The flasks were returned to the bath and shaken for an additional 15 minutes. The flasks were then removed from the water bath to a rack above the bath, continually flushed with CO_2 and allowed to cool. Enough ascorbic acid was added to stop the reaction (color changed from bright yellow to pale yellow or white). Each sample was filtered on a sintered glass crucible, and the residue obtained was termed holocellulose (HOLO); the soluble portion was termed lignin (LIG).

The holocellulose residues were further fractionated to cellulose and hemicellulose by the use of KOH as suggested by Whistler and Gaillard (1961), Bailey et al. (1976) and Wilkie (1979). Holocellulose residues were transferred to 100 ml glass bottles with plastic screw-on caps. Weights were determined by difference, as for NDF. Fifty milliliters of 5% KOH was added and the bottles flushed with N_2. Up to 20 samples at a time were shaken intermittently in an Eberbach shaker for 24 hrs. After 24 hrs, an additional 9.5 gm KOH was added to each bottle (to bring the final solution up to 24% KOH), the bottles were again flushed with N_2 and shaken an additional 24 hrs. The samples were filtered on sintered glass crucibles fitted to 250 ml side-arm Erlenmeyer flasks. The residue was washed with 10% acetic acid and water and was termed cellulose (CELL). The filtrate was immediately neutralized to pH 7 by the addition of acetic acid and refrigerated. The filtrate contained the hemicellulosic portion of the cell walls (HC). CW components were expressed as the percentage of NDF.

A list of abbreviations is included in Table 1. Descriptions of hays and composition data are listed in Appendix Tables 14 - 17. Statistical analysis (S.A.S., 1979) included the general linear model procedure and simple correlations.

Results and Discussion

Analyses of variance for the chemical composition of the hays are presented in Table 2. Cultivar and age were the main effects. Age generally did not have a consistent linear effect on chemical parameters. For all parameters, hay (cultivar) and age were significant. Significant interactions of hay x age occurred only for NDF, NDF(H), HC, HEMV, CELL and PLIG. Except for HOLO and LIG (which sum to 100 for each sample), the R^2 values were .89 - .94, showing that the effects of hay and age explained most of the variation in chemical composition.

One of the objectives of this experiment was to determine if the HC, CELL and LIG values were different from HEMV, CELLV and PLIG or, in other words, was the $NaClO_2$-KOH method different from the Van Soest analyses. The two procedures ($NaClO_2$-KOH vs. Van Soest) were compared by analysis of variance of hemicellulose, cellulose and lignin (Table 3). For all 3 variables, methods were different. The overall means for $NaClO_2$-KOH vs. Van Soest's analysis, respectively, were hemicellulose 51.8 vs. 45.2; cellulose 40.8 vs. 43.5; and lignin 7.4 vs. 8.6 (% of NDF).

The correlation between values obtained for the various chemical fractions by the two methods are in Table 4. High correlations

Table 1. Abbreviations used in the dissertation.

ADF	Acid detergent fiber, % of DM
CC	Cell contents, % of DM
CELL	Alkali-insoluble cellulose, % of NDF
CELLD	Cellulose digestibility, *in vivo*, %
CELLV	Van Soest cellulose (ADF - PLIG), % of NDF
CP	Crude protein, % of DM
CWC	Cell wall constituents, % of DM
DM	Dry matter, %
DNDF	Digestible neutral detergent fiber, % of DM
DOM	Digestible organic matter, % of DM
DOMI	Digestible organic matter intake, g/kg $W^{.75}$/day
HC	Alkali soluble hemicellulose, % of NDF
HCD	Hemicellulose digestibility, *in vivo*, %
HEMV	Van Soest hemicellulose (NDF - ADF), % of NDF
HOLO	Holocellulose, % of NDF
HOLOD	Holocellulose digestibility, *in vivo*, %
IVCELLD	*In vitro* cellulose digestion, rumen fluid only, %
IVCELLDP	*In vitro* cellulose digestion, rumen fluid plus pepsin, %
IVHCD	*In vitro* hemicellulose digestion, rumen fluid only, %
IVHCDP	*In vitro* hemicellulose digestion, rumen fluid plus pepsin, %
IVHOLOD	*In vitro* holocellulose digestion, rumen fluid only, %
IVHOLODP	*In vitro* holocellulose digestion, rumen fluid plus pepsin, %
IVNDFD	*In vitro* neutral detergent fiber digestion, rumen fluid only, %
IVNDFDP	*In vitro* neutral detergent fiber digestion, rumen fluid plus pepsin, %

Table 1 - continued.

LIG	Lignin (100 - HOLO), % of NDF
NDF	Neutral detergent fiber, % of DM
NDFD	Neutral detergent fiber digestibility, *in vivo*, %
NDF(H)	Neutral detergent fiber (Hartadi, 1980), % of DM
NDFI	Neutral detergent fiber intake, g/kg $W^{.75}$/day
OM	Organic matter, % of DM
OMD	Organic matter digestibility, *in vivo*, %
OMI	Organic matter intake, g/kg $W^{.75}$/day
PLIG	Permanganate lignin, % of DM

Table 2. Analysis of variance of chemical composition of forty hays.[a]

		P Values								
	df	NDF[b]	NDF(H)	HOLO	HC	HEMV	CELL	CELLV	LIG	PLIG
Hay	4	.0001	.0001	.0679	.0001	.0001	.0001	.0001	.0679	.0005
Age	3	.0001	.0001	.0655	.0002	.0001	.0001	.0001	.0655	.0001
H x A	12	.0094	.0088	.1588	.0239	.0070	.0023	.5491	.1588	.0285
R^2		.94	.96	.66	.91	.91	.92	.94	.66	.89
C.V.		1.5	1.3	.8	2.2	3.0	2.4	3.0	9.4	7.7
S.D.		1.2	1.0	.7	1.1	1.4	1.0	1.3	.7	.7
Mean		79.7	76.7	92.6	51.8	45.2	40.8	43.5	7.4	8.6

[a]Values expressed as percentage of NDF

[b]Abbreviations may be found in Table 1.

Table 3. Analysis of variance of method differences.[a]

		P Values		
	df	Hemicellulose[b]	Cellulose	Lignin
Hay (H)	4	.0001	.0001	.1364
Age (A)	3	.0001	.0001	.0001
H x A	12	.0001	.0034	.0193
Method (M)	1	.0001	.0001	.0001
H x M	4	.0001	.0029	.0001
A x M	3	.0111	.0026	.0001
A x H x M	12	.2122	.3154	.0836
C.V.		2.6	2.7	8.4
S.D.		1.3	1.2	.7
Mean		48.5	42.1	8.0

[a]The two methods are $NaClO_2$-KOH and the Van Soest analysis.

[b]Values expressed as percentage of NDF.

Table 4. Correlation coefficients (r) between variables obtained by two methods.[a,b]

	$NaClO_2$-KOH				
	NDF[c]	HOLO	HC	CELL	LIG
Van Soest					
NDF(H)[d]	.96***	.15	.10	-.06	-.15
HEMV	.03	.12	.71***	-.73***	-.12
CELLV	-.04	-.24	-.86***	.86***	.24
PLIG	.55***	.03	-.01	.02	-.03

[a]The two methods are $NaClO_2$-KOH and the Van Soest analysis.

[b]Abbreviations are found in Table 1.

[c]Analyzed by Van Soest method and recovered on sintered glass.

[d]Analyzed by Van Soest method and recovered using glass wool.

*** P<.001

existed between the two hemicellulose fractions (r=.71) and the two cellulose fractions (r=.86) but there was no relationship between the lignin values (r=-.03).

Differences due to method of analysis are more readily apparent when presented in graphic form. The actual numerical values for the means of each chemical analyses, grouped by age and cultivar, may be found in Appendix Tables 18 - 22.

Neutral Detergent Fiber

NDF values were higher than NDF(H) values (Figures 1 and 2), presumably due to differences in procedure. NDF(H) was filtered on Gooch crucibles packed with glass wool, which is more rapid than filtering on sintered glass crucibles, as the NDF samples were. NDF and NDF(H) analyses were conducted by two different analysts, at different times. Disregarding the numerical values of the two determinations, it can be seen that the lines follow the same pattern, all cultivars increasing in NDF from 2 to 4 weeks, then leveling off or decreasing. Overall, in both cases, the two digitgrasses are different from the bahiagrasses and the bermudagrass. The two analyses were highly correlated (Table 4). The NDF residues were used for subsequent fractionation in the present study.

It has often been stated that maturity is the greatest factor affecting forage quality. In many instances, the effect of age is linear; i.e., with increasing maturity, some factor increases and/or decreases. Well-known effects of maturity have been the increase in cell wall components and the decrease in crude protein and digestibility. The data presented here, however, generally do not exhibit

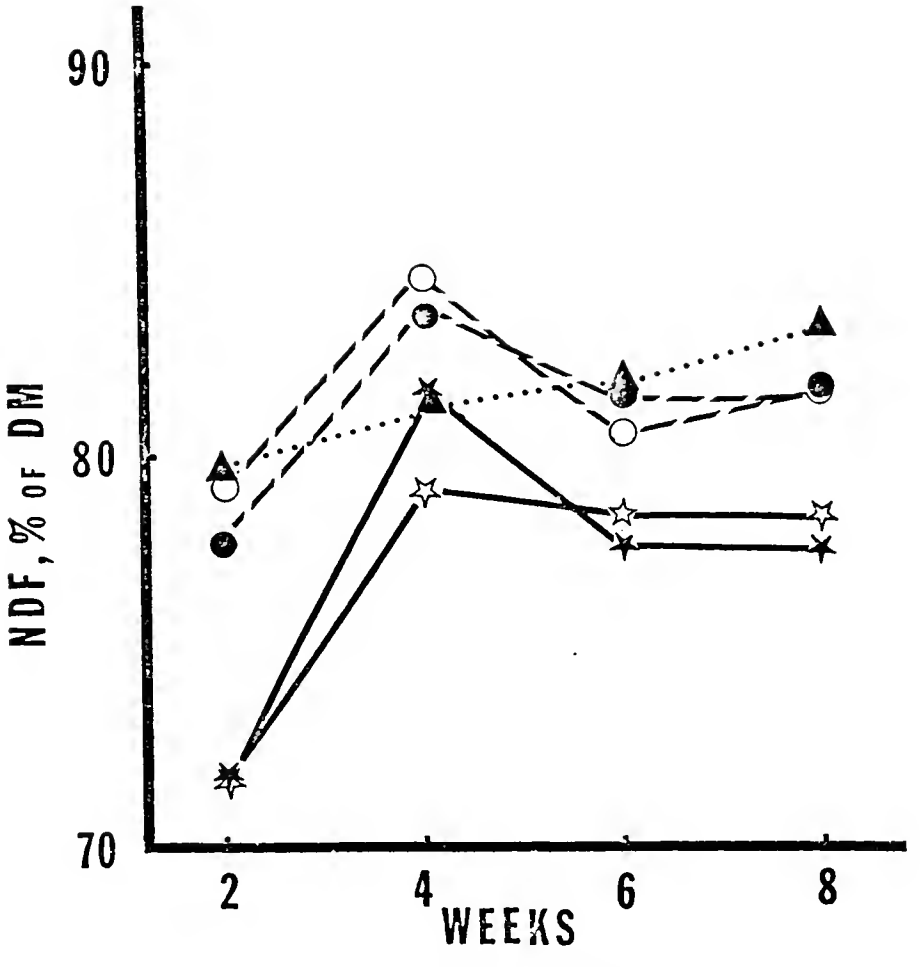

Figure 1. Change in NDF content by weeks and hay.

○- - -○ Argentine bahiagrass, ●- - -● Paraguay bahiagrass,

★——★ X46-2 digitgrass, ☆——☆ X124-4 digitgrass,

▲. . ▲ Coastcross-1 bermudagrass.

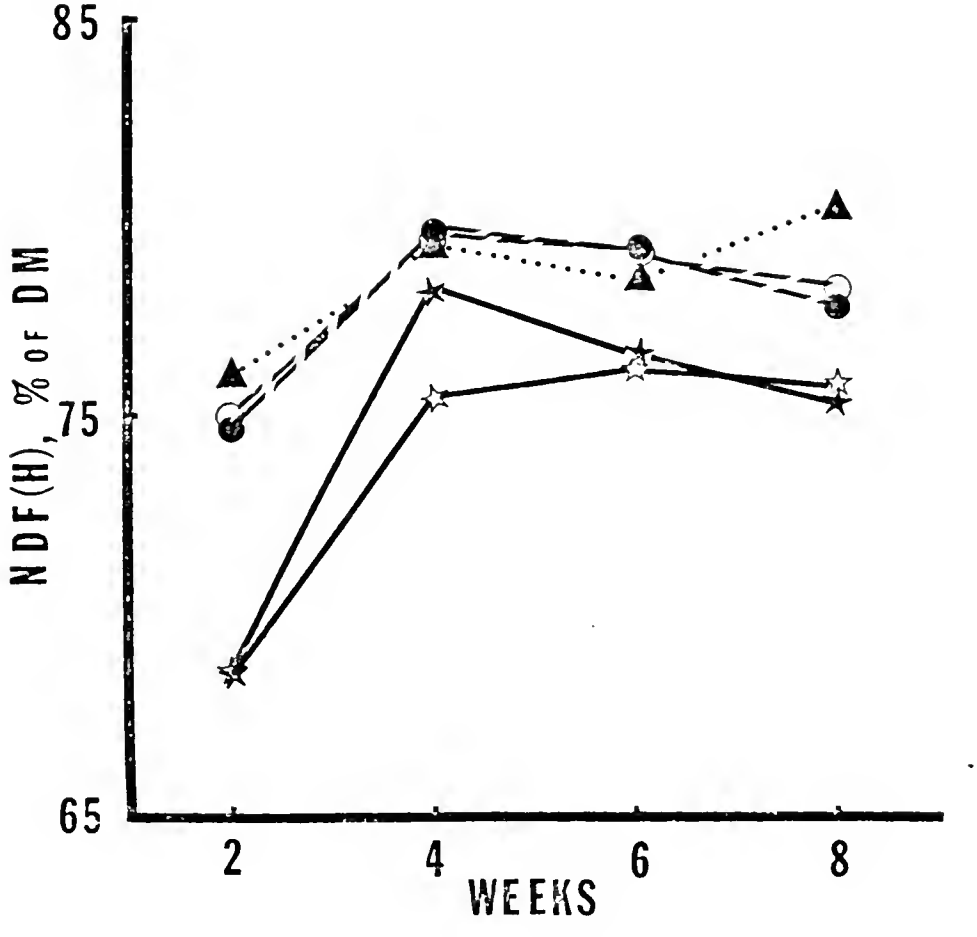

Figure 2. Change in NDF(H) content by weeks and hay.

○- - -○ Argentine bahiagrass, ●- - -● Paraguay bahiagrass,

★——★ X46-2 digitgrass, ☆——☆ X124-4 digitgrass,

▲. . .▲ Coastcross-1 bermudagrass.

a linear trend with maturity. One known event, extensive insect damage (striped grass looper, Mocis latipes) at 4 weeks to the digitgrasses and bahiagrass, may provide partial explanation for lack of linearity.

Holocellulose

Holocellulose, the delignified plant cell wall composed primarily of hemicellulose and cellulose, made up the major portion of the NDF fraction, ranging from 90.45 - 94.36% of NDF (Appendix Table 19). Holocellulose values, and all subsequent chemical composition values, were expressed as percentage of NDF rather than DM.

Holocellulose content of X46-2, X124-4 and Argentine cultivars were not different; Coastcross-1 was different from the former three cultivars and Paraguay fell between Coastcross-1 and the rest (Figure 3). Four of the cultivars showed an eventual decline in holocellulose content from 2 to 8 weeks but the interim pattern was erratic.

Earlier plant analysts felt that lignin interfered with the analysis of structural carbohydrates (Whistler et al., 1948). Adaption of wood chemistry methods led to the use of $NaClO_2$-KOH to de-lignify plant material. Packett et al. (1965) indicated that polysaccharide structures are left intact after delignification provided the procedure is not continued to reduce lignin values to 2% or less. Wilkie (1979), however, stated that delignification may oxidize some reducing-end residues and cause partial depolymerization. Whether or not hemicellulose is bonded to lignin is still uncertain although work by Albersheim et al.(Bauer et al., 1973; Keegstra et al., 1973; Talmadge et al., 1973) on dicotyledonous

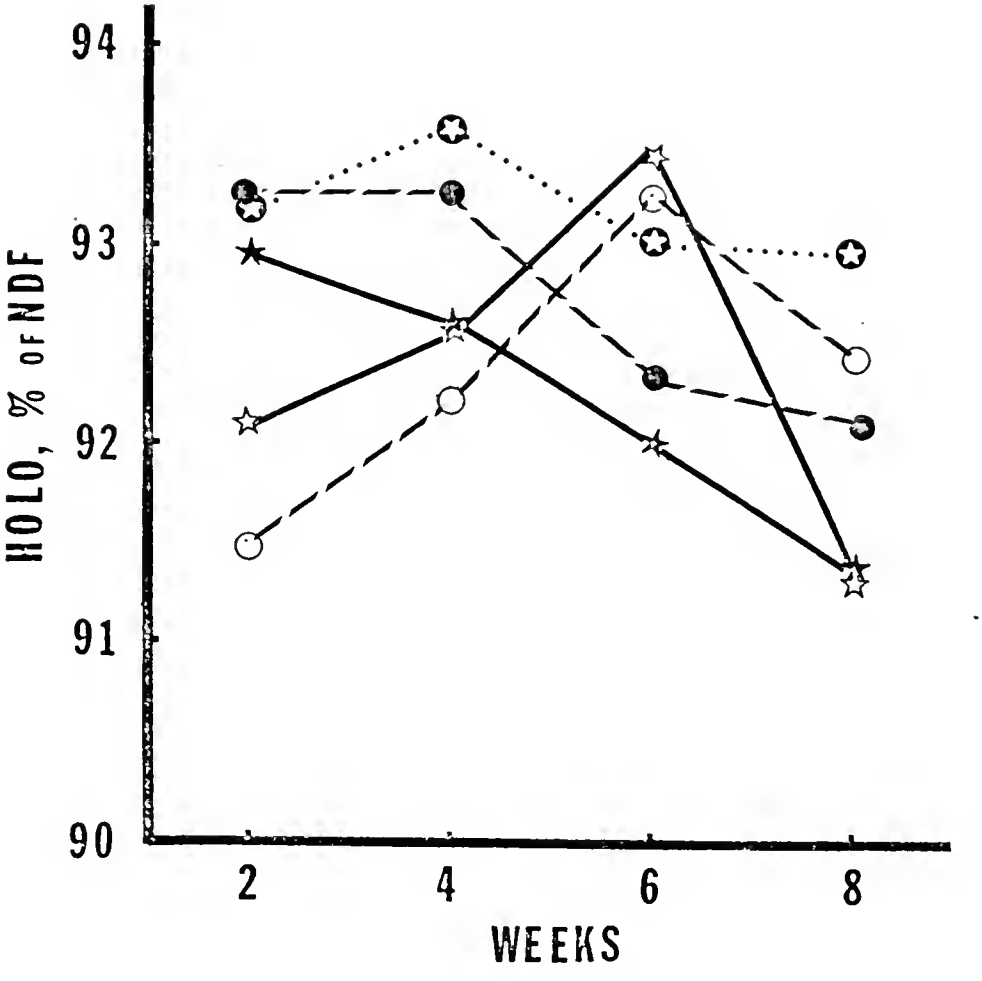

Figure 3. Change in holocellulose content by weeks and hay.

○- - -○ Argentine bahiagrass, ●- - -● Paraguay bahiagrass,

★——★ X46-2 digitgrass, ☆——☆ X124-4 digitgrass,

✪ · ✪ Coastcross-1 bermudagrass.

plants suggest that there is bonding between hemicellulose and lignin. Hemicellulose has been extracted from plants without prior delignification (Bailey et al., 1976) but the completeness of the extraction is unclear (Gaillard, 1958). It was felt on these tropical grass hays, which are high in CWC and lignin, the best procedure would be delignification prior to further analyses.

The holocellulose values, as an end in themselves, did not give a clearer or better picture of the plant than did NDF values, which are easier to obtain. The erratic pattern with maturity suggests some caution should be used in interpretation of the LIG data (100-HOLO).

Hemicellulose

Two methods of analyzing for hemicellulose were used - $NaClO_2$-KOH and the Van Soest detergent system. Figures 4 and 5 present the changes observed in hemicellulose content by cultivar and age (see Appendix Table 20 for means). HC values, except in the case of Coastcross-1, decreased only slightly with maturation which agrees with other work on hemicellulose in tropical grasses (Bailey, 1973). HEMV values, on the other hand and again excepting Coastcross-1, showed an overall greater decline with maturity.

HC values were higher as a percentage of NDF than were HEMV values. With both methods, X124-4 was different from the other grasses. The two bahiagrasses and X46-2 were similar in HC content compared to the other grasses while Coastcross-1 was different from the rest. The bahiagrasses and Coastcross-1 formed a similar group with respect to HEMV values, with the digitgrasses differing from each other and from the rest of the grasses.

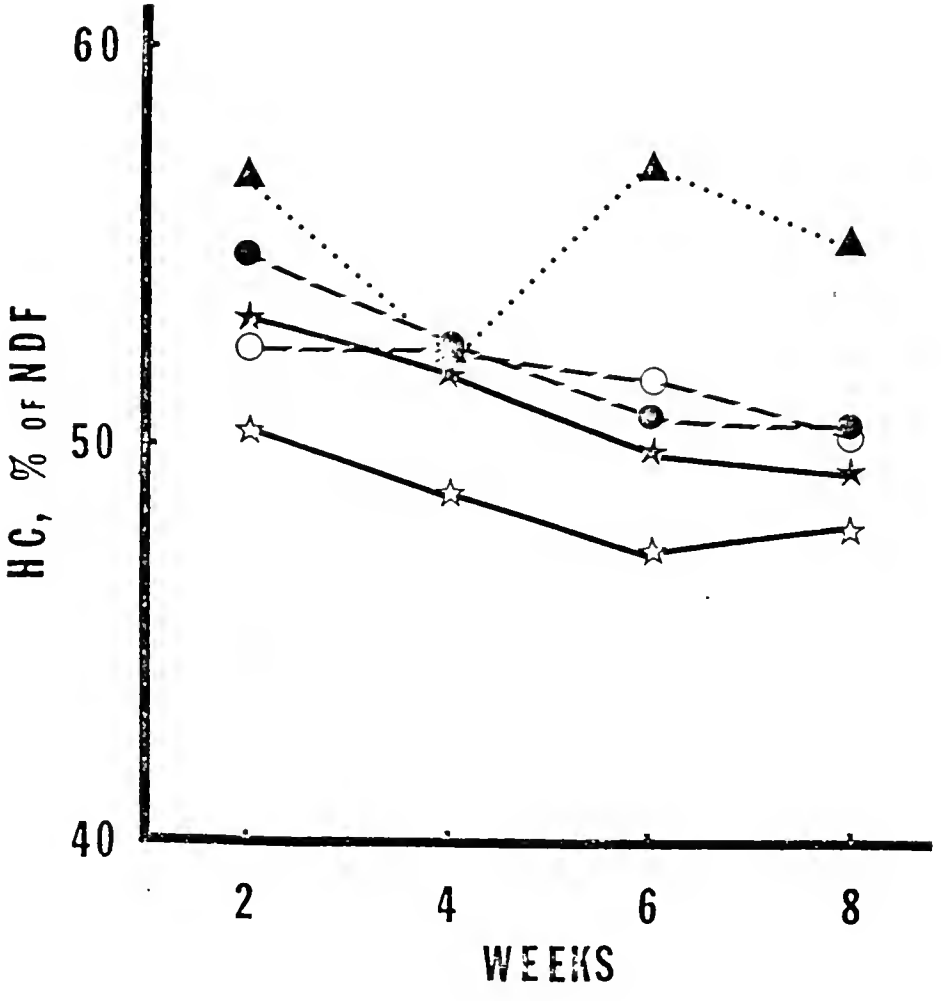

Figure 4. Change in alkali-soluble hemicellulose by weeks and hay.

○- - -○ Argentine bahiagrass, ●- - -● Paraguay bahiagrass,

★——★ X46-2 digitgrass, ☆——☆ X124-4 digitgrass,

▲· · ·▲ Coastcross-1 bermudagrass.

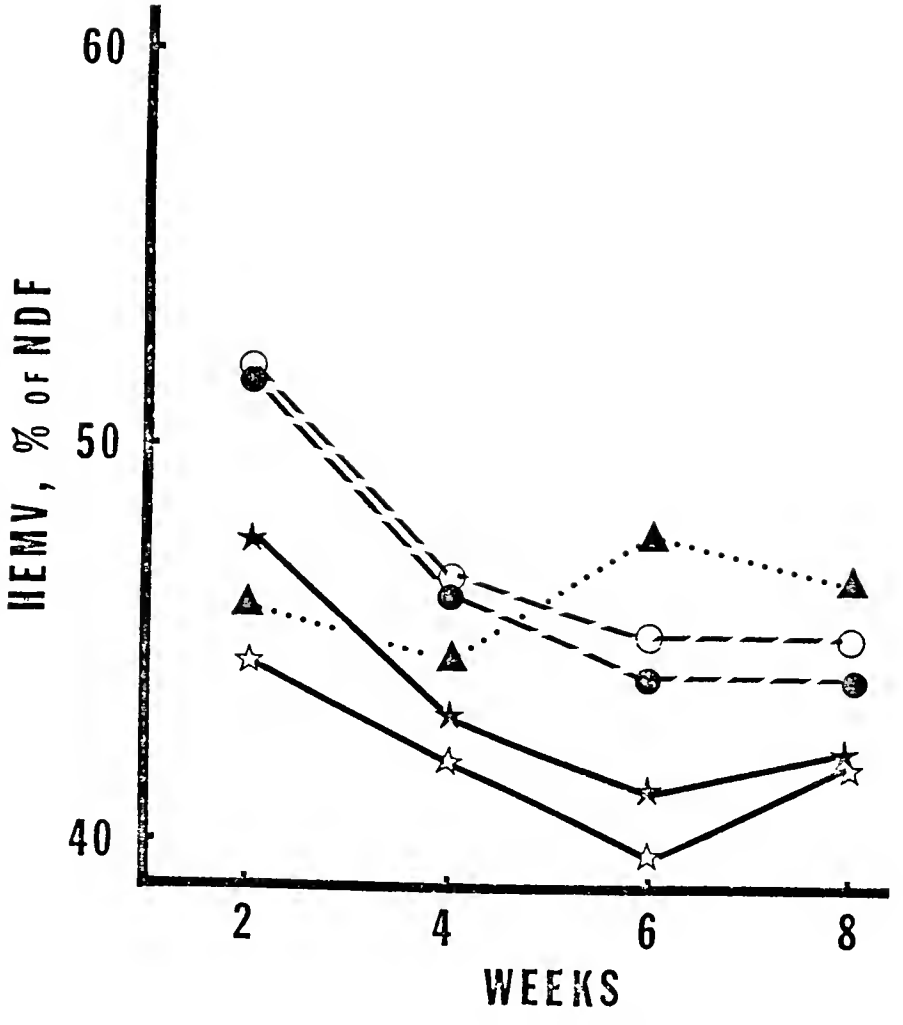

Figure 5. Change in Van Soest hemicellulose by weeks and hay.

○- - -○ Argentine bahiagrass, ●- - -● Paraguay bahiagrass,

★——★ X46-2 digitgrass, ☆——☆ X124-4 digitgrass,

▲· · ·▲ Coastcross-1 bermudagrass.

Hemicellulose has been defined as the cell wall and intercellular polysaccharides that can be extracted from higher land plant tissues that are, or were, lignified (Wilkie, 1979). More simply, hemicellulose refers to all of the types of polysaccharides found in plants other than cellulose, starch and fructans.

The preponderance of literature on hemicellulose deals with the Van Soest hemicellulose obtained by subtracting ADF from NDF. Work as early as 1970 (Bailey and Ulyatt) points out the problems inherent in this definition, viz., that there are hemicellulosic sugars present in the ADF residue (see also Morrison, 1980).

The hemicellulose content of a plant changes with growth and maturity and is expected to increase with maturity. Bailey (1973) reported on several studies with temperate grasses where the increase in hemicellulose content with age was associated with an increase in stem tissue. For tropical grasses, however, seasonal changes are not as marked, the hemicellulose content remaining about the same in spite of increasing CWC and increasing proportion of stems.

Hemicellulosic materials extracted by alkali may precipitate on neutralization and mild acidification at 0 - 2 C to form hemicellulose-A. The polysaccharides remaining in solution can be precipitated with ethanol to form hemicellulose-B. Repeated attempts to obtain a hemicellulose-A fraction failed. Scant information in the literature as reviewed by Bailey (1973) showed that hemicellulose-A comprises 1.0 - 19% of DM in Heteropogon contortus leaves and stems respectively, 1.4 - 3.2% of DM in Digitaria decumbens and .1 - .4% of DM in Setaria sphacelata. It would seem then, that the hemicellulose-A fraction is generally low in tropical grasses, thus providing

partial explanation for our failure to obtain the fraction. HC values presented here are representative of the hemicellulose-B fraction which consists primarily of xylans.

Cellulose

Cellulose obtained by either method (Figures 6 and 7) showed remarkably similar patterns (see Appendix Table 21 for means). In both instances, Coastcross-1 was very different (having the lowest amount of cellulose) from the other grasses; X46-2, Paraguay and Argentine cultivars were not different, and X124-4 had the highest amount of cellulose. CELL, collected on the sintered glass crucibles, was a pure white fibrous substance very similar in appearance to CELLV obtained by the ADF procedure.

Lignin

Lignin values (Figure 8) were calculated as 100 - HOLO. These lignin values are very different from the PLIG values (Figure 9; Appendix Table 22). Lignin, like hemicellulose, is defined by the method used to isolate it. The completeness of delignification of NDF was tested by staining holocellulose with phloroglucinol-HCl (a test for lignin). No stain was present in the holocellulose but it is likely that the material oxidized by the $NaClO_2$ included more than lignin. Obtaining a lignin value in this manner is not recommended due to the possibility of additional products forming during the oxidation of lignin from the cell walls by $NaClO_2$. Only Coastcross-1 had a different amount of LIG than the other grasses while PLIG amounts were different for each cultivar.

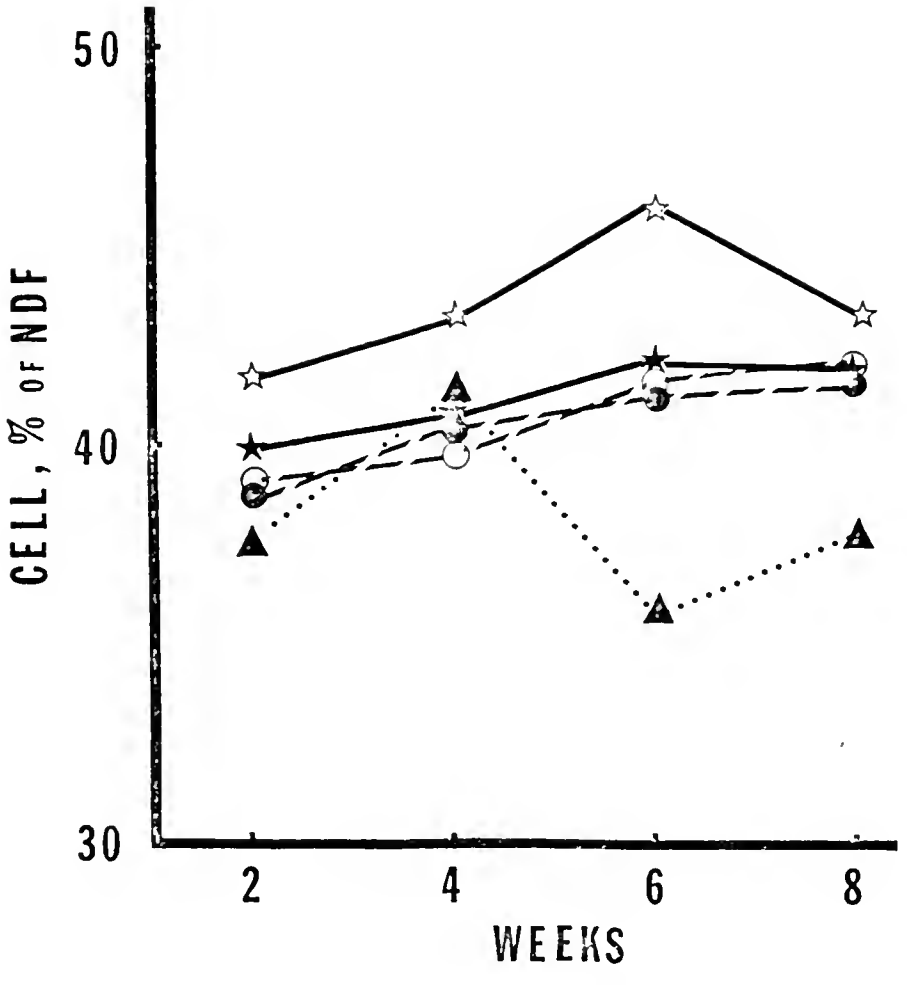

Figure 6. Change in alkali-insoluble cellulose by weeks and hay.

○- - -○ Argentine bahiagrass, ●- - -● Paraguay bahiagrass,

★——★ X46-2 digitgrass, ☆——☆ X124-4 digitgrass,

▲. . .▲ Coastcross-1 bermudagrass.

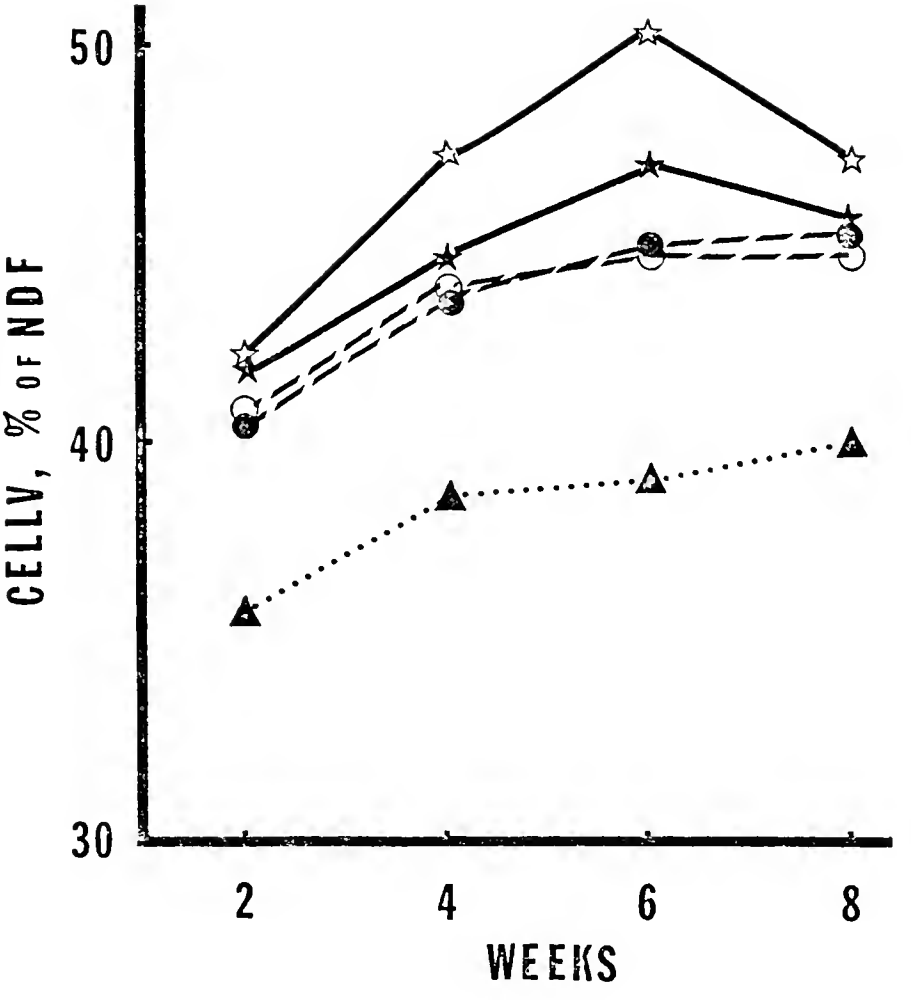

Figure 7. Change in Van Soest cellulose by weeks and hay.

○- - -○ Argentine bahiagrass, ●- - -● Paraguay bahiagrass,

★——☆ X46-2 digitgrass, ☆——☆ X124-4 digitgrass,

▲. . .▲ Coastcross-1 bermudagrass.

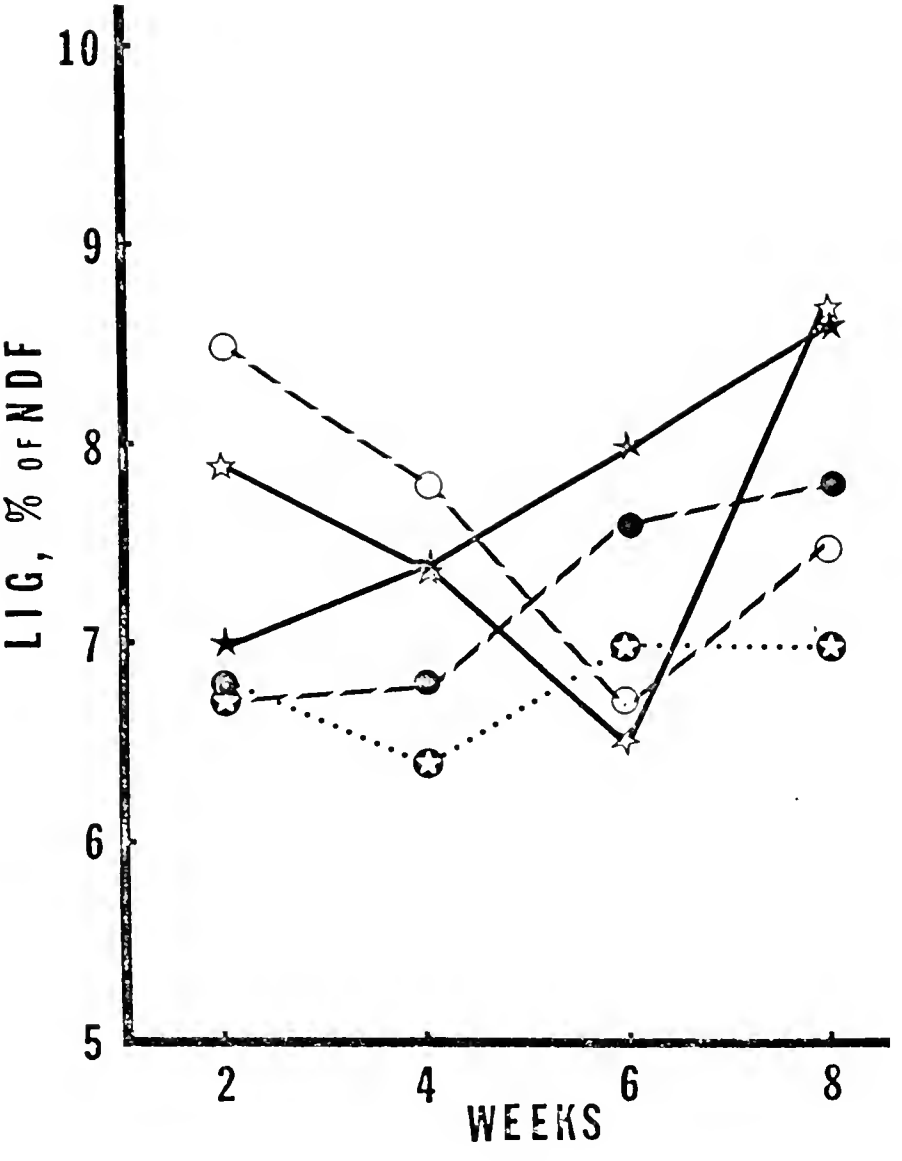

Figure 8. Change in chlorite lignin by weeks and hay.

○- - -○ Argentine bahiagrass, ●- - -● Paraguay bahiagrass,

★——★ X46-2 digitgrass, ☆——☆ X124-4 digitgrass,

✪ . ✪ Coastcross-1 berumudagrass.

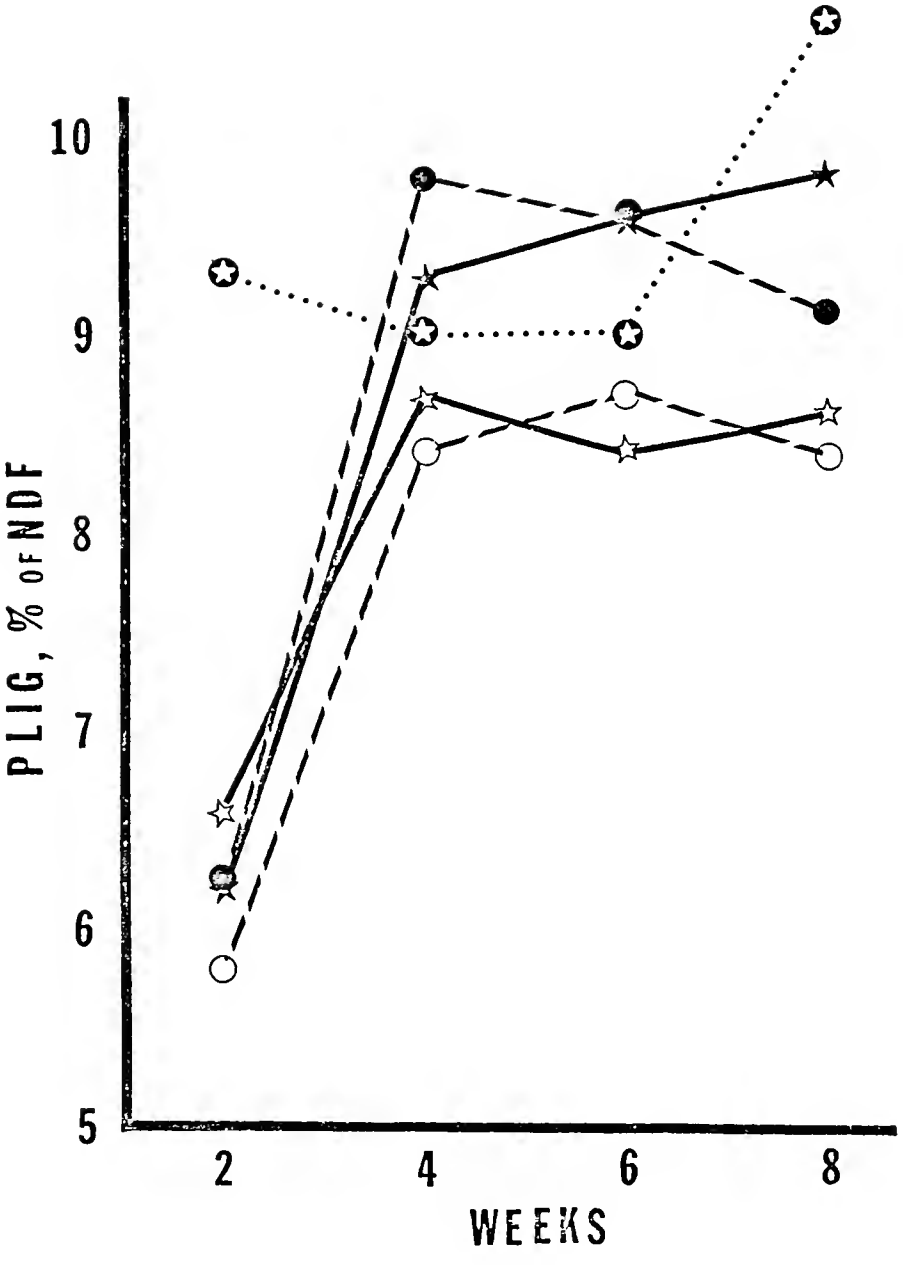

Figure 9. Change in permanganate lignin by weeks and hay.

○- - -○ Argentine bahiagrass, ●- - -● Paraguay bahiagrass,

★——★ X46-2 digitgrass, ☆——☆ X124-4 digitgrass,

✪. . .✪ Coastcross-1 bermudagrass.

Summary

One cultivar of Cynodon dactylon (Coastcross-1), two experimental lines of Digitaria species (X46-2 and X124-4) and two cultivars of Paspalum notatum (Argentine and Paraguay) cut at four ages each (2, 4, 6 and 8 weeks regrowths) were evaluated by chemical analyses. Composite samples for each hay were analyzed for organic matter and neutral detergent fiber. NDF fractions (cellulose, hemicellulose, lignin) were determined by two methods: one, the conventional Van Soest detergent analysis (CELLV, HEMV, PLIG); the other a classical fractionation using hypochlorite and alkali (HOLO, CELL, HC, LIG).

Values obtained by the two methods used in the analyses were significantly different. As percentages of NDF, HC values remained the same or decreased slightly with age for each cultivar while HEMV values showed a greater decline with maturity. CELL and CELLV values had similar patterns, increasing up to 6 weeks, then decreasing slightly. Correlation between HC and HEMV was .71 and between CELL and CELLV was .86. Further studies of these fractions to determine their digestibility and value in prediction equations are in subsequent chapters.

CHAPTER IV
IN VITRO AND *IN VIVO* DIGESTIBILITY OF TROPICAL GRASS HAYS

Introduction

Ruminants derive a great portion of their dietary energy from the digestion of cell wall polysaccharides when they are fed forage diets. The digestibility and intake of CWC is crucial in determining forage quality. Differences in breakdown of the CWC in the rumen are due, in part, to differences in chemical structure of the polysaccharides.

Early *in vitro* work on hemicellulose degradation in the rumen used isolated fractions to determine digestibility of hemicellulose. Two general theories arose from these studies. One theory attributes low hemicellulose digestibility to chemical heterogeneity of the hemicellulose structure (Gaillard, 1965; Sullivan, 1966; Dekker and Richards, 1973). Other work has shown that rather than the chemical heterogeneity of the hemicellulose being responsible for lower digestion, that the incomplete digestion was due to the presence of lignin (Bailey and MacRae, 1970; Beveridge and Richards, 1973). Morrison (1979) suggests that both theories may be involved, i.e., in young plants the chemical structure of the hemicellulose interferes with digestion processes while in older plants, increasing lignification causes lower digestibility.

Studies on isolated hemicelluloses and its components give only a limited idea of what is occurring in the rumen. Beever et al. (1971) in *in vivo* studies, showed that 70% of the hemicellulose digestion occurred before entering the small intestine, very little hemicellulose digestion occurred in the small intestine and approximately 30% of the hemicellulose digestion occurred in the cecum and colon. A review of several digestion studies (Ulyatt et al., 1975) found that the proportion of hemicellulose digested in the large intestine was higher than that of cellulose. Bailey et al. (1976) found that as hemicellulose content increased, the percent digested in the rumen decreased. Ulyatt and Egan (1979) found 77 to 94% of digestible hemicellulose apparently digested in the rumen, and 6 to 23% in the intestines. Some explanation of the confusing *in vivo* results may be provided by Gaillard and Richards (1975) who suggest the presence of a lignin-carbohydrate complex which passes from the rumen in solution, is precipitated in the lower pH of the abomasum, undergoes some redissolution at later stages in the lower gastrointestinal tract, and emerges in the feces.

An intermediate step, then, between studying the digestibility of isolated hemicellulose and other CWC fractions and the complex *in vivo* studies appears to be *in vitro* studies on whole plant tissue, followed by analyses of the various fractions.

The objectives of this study were to determine the *in vitro* and *in vivo* digestibilities of various fractions of tropical grass hays.

Experimental Procedures

One cultivar of Cynodon dactylon (Coastcross-1 bermudagrass), two experimental lines of Digitaria (X124-4 and X46-2) and two cultivars of Paspalum notatum (Paraguay and Argentine bahiagrass) harvested after 2, 4, 6 and 8 weeks of regrowth with two field replications were used in this experiment (Abrams, 1980). The grasses were artificially dried and chopped.

The grasses were fed ad libitum as chopped hay to mature wethers to determine simultaneously voluntary intake and digestibility of organic matter (OM) and neutral detergent fiber (NDF) (Abrams, 1980). Voluntary intakes were reported on the basis of metabolic body weight (g/kg $W^{.75}$/day). Each sample was pooled by animal, ground to pass a 4 mm screen in a Wiley mill, mixed and a representative portion reground through a 1 mm screen. A composite hay sample was made by mixing proportional amounts from each animal fed that specific hay. Feces were collected in canvas bags, weighed daily and 20% kept for further analyses. The composite hay samples and some individual feces samples were used for the analyses in this study.

Half gram samples of each hay were fermented in rumen fluid and buffer for 48 hr, followed by 48 hr in acid-pepsin (Moore et al., 1972; Moore and Mott, 1974; 1976), refluxed in neutral detergent solution (Goering and Van Soest, 1970) and filtered on sintered glass crucibles. The NDF residues were saved for further analyses. This treatment was termed the rumen fluid plus pepsin (RF+P) treatment. Another set of hay samples (.5 gm each) were fermented in rumen fluid

for 48 hr, refluxed in neutral detergent solution (Goering and Van Soest, 1970), filtered on sintered glass crucibles and the NDF residue saved for further analyses. This treatment was termed the rumen fluid (RF) treatment. Each of the RF and RF+P residues were analyzed for holocellulose (HOLO), hemicellulose (HC), cellulose (CELL) and lignin (LIG) as outlined in Chapter III.

Fecal samples from the *in vivo* studies of 2 wk and 8 wk Argentine bahiagrass, Coastcross-1 bermudagrass and X46-2 digitgrass were analyzed for NDF, HOLO, HC, CELL and LIG (Chapter III), and acid detergent fiber (ADF) (Goering and Van Soest, 1970). *In vivo* digestion coefficients were calculated for these fractions. Abbreviations are listed in Table 1. Descriptions of hays and *in vivo* data are listed in Appendix Tables 14 - 17.

Statistical analyses were performed using the statistical analysis system (SAS, 1979) computer package for general linear models, t-tests and correlation procedures.

Results and Discussion

In Vitro Digestibility

Analysis of variance of the *in vitro* data is presented in Table 5. Values for *in vitro* digestion of individual hays are in Appendix Table 15. *In vitro* digestibility of all fractions decreased with increasing maturity in all hays. RF and RF+P digestibilities were compared by t-tests (SAS, 1979) (H_0:RF+P - RF = 0) for each fraction (NDF, HOLO, HC and CELL) by hay and weeks of regrowth (Table 6).

Table 5. Analysis of variance of *in vitro* data.

		P Values							
	df	IVNDFD[a]	IVNDFDP	IVHOLOD	IVHOLODP	IVHCD	IVHCDP	IVCELLD	IVCELLDP
Hay	4	.0001	.0001	.0001	.0001	.0001	.0001	.0001	.0015
Age	3	.0001	.0001	.0001	.0001	.0001	.0001	.0001	.0001
H x A	12	.0001	.0002	.0003	.0161	.0768	.0390	.0001	.1108
C.V.		3.6	3.0	4.8	4.5	3.6	5.2	4.1	6.7
S.D.		2.0	1.7	2.8	2.6	1.7	3.0	2.5	4.1
Mean		56.3	57.9	57.8	59.0	47.4	57.4	60.6	60.9

[a]Abbreviations are found in Table 1.

Table 6. Comparison of *in vitro* digestion in rumen fluid and in rumen fluid plus pepsin.

	Hays									
	X46-2		X124-4		Paraguay		Argentine		Coastcross-1	
Fraction	t	P>|t|	t	P>|t|	t	P>|t|	t	P>|t|	t	P>|t|
NDF[a]	4.4	.003	3.3	.01	2.2	.07	5.7	.001	2.8	.02
HOLO	-.6	.60	.6	.55	2.6	.04	.5	.61	2.7	.03
HC	.02	.98	2.1	.08	2.4	.04	2.4	.05	3.0	.02
CELL	-.9	.38	-1.6	.16	2.0	.09	.8	.42	3.1	.02

	Weeks							
	2		4		6		8	
Fraction	t	P>|t|	t	P>|t|	t	P>|t|	t	P>|t|
NDF	3.4	.007	4.4	.002	5.0	.0007	1.6	.14
HOLO	.6	.59	.8	.45	1.7	.13	1.4	.18
HC	5.2	.0006	1.8	.10	1.7	.12	1.0	.4
CELL	.8	.4	.0	1.0	.3	.8	-.1	.9

[a]Abbreviations may be found in Table 1.

NDF digestibility was greater (P values ranged from .14 to .0007, Table 6) in RF+P for all hays and regrowths. The standard method of determining NDF digestibility (Goering and Van Soest, 1970) uses only rumen fluid for 48 hr. While the overall increase in NDF digestibility ranged from 2.2 to 5.7 percentage units (Table 7), for individual hays there were differences in NDF digestibility of up to 7 percentage units. In most instances, CELL was more digestible than HC, whether digested in RF or RF+P. HOLO and CELL digestibility increased in RF+P only for Paraguay and Coastcross-1 and either decreased or showed minimal change in the other grasses. HC digestibility was not different for X46-2 but showed an increase of 2.1 to 3.0 percentage units for the other grasses. For individual hays, there were increases in HC digestibility of up to 12 units by use of acid-pepsin.

By weeks of regrowth, NDF digestibility increased with RF+P except at 8 weeks. HOLO and CELL showed no significant difference in digestibility. HC exhibited the greatest difference in digestibility at 2 weeks; the difference decreased with increasing maturity. NDF and HC digestibility decreased with age (Table 7 and Appendix Table 16) which is consistent with work on temperate grasses (Gaillard, 1965; Waite et al., 1964; Bailey, 1967).

It seems, then, that further digestion *in vitro* of the CWC occurs under acid-pepsin conditions. This digestion, for the most part, is not attributable to further cellulose digestion but rather, to hemicellulose digestion and dissolution of lignin. Whether this increase in hemicellulose digestibility is due to solubilization by

Table 7. Digestion coefficients of chemical fraction *in vitro* and *in vivo*.

	NDF[a]			HOLO			HC			CELL		
	IVNDFD	IVNDFDP	NDFD	IVHOLOD	IVHOLODP	HOLOD	IVHCD	IVHCDP	HCD	IVCELD	IVCELDP	CELD
2 weeks												
X46-2	64.3	67.1	67.6	64.6	67.3	69.8	60.6	64.6	65.8	70.1	71.1	75.1
Argentine	76.2	76.7	74.4	77.6	77.6	75.4	76.1	77.7	73.5	79.7	77.4	78.0
Coastcross-1	50.4	57.5	60.8	51.5	60.7	63.2	52.1	61.5	62.8	50.7	59.7	63.9
4 weeks												
X46-2	60.0	60.5		62.4	57.0		60.4	56.4		64.8	58.8	
Argentine	46.5	50.1		46.6	51.5		43.4	49.6		50.7	53.9	
Coastcross-1	52.7	54.0		54.6	54.6		48.0	50.8		59.2	59.7	
6 weeks												
X46-2	58.6	59.5		59.8	60.7		57.4	58.5		62.6	63.3	
Argentine	48.4	50.4		49.6	53.3		46.7	54.3		53.2	52.0	
Coastcross-1	54.8	56.2		55.5	58.6		55.1	58.0		56.1	59.4	
8 weeks												
X46-2	49.1	49.6	60.2	52.5	50.8	63.5	49.0	46.4	57.6	56.6	56.1	70.4
Argentine	55.0	55.7	58.1	56.1	57.5	60.0	56.1	57.8	58.1	56.2	57.2	62.5
Coastcross-1	47.8	48.2	56.3	48.4	50.0	57.6	47.4	48.2	56.8	49.8	52.4	58.8

[a]Abbreviations may be found in Table 1.

the acidic conditions and whether the hemicellulose would re-precipitate under neutral conditions (as suggested by Gaillard and Richards, 1975) is not known. The majority of the *in vivo* studies have used HEMV as the measure of hemicellulose. Our earlier experiments suggest that HEMV and HC may not be the same entity. Thus, studies showing that the greatest proportion of "hemicellulose" digestion occurs in the rumen may be misleading, as indicated by these *in vitro* results.

In Vivo Digestibility

Fecal samples collected during the feeding trials of these tropical grass hays were used in further analyses. Argentine bahiagrass, Coastcross-1 bermudagrass and X46-2 digitgrass were chosen from each of the three genera at 2 wk and 8 wk of age. NDF, HOLO, HC, CELL, LIG and ADF values are found in Table 8. Analysis of variance of the chemical composition of the fecal samples is in Appendix Table 24.

Compared to the chemical composition of the hays (Appendix Table 14), fecal samples were lower in NDF, HOLO and CELL, higher in LIG and were about the same or slightly higher in HC content.

In vivo intake information was used in conjunction with the feces, orts and waste information to calculate digestion coefficients for NDF, HOLO, HC and CELL (Table 7; Abrams, 1980). Analysis of variance for the digestion coefficients is in Appendix Table 23. The values are compared with in vitro digestibility data on the same hays in RF and RF+P (Table 7). At 2 weeks, *in vivo* digestion coefficients were similar to *in vitro* for X46-2 digitgrass and Argentine bahiagrass. For these two grasses, also, digestion coefficients were higher for

Table 8. Chemical composition of feces.[a]

	NDF[b]	HOLO	ADF	HC	CELL	LIG
2 Weeks						
X46-2	61.3c	89.9a	37.5b	57.2a	34.4a	10.1b
Argentine	67.8b	86.2b	38.9b	56.2a	30.1a	13.8a
Coastcross-1	73.4a	86.7ab	43.4a	53.9a	32.8a	13.3ab
8 Weeks						
X46-2	73.9c	86.6b	44.2a	50.5a	46.1a	13.4a
Argentine	76.5b	84.3b	44.3a	49.4a	31.5b	15.7a
Coastcross-1	82.1a	90.3a	45.5a	54.2a	36.0ab	9.7b

[a]Numbers within a column at each age followed by different letters are significantly different from each other (Duncan's multiple range).

[b]Abbreviations may be found in Table 1.

cellulose than for hemicellulose at 2 and 8 weeks. The Coastcross-1 bermudagrass had higher *in vivo* digestion coefficients. Digestibility of the various fractions was high at an early stage of growth. Not unusually, the situation changed with maturity; at 8 weeks, the *in vivo* digestion coefficients were higher than the *in vitro*, ranging from at least 2 to 14 percentage points higher. The degree of relationship (or correlation) between the *in vivo* and *in vitro* values was high (Table 9) despite lower *in vitro* values at 8 weeks. Implications are that *in vitro* digestibility information is more representative of *in vivo* performance at early stages of plant growth and that with maturity, the *in vitro* digestibility values still accurately reflect *in vivo* but the absolute values will be lower than *in vivo* values. The correlation of HCD with IVHCDP was higher than with IVHCD, implying that acid-pepsin may have some effect *in vivo*.

Summary

One cultivar of *Cynodon dactylon* (Coastcross-1), two cultivars of *Digitaria* species (X46-2 and X124-4) and two cultivars of *Paspalum notatum* (Argentine and Paraguay) cut at four ages each 2, 4, 6 and 8 weeks of regrowth, were evaluated by *in vitro* digestion of neutral detergent fiber (NDF) in rumen fluid (RF) and RF plus acid-pepsin (RF+P) and by chemical analyses of NDF fractions. In addition, sheep feeding trials were run. Composite samples of each hay were analyzed for NDF, holocellulose (HOLO), hemicellulose (HC), cellulose (CELL) and lignin (LIG). Fecal samples of 2 and 8 week Coastcross-1,

Table 9. Relationship (r^2) between *in vivo* and *in vitro* digestion coefficients for six hays.

Variables in Model	r^2	P
NDFD[a] vs. IVNDFD	.91	.0033
NDFD vs. IVNDFDP	.92	.0025
HOLOD vs. IVHOLOD	.88	.0058
HOLOD vs. IVHOLODP	.84	.0094
HCD vs. IVHCD	.87	.0065
HCD vs. IVHCDP	.91	.0034
CELD vs. IVCELD	.84	.0100
CELD vs. IVCELDP	.79	.0180

[a]Abbreviations may be found in Table 1.

Argentine and X46-2 grasses were analyzed for NDF, HOLO, HC, CELL, LIG and acid detergent fiber (ADF).

In vitro digestibility in RF and RF+P of NDF, HOLO, HC and CELL decreased with increasing maturity in all hays. In vitro digestion of cellulose was generally greater than that of hemicellulose. In vitro digestibility of NDF and hemicellulose was increased by further digestion in RF+P while holocellulose and cellulose digestibility showed minimal change.

Fecal samples were lower in NDF, HOLO and CELL, higher in LIG and about the same or slightly higher in HC content. In vivo digestion coefficients were calculated for each of the fractions (NDF, HOLO, HC and CELL) and showed trends similar to in vitro digestibility.

CHAPTER V
PREDICTION OF FORAGE QUALITY FROM CHEMICAL AND *IN VITRO* ANALYSES

Introduction

Forage quality must be related to animal performance. Moore and Mott (1973) defined forage quality as output per animal and as being a function of voluntary intake and digestibility of nutrients when the forage is fed alone, *ad libitum*, to a specified animal. When long-term production trials are not feasible as is often the case, researchers turn to laboratory analyses of forage quality. Many and varied are the laboratory procedures devised to predict forage quality. These may be grouped into chemical and *in vitro* methods. Thus far, chemical methods have not been overly successful in predicting forage quality but the *in vitro* methodology, especially with respect to predicting *in vivo* digestibility, has been more successful. In a review of the many forage evaluation methods in popular use, Barnes and Marten (1979) reported that the *in vitro* system was superior to chemical analyses for predicting *in vivo* digestibility of forages. Intake is much more difficult to predict from laboratory analyses but Rohweder et al. (1976) have suggested the use of neutral detergent fiber (NDF) to predict intake.

This study was conducted to examine the relationship between the Van Soest chemical analyses, another chemical analysis procedure ($NaClO_2$-KOH, see Chapter III) and/or *in vitro* digestion with *in vivo*

intake and digestibility of organic matter (OM) and NDF in 39 tropical grass hays of three genera harvested after 2, 4, 6 and 8 weeks of regrowth.

Experimental Procedure

One cultivar of Cynodon dactylon (Coastcross-1 bermudagrass), two experimental cultivars of Digitaria (X46-2 and X124-4 digitgrass) and two cultivars of Paspalum notatum (Argentine and Paraguay bahiagrass) harvested after 2, 4, 6 and 8 weeks of regrowth were used in this experiment. Hays were chopped and fed ad libitum to mature wethers to determine intake and digestibility as outlined in Chapter III. A composite hay sample was made by mixing proportional amounts of hay from each animal fed that particular hay, was ground through 4 mm and 1 mm screens of a Wiley mill and was used for the laboratory analyses.

The composite hay samples were analyzed for DM, OM, NDF, holocellulose (HOLO), hemicellulose (HC, HEMV), cellulose (CELL, CELLV) and lignin (LIG, PLIG) as outlined in Chapter III. In vitro fermentation in rumen fluid (RF) for 48 hrs and in vitro fermentation in RF for 48 hrs followed by 48 hrs in acid pepsin (RF+P) were both followed by refluxing in neutral detergent solution to determine in vitro NDF, HOLO, HC and CELL digestion as outlined in Chapter IV. A list of abbreviations is presented in Table 1.

Statistical analyses were performed using the statistical analysis system (SAS, 1979) computer package for general linear models and correlation procedures.

Results and Discussion

Intake and Digestibility

Individual values of *in vivo* intake and digestibility are in Appendix Tables 17, 24 and 25. Chaves (1979), Abrams (1980) and Hartadi (1980) presented discussions of the relationship between intake and digestibility *in vivo* of these hays. They suggested that digestibility was not the primary factor controlling intake of these hays. Furthermore, there was a poor relationship between intake and digestibility (OMI vs. OMD, r = .47; Chaves, 1979). Therefore, it is unlikely that a single laboratory procedure will prove to be a good predictor of *in vivo* performane.

Another reason for skepticism concerning the ability of a single laboratory procedure to predict forage quality lies in the effect of maturity on the *in vivo* values compared to the effect of maturity on the laboratory values. In Chapter III, it was reported that maturity did not have a consistent effect on the chemical parameters, i.e., the effect of age was not linear. Expectations are that, with maturity, some factors decrease (e.g., crude protein) and some increase (e.g., NDF) but the hays used in this study did not follow the expected pattern. However, the *in vitro* and *in vivo* values did follow the expected pattern of decline with maturity in most of the hays (see Appendix Tables 15 and 16). Argentine bahiagrass and Coastcross-1 bermudagrass did not change in OMI and changed very little in OMD but the other three grasses decreased from 2 - 8 weeks in OMI by 4 - 8 units (g/kg $W^{.75}$/day) and decreased in OMD by 10 - 12 percentage units (Abrams, 1980).

Prediction of Forage Quality from Laboratory Analyses

Simple correlations between *in vivo* data and laboratory analyses were conducted (SAS, 1979). Only the best correlations for each *in vivo* parameter are presented in Table 10. The highest correlation obtained between OMI and all laboratory analyses was with CP (r^2=.20). DOMI had the highest correlation with NDF (r^2=.46) and NDFI had the highest correlation with PLIG and CP (r^2=.06). Intake has always been difficult to predict by laboratory analyses alone. Differences in voluntary intake are often attributed to rate of digestion and *in vitro* digestibility systems generally are more accurate at predicting intake than are chemical analyses. Table 11 presents correlations between *in vivo* and *in vitro* values. Two *in vivo* intake variables, OMI and DOMI, had significant correlations with all of the *in vitro* analyses; r^2 values ranging from .13 to .56 with significance probabilities of $P \leq .10$ to $\leq .001$, respectively. NDFI, which did not vary (Appendix Table 24), was not predicted well, or at all, by any of the *in vitro* analyses.

OMD and NDFD had the highest correlations with NDF (r^2=.59 and .56, respectively) while DNDF had the highest correlation with PLIG (r^2=.23) (Table 10). Aside from NDF and PLIG, there were no chemical analyses which accurately predicted *in vivo* digestibility. However, the *in vitro* analyses generally gave highly significant correlations with OMD and NDFD; r^2 values ranging from .61 to .81 (Table 11). Correlations between *in vitro* analyses and DNDF, although significant, were lower.

Table 10. Relationship (r^2) between in vivo values and laboratory analyses, obtained with thirty-nine different hays.

Variables in Model	r^2	P
OMI[a] vs. NDF	.18	.0076
OMI vs. PLIG	.19	.0053
OMI vs. CP	.20	.0039
OMD vs. NDF	.59	.0001
OMD vs. PLIG	.40	.0001
OMD vs. CP	.22	.0026
OMD vs. HC	.03	.2725
OMD vs. CELL	.03	.2840
DOMI vs. NDF	.46	.0001
DOMI vs. PLIG	.37	.0001
DOMI vs. CP	.28	.0005
NDFI vs. PLIG	.06	.1379
NDFI vs. CP	.06	.1166
NDFD vs. NDF	.56	.0001
NDFD vs. PLIG	.42	.0001
NDFD vs. CP	.23	.0022
DNDF vs. NDF	.07	.0921
DNDF vs. HC	.17	.0097
DNDF vs. CELL	.19	.0056
DNDF vs. CELLV	.17	.0099
DNDF vs. PLIG	.23	.0010

[a]Abbreviations may be found in Table 1.

Table 11. Relationships (r^2) between *in vivo* values and *in vitro* analyses, obtained with thirty-nine different hays.[a]

	In vivo Variables					
	OMI	OMD	DOMI	NDFI	NDFD	DNDF
IVNDFD[a]	.18**	.77***	.54***	.02	.80***	.32***
IVNDFDP	.20***	.79***	.56***	.02	.81***	.28***
IVHOLOD	.15*	.72***	.47***	.01	.75***	.31***
IVHOLODP	.19**	.71***	.52***	.01	.70***	.18**
IVHCD	.16**	.73***	.49***	.01	.74***	.25***
IVHCDP	.20***	.67***	.52***	.02	.65***	.14*
IVCELLD	.17**	.71***	.50***	.02	.78***	.38***
IVCELLDP	.13*	.61***	.42***	.004	.63***	.20***

* $P \leq .10$
** $P \leq .01$
*** $P \leq .001$

[a]Abbreviations may be found in Table 1.

Interestingly, in almost every instance, r^2 values for each chemical fraction's *in vitro* digestibility in RF and RF+P (IVNDFD and IVNDFDP, for example) were not different. In Chapter III, it was shown that there was a significant increase in digestion in the RF+P treatment for the NDF and hemicellulose fractions, while holocellulose and cellulose digestibility showed minimal change. The similar *in vivo* correlations with the RF and RF+P *in vitro* data make laboratory decision making easier. Since there is little difference in the two methods (RF vs. RF+P) insofar as predicting *in vivo* data is concerned, the method of choice would be RF, which is less time, equipment and reagent consuming. Furthermore, there was no advantage to using a more complicated procedure for residue analysis than the NDF procedure.

Tables 12 and 13 present the models obtained by general linear models procedure (SAS, 1979) that best fitted the *in vivo* intake and digestibility data. A rational, rather than an empirical or multiple regression, approach was used to choose independent variables for each model. Independent variables were chosen on the basis of significant correlation with the *in vivo* measurement of interest (Tables 10 and 11). In most cases, *in vitro* digestion values in RF only were used unless RF+P values were much higher. In addition, some variables were included on the basis of their presumed biological role in the structure of the cell wall. Cell wall structure and components play a crucial role in determining intake and digestibility of tropical grasses.

Table 12. Coefficient of determination (R^2), probability (P) and standard error of the estimate ($s_{y.x}$) for the regression of in vivo intake values on several independent variables.

Dependent Variable	Independent Variable(s)	R^2	P	$s_{y.x}$
OMI[a,b]	NDF PLIG	.24	.0078	6.42
OMI	NDF HC PLIG	.25	.0166	6.44
OMI	NDF CELL PLIG	.26	.0150	6.43
OMI	NDF HC CELL PLIG	.25	.0347	6.51
OMI	NDF HC HC^2	.23	.0269	6.54
OMI	HC CP PLIG	.25	.0185	6.47
OMI	IVNDFDP IVHOLODP	.20	.0183	6.57
OMI	IVNDFDP IVHOLODP IVHCDP	.21	.0366	6.61
OMI	IVNDFDP IVHOLODP IVHCDP IVCELD	.21	.0779	6.70
DOMI	NDF PLIG	.53	.0001	4.89
DOMI	NDF HC PLIG	.53	.0001	4.94
DOMI	NDF CELL PLIG	.53	.0001	4.94
DOMI	NDF HC HC^2	.47	.0001	5.30
DOMI	HC CP PLIG	.42	.0002	5.47
DOMI	IVNDFDP PLIG HC	.58	.0001	4.71
DOMI	IVNDFD IVHOLOD	.55	.0001	4.81
DOMI	IVNDFD IVHOLOD IVHCD	.55	.0001	4.87
DOMI	IVNDFD IVHOLOD IVCELD	.55	.0001	4.93
NDFI	HC PLIG	.07	.2824	4.85
NDFI	HC CP PLIG	.08	.4106	4.90
NDFI	IVNDFDP HC PLIG	.07	.4677	4.92

[a]Abbreviations may be found in Table 1.

[b]Intake expressed as g/kg $W^{.75}$/day.

Table 13. Coefficient of determination (R^2), probability (P) and standard error of the estimate ($s_{y.x}$) for the regression of in vivo digestibility values on several independent variables.

Dependent Variable	Independent Variable(s)	R^2	P	$s_{y.x}$
OMD[a,b]	NDF CP	.61	.0001	3.62
OMD	NDF CP PLIG	.65	.0001	3.47
OMD	NDF PLIG	.65	.0001	3.42
OMD	NDF HC	.59	.0001	3.69
OMD	NDF CELL	.60	.0001	3.68
OMD	NDF HC PLIG	.66	.0001	3.44
OMD	NDF HC CELL	.43	.0001	3.74
OMD	NDF HC CELL PLIG	.66	.0001	3.49
NDFD	CP PLIG	.43	.0001	5.14
NDFD	NDF CP	.59	.0001	4.38
NDFD	NDF PLIG	.64	.0001	4.08
NDFD	NDF CP PLIG	.64	.0001	4.14
NDFD	CP PLIG HC	.54	.0001	4.67
NDFD	NDF HC PLIG	.66	.0001	4.02
NDFD	NDF HC CELL PLIG	.67	.0001	4.05
NDFD	NDF HC HC^2	.58	.0001	4.46
DNDF[c]	HC CELL	.19	.0225	3.50
DNDF	HC PLIG	.40	.0001	3.02
DNDF	HC CELL PLIG	.42	.0002	2.99
DNDF	PLIG CELL	.42	.0001	2.96
DNDF	CP PLIG	.32	.0011	3.22
DNDF	CP PLIG HC	.40	.0004	3.05
DNDF	NDF CP PLIG	.32	.0035	3.26
DNDF	NDF HC CELL PLIG	.43	.0006	3.02

[a]Abbreviations may be found in Table 1.

[b]Digestibility expressed as %.

[c]Digestible NDF expressed as % of DM.

Going from a model containing one independent variable to a model containing more than one independent variable did not produce better or more acceptable predictions of intake. OMI ranged from 46.7 to 79.7 g/kg $W^{.75}$/day (Appendix Table 24). Four of the "best" equations for predicting OMI included NDF and PLIG. R^2 values and standard error of the estimate, respectively, were .24 and 6.42 (NDF and PLIG), .25 and 6.44 (NDF, HC and PLIG), .26 and 6.43 (NDF, CELL and PLIG) and .25 and 6.51 (NDF, HC, CELL and PLIG). DOMI (ranging from 25.6 to 54.0 g/kg $W^{.75}$/day) was best predicted by two and three variable models that included an in vitro value in the model, as to be expected for the prediction of digestible OM intake. The best equation for predicting DOMI included IVNDFDP, PLIG and HC (R^2= .58 and $s_{y\cdot x}$ = 4.71), but this three variable equation was not much better than the equation using only IVNDFDP (R^2=.56).

NDFI was still predicted poorly by any equation, be it single or multiple variable, probably because there is little variation in NDFI (see Appendix Table 24). As R^2 values increased slightly, standard error of the estimate increased so that the best predictor of NDFI remained crude protein alone (Table 10).

OMD and NDFD were predicted quite well by any and all of the single variable in vitro equations (Table 11). Adding more variables to the model decreased the R^2 and increased $s_{y\cdot x}$ for OMD and NDFD. Single variable models of in vitro data used for predicting DNDF had R^2 values ranging from .14 to .38. The best two variable model for predicting DNDF included PLIG and CELL (R^2=.42) and was not improved by adding another variable to the model (HC, CELL and PLIG, R^2=.42).

The best four variable model included NDF, HC, CELL and PLIG (R^2=.43) but the standard error of the estimate increased with the addition of more variables to the model statements.

Conclusions

The results of the studies indicated that NDF, CP and PLIG were the chemical analyses most closely associated with the digestibility and intake of tropical grasses. New methodology developed to analyze cell walls did not contribute to the prediction of *in vivo* performance. Most of the *in vitro* procedures gave fair to good predictions of OMI, OMD, DOMI, NDFD and DNDF but not of NDFI. Two, three and four variable models increased the R^2 values for OMI, OMD and DOMI and decreased the R^2 values slightly for DNDF and more so for NDFD. NDFI could not be predicted by any single or multiple variable model. It is concluded that we still do not have an accurate picture of the structure and function of the cell wall in tropical grasses. The difficulty of predicting *in vivo* animal performance remains a great challenge in evaluation of forage quality. Twenty of these hays were analyzed at the Pennsylvania State University by near infrared reflectance spectroscopy (NIRS) (Russo, 1981, unpublished data). Using large multiple regression models and two wavelengths, NIRS gave better estimates of forage quality than any of the chemical and *in vitro* analyses conducted in this study.

Summary

One cultivar of *Cynodon dactylon* (Coastcross-1 bermudagrass), two cultivars of *Digitaria* species (X46-2 and X124-4) and two cultivars of *Paspalum notatum* (Argentine and Paraguay bahiagrass) cut at four ages each (2, 4, 6 and 8 week regrowths) were evaluated by chemical analyses and *in vitro* NDF digestibility in rumen fluid (RF) and RF plus acid pepsin (RF+P). In addition, sheep feeding trials were conducted. Composite samples for each hay were analyzed for organic matter, NDF and holocellulose (HOLO). Hemicellulose (HC, HEMV), cellulose (CELL, CELLV) and lignin (LIG, PLIG) were determined by two different methods. Information obtained was used to generate prediction equations for sheep *in vivo* intake and digestibility.

NDF, CP and PLIG were the best single chemical analyses for prediction of intake and digestibility. OMI and NDFI were not predicted as well as DOMI. Any of the *in vitro* analyses were better for prediction of *in vivo* digestibility (OMD, NDFD and DNDF) than were chemical analyses. Two and three variable models which included NDF and PLIG improved prediction of OMI and DOMI.

CHAPTER VI
GENERAL DISCUSSION

Forage quality reflects the potential of animal production from forage by use of the available nutrients in the forage. Forage quality may be defined as animal performance (Moore and Mott, 1973). Analysis of forage quality in the laboratory has almost a hundred year history and gaps still remain in our knowledge of how the forage plant is put together, how it functions and how it affects animal performance.

Essentially all of the forage evaluation procedures have been developed in temperate climates using temperate forages. Not unlike the problems associated with transferring Western agricultural expertise to developing countries is the problem of transferring forage evaluation schemes generated on temperate forages to tropical forages. Sometimes it works, sometimes it does not. Tropical grasses are rather different from temperate grasses. Tropical grasses have a much greater photosynthetic capability, are more efficient in water use, have different carbon pathways and have different anatomies. The first product of photosynthesis is carbohydrates and tropical grasses have specialized plastids in the sheath surrounding the vascular bundles for starch conversion and storage. Tropical grasses are higher in cell wall and cell wall components than temperate grasses.

What is the cell wall? It is a highly ordered macromolecular multilayered network made up of structural carbohydrates (cellulose and hemicellulose), lignin and protein. Dividing plants into cell contents and cell walls (as does the Van Soest forage evaluation system) provides a rational basis for discussion of forage quality. Cell contents are essentially completely digestible by the ruminant, cell walls have incomplete digestibilities. A number of cell wall models have been proposed. Albersheim and others (Bauer et al., 1973; Keegstra et al., 1973; Talmadge et al., 1973) propose that the cell wall is made up of long crystalline microfibrils (cellulose) surrounded by hemicellulose, which is hydrogen bonded to the cellulose and covalently bonded to the other cell wall components (primarily lignin). Covalent bonds are more difficult to break than are hydrogen bonds. Bailey and others (1976) propose that all of the crystalline polymer need not be cellulose, but could be hemicellulosic xylans.

The role of hemicellulose in the plant has been established as structural but hemicellulose may exist not only in the cell wall and have been found in the endosperm of cereal plants, for example (Wilkie, 1979). Hemicellulose is added to the wall continuously and is increased by increases in temperature and light. While cellulose composition and structure does not change with maturity or under environmental influences, hemicellulose does. Not enough information exists, but it is likely that each genera, each cultivar, perhaps even each plant, and certainly, different parts within a plant have different kinds and amounts of hemicellulose. Hemicellulose content and concentrations are variable, not constant.

The results of this research indicate that "hemicellulose" is an elusive term. Van Soest hemicellulose is certainly not the same hemicellulose obtained in the $NaClO_2$-KOH procedure despite presumed similarities in the cellulose fraction. Lack of expertise prevented further examination of the sugars making up the hemicellulose but some initial observations were made. One of the experimental digitgrasses, X46-2, which is "high" in forage quality by most standards, appeared very low in xylans. Xylans are usually highly ordered, even crystalline and difficult to digest (Daughtry, 1976). Tropical grasses are generally higher in xylans than are temperate grasses (Ojima and Isawa, 1968; Bailey, 1973) and xylans may be responsible for lower quality of tropical grasses. This experimental line of digitgrass should be examined for xylan content and compared with other grasses used in this study.

In vitro digestibility results were illuminating. NDF and hemicellulose digestibility increased in acid-pepsin conditions, cellulose digestibility did not increase. Further breakdown of the cell wall, then, in the lower gastrointestinal tract, could be due to breakdown of the hemicellulose. Hemicellulose has generally been presumed to have a low digestibility, with lignification postulated as the limiting factor but a distinction should be made between hemicellulose unavailability due to structural organization of the cell wall carbohydrates or interference by cellulose, lignification, acetylation, etc. (Morrison, 1979). McLeod and Minson (1974) propose that the cell wall carbohydrates have two fractions - lignin-free and lignified - the lignin-free being totally digestible,

the lignified fraction being undigestible. Other digestion models (Waldo et al., 1972; Abrams, 1980) are similar. Accurate procedures for determining hemicellulose and lignin must be developed in order to answer some of these questions and to prove some of these theories. It would seem that if the proposed cell wall models and digestion models were fairly accurate, using both models would allow one to state that hemicellulose can be "digested" from cellulose and within the hemicellulose polymer itself but that pieces of the hemicellulose polymer remain covalently bonded to lignin and are more resistant to digestion. Bailey and Ulyatt (1970) and Morrison (1980) intimate these conclusions also. To extrapolate further, Chaves (1979) suggests that bridges are formed between the cell wall carbohydrates and the lignin by phenolic acids which can deleteriously affect microbial digestion.

In tropical grasses, hemicellulose, lignin and phenolic acids are higher than in temperate grasses. Are these, then, the components responsible for low quality in tropical grasses? Can the plant breeder do anything about it? Breeding for low lignin brought about the development of brown midrib corn, academically interesting but of little use to the farmer. Is low hemicellulose or low lignin content really desirable in a forage plant?

Forage quality analysis and prediction of intake and digestibility still has researchable areas. This research should be carried further to determine monosaccharide composition, relate the composition to animal performance and then devise a rapid method of cell wall monosaccharide determination. The physical and chemical forces

holding the various cell wall components together and what it takes in terms of energy, microbes, enzymes, acids or whatever to break those bonds could be examined. Anatomy and histology of forages as related to intake and digestibility should be examined and the polysaccharides and monosaccharides in the various structures be determined. Infrared reflectance spectroscopy may alter procedures used by many forage testing laboratories as it becomes more accurate and accessible for routine use. That may be for the better, allowing more basic research to be conducted on forage plants.

APPENDIX

Appendix Table 14. Chemical composition of individual hays.[a]

Hay	Genus	Cultivar	Age	DM	OM	NDF	HOLO	HC	CELL	LIG
2	Digitaria	X46-2	2	91.02	89.88	71.67	92.34	52.22	40.11	7.66
5	Paspalum	Paraguay	2	90.80	93.78	77.96	92.94	55.12	37.82	7.06
7	Paspalum	Argentine	2	91.06	93.99	78.40	92.50	53.31	39.20	7.50
8	Digitaria	X124-4	2	91.62	88.41	72.41	92.06	50.33	41.73	7.94
9	Digitaria	X46-2	2	91.00	91.26	71.68	93.56	53.97	39.60	6.44
10	Paspalum	Argentine	2	91.32	94.04	79.96	90.45	51.63	38.82	9.55
11	Paspalum	Paraguay	2	91.50	93.64	77.90	93.60	54.19	39.41	6.40
13	Digitaria	X124-4	2	91.44	89.15	70.85	92.12	50.52	41.61	7.88
23	Digitaria	X46-2	4	90.46	91.68	82.66	92.25	52.30	39.95	7.75
25	Paspalum	Paraguay	4	90.32	94.82	84.92	93.26	53.26	40.00	6.74
27	Paspalum	Argentine	4	90.44	94.99	85.32	91.76	52.43	39.32	8.24
28	Digitaria	X124-4	4	91.12	91.03	78.37	92.72	51.51	41.21	7.28
30	Digitaria	X124-4	4	91.22	92.60	79.98	92.38	46.30	45.21	7.62
32	Paspalum	Paraguay	4	90.91	95.22	82.72	93.22	52.21	41.03	6.78
33	Paspalum	Argentine	4	90.32	94.80	83.72	92.72	52.44	40.28	7.28
34	Digitaria	X46-2	4	90.41	92.22	80.76	92.98	51.42	41.57	7.02
35	Cynodon	Coastcross-1	2	91.50	88.42	81.28	93.78	56.28	37.49	6.22
36	Cynodon	Coastcross-1	2	91.44	86.92	78.35	92.60	56.89	35.71	7.40
44	Digitaria	X46-2	6	92.10	91.52	79.97	92.18	50.32	41.85	7.82
46	Paspalum	Paraguay	6	92.28	94.02	82.37	91.94	50.90	41.04	8.06
48	Paspalum	Argentine	6	92.50	94.44	80.90	93.50	52.04	41.47	6.50
49	Digitaria	X124-4	6	92.78	92.78	77.70	94.24	48.34	45.90	5.76
51	Digitaria	X124-4	6	91.50	92.86	79.32	92.75	46.42	46.33	7.25
53	Paspalum	Paraguay	6	92.50	90.87	80.62	92.76	50.87	41.51	7.24
54	Paspalum	Argentine	6	92.86	94.42	80.32	93.06	51.26	41.80	6.94
55	Digitaria	X46-2	6	92.14	93.46	75.91	91.86	49.57	42.30	8.14
56	Cynodon	Coastcross-1	4	92.68	86.76	82.43	92.86	51.91	40.96	7.14
57	Cynodon	Coastcross-1	4	90.04	87.60	80.28	94.36	52.55	41.81	5.64
66	Digitaria	X46-2	8	91.68	92.38	78.52	91.92	48.32	43.59	8.08

Appendix Table 14 - continued.[a]

Hay	Genus	Cultivar	Age	DM	OM	NDF	HOLO	HC	CELL	LIG
68	Paspalum	Paraguay	8	91.18	93.97	81.33	92.54	50.98	41.56	7.46
70	Paspalum	Argentine	8	91.33	94.32	81.81	91.99	49.66	42.33	8.01
71	Digitaria	X124-4	8	91.32	93.76	78.08	91.12	47.88	43.24	8.88
73	Digitaria	X124-4	8	91.68	93.66	78.94	91.54	47.86	43.67	8.46
75	Paspalum	Paraguay	8	91.42	94.48	82.02	91.78	50.14	41.64	8.22
76	Paspalum	Argentine	8	91.26	94.70	81.48	92.94	51.09	41.84	7.06
77	Digitaria	X46-2	8	91.39	94.44	77.11	90.86	50.49	40.37	9.14
84	Cynodon	Coastcross-1	6	92.05	92.33	82.13	92.34	56.32	36.03	7.66
85	Cynodon	Coastcross-1	6	91.60	93.24	81.40	93.76	57.93	35.83	6.24
86	Cynodon	Coastcross-1	8	91.64	94.90	83.22	92.98	55.62	37.36	7.02
87	Cynodon	Coastcross-1	8	91.69	91.96	83.11	92.96	54.59	38.37	7.04

[a]Refer to Table 1 for explanation of abbreviations and units of expression.

Appendix Table 15. *In vitro* digestion of individual hays.[a]

Hay	IVNDFD	IVNDFDP	IVHOLOD	IVHOLODP	IVHCD	IVHCDP	IVCELD	IVCELDP
2	74.04	75.48	75.14	75.28	72.48	75.13	78.58	75.41
5	68.66	69.09	69.23	69.72	66.15	67.54	73.69	72.84
7	64.26	67.06	64.64	67.33	60.62	64.55	70.10	71.07
8	71.42	71.54	72.31	72.95	70.34	72.72	74.68	73.16
9	76.24	76.60	77.63	77.59	76.13	77.74	79.68	77.39
10	61.68	63.30	76.55	64.06	57.74	62.37	66.53	67.02
11	60.28	62.30	61.97	64.76	58.64	63.39	66.53	66.65
13	69.92	73.12	71.48	74.31	70.99	75.36	72.06	73.02
23	59.64	59.66	63.98	50.86	62.92	51.52	65.35	49.96
25	45.45	47.84	45.84	49.86	42.72	48.43	49.96	51.63
27	46.98	49.78	46.55	50.89	43.42	49.73	50.67	52.41
28	63.80	65.34	65.10	66.66	62.39	67.07	68.02	66.17
30	63.26	65.20	63.72	66.16	59.03	63.20	68.00	68.55
32	44.55	49.62	44.75	51.58	40.45	49.22	50.21	54.57
33	46.04	50.44	46.60	52.05	43.41	49.39	50.73	55.48
34	60.41	61.40	60.76	63.08	57.89	61.38	64.32	67.66
35	54.50	58.19	56.15	59.45	56.68	59.42	53.91	59.57
36	50.45	57.54	51.50	60.74	52.07	61.49	50.71	59.72
44	60.54	61.72	61.26	63.19	57.82	61.83	65.41	64.83
46	46.08	49.42	46.02	50.73	40.78	47.51	52.06	54.73
48	46.47	49.14	47.19	53.43	43.06	59.00	52.39	46.50
49	62.52	62.52	63.52	57.12	61.17	57.32	66.08	56.93
51	59.68	61.36	60.28	63.10	57.76	60.77	62.87	65.45
53	49.97	51.25	50.76	52.22	47.06	47.81	55.25	57.61
54	50.26	51.66	51.97	53.10	50.32	49.56	53.97	57.46
55	56.76	57.28	58.31	58.21	56.95	55.09	59.91	61.82
56	53.48	54.96	55.29	54.93	44.62	50.73	61.44	60.41
57	51.86	53.08	53.89	54.32	51.43	50.77	57.05	58.94
66	55.84	56.38	57.41	57.13	54.05	54.21	61.14	60.85

Appendix Table 15 - continued.

Hay	IVNDFD	IVNDFDP	IVHOLOD	IVHOLODP	IVHCD	IVHCDP	IVCELD	IVCELDP
68	49.85	49.13	51.56	50.36	48.08	47.13	55.85	54.36
70	49.10	49.58	52.49	50.82	48.98	46.36	56.60	56.07
71	58.31	60.10	60.11	61.19	60.65	60.14	68.64	62.36
73	57.66	57.85	59.54	59.92	58.69	61.65	60.46	58.09
75	50.14	49.00	50.88	51.23	45.90	44.62	56.88	59.15
76	49.06	51.90	51.40	54.84	47.76	51.67	55.84	58.63
77	55.02	55.69	56.13	57.52	56.12	57.80	56.17	57.16
84	55.72	57.13	56.16	58.99	55.79	58.39	56.75	59.83
85	53.80	55.36	54.93	58.13	54.47	57.68	55.52	58.87
86	48.76	49.52	49.04	50.93	48.04	50.17	50.49	52.08
87	47.83	48.24	48.44	49.96	47.42	48.25	49.79	52.39

[a]Refer to Table 1 and Appendix Table 14 for explanation of abbreviations and units of expression.

Appendix Table 16. Chemical composition and *in vitro* digestion of individual hays.[a,b]

Hay	CP	NDF(H)	ADF	HEMV	CELLV	PLIG	IVNDFD	IVOMD
2	10.82	69.21	37.46	31.75	29.64	4.29	77.18	76.12
5	13.66	74.55	35.06	39.49	29.42	4.45	68.99	62.49
7	12.25	74.36	35.15	39.21	29.83	4.23	65.52	62.88
8	10.23	69.16	38.37	30.79	28.37	4.81	74.26	71.21
9	10.75	67.61	33.87	33.74	27.66	4.18	77.80	74.22
10	11.71	75.58	36.76	38.82	31.24	4.52	63.05	58.57
11	11.22	75.11	37.33	37.78	31.18	4.89	63.27	60.27
13	11.17	67.91	37.51	30.40	29.50	4.24	73.80	72.37
23	8.43	79.11	44.03	35.08	33.95	7.67	61.66	61.29
25	6.63	80.39	42.88	37.51	34.25	8.01	46.93	47.27
27	7.06	80.12	42.69	37.43	34.88	7.07	45.72	45.72
28	7.33	74.97	42.25	32.72	33.99	6.34	66.65	64.67
30	6.78	76.36	45.39	30.97	37.44	6.82	64.34	63.77
32	6.33	79.16	42.86	36.30	35.59	6.82	47.34	47.96
33	6.67	76.09	42.06	37.00	35.22	6.29	45.32	46.61
34	6.73	77.22	44.45	32.67	36.11	7.63	58.73	59.57
35	11.22	77.51	40.88	36.63	28.38	6.14	57.51	57.79
36	13.83	74.29	41.03	33.26	26.05	7.98	57.92	58.94
44	6.98	77.93	45.47	32.46	36.35	7.21	60.35	59.60
46	6.14	80.05	45.23	34.82	36.05	8.09	49.87	47.23
48	6.35	79.15	43.66	35.49	35.29	7.34	46.83	47.31
49	5.36	76.18	45.18	31.00	37.85	6.11	65.29	62.61
51	5.04	76.37	46.94	29.43	39.24	6.77	59.50	61.46
53	6.85	78.49	43.18	35.31	35.37	7.18	48.72	51.85
54	6.83	79.15	43.13	36.02	35.73	6.40	50.78	49.10
55	5.23	75.37	44.35	31.02	35.74	7.50	58.09	59.58
56	8.12	80.11	43.87	36.24	30.49	6.94	56.38	56.41
57	8.45	78.75	43.94	34.81	31.04	7.38	55.65	54.76
66	6.34	76.49	45.51	31.08	35.85	7.45	55.26	58.21

Appendix Table 16 - continued.[a,b]

Hay	CP	NDF(H)	ADF	HEMV	CELLV	PLIG	IVNDFD	IVOMD
68	6.23	77.60	43.77	33.83	35.41	7.24	47.01	49.36
70	6.11	78.20	42.84	35.36	35.20	6.43	46.99	48.63
71	5.11	75.66	43.34	32.32	35.67	6.24	61.74	62.35
73	5.06	76.00	44.44	31.56	36.06	6.79	59.56	58.89
75	6.69	78.49	43.29	35.20	35.45	7.02	46.43	47.36
76	6.78	78.61	42.72	35.89	35.23	6.68	48.66	48.41
77	4.29	74.65	41.70	32.95	33.46	7.29	54.80	58.77
84	6.30	78.93	41.03	37.90	30.33	6.79	56.47	57.57
85	6.97	78.01	40.76	37.25	31.08	7.41	54.40	57.41
86	5.08	80.76	42.32	38.44	32.94	8.49	45.15	50.65
87	4.89	80.06	43.57	36.49	31.60	8.60	46.08	47.34

[a]Refer to Table 1 and Appendix Table 14 for explanation of abbreviations; composition values are expressed on dry matter basis.

[b]Hartadi, 1980.

Appendix Table 17. *In vivo* intake and digestibility data.[a]

Hay[b,c]	OMI	OMD	DOM	DOMI	NDFI	NDFD	DNDF
2	74.3	72.7	65.0	54.0	53.6	77.3	49.8
5	66.9	67.1	62.8	44.9	52.1	71.8	52.5
7	63.8	64.5	60.6	41.3	50.1	68.9	51.0
8	79.7	71.0	62.3	56.5	57.1	76.2	48.1
9	65.3	71.9	65.4	47.1	48.3	77.1	51.9
10	57.5	63.3	59.5	36.4	45.3	67.0	49.7
11	55.8	62.2	58.1	34.8	44.2	66.5	49.2
13	64.2	69.7	62.1	44.8	45.1	73.3	46.1
23	61.3	61.4	56.2	37.5	50.4	66.6	50.3
25	62.7	54.6	51.7	34.2	52.2	57.5	45.8
27	62.4	56.6	53.8	35.4	52.5	60.1	48.0
28	55.7	67.2	61.4	41.9	43.9	72.5	52.4
30	55.0	63.4	58.6	34.8	44.1	67.9	50.6
32	55.7	54.3	51.6	30.3	45.5	56.6	44.1
33	58.0	53.7	51.1	31.4	47.3	55.3	43.0
34	53.8	55.2	51.1	29.9	43.6	59.8	44.9
35	--	--	--	--	--	--	--
36	58.0	59.6	49.6	34.5	43.9	61.0	38.1
44	56.5	63.6	58.8	35.9	45.6	68.0	51.2
46	61.2	60.0	56.8	36.8	51.2	62.6	49.7
48	62.7	52.6	50.1	33.0	51.1	54.2	42.3
49	53.7	67.2	62.9	36.1	43.4	71.9	54.6
51	58.0	69.7	65.3	40.7	46.5	74.2	56.0
53	48.0	53.7	50.9	26.0	39.4	56.8	44.2
54	59.5	57.8	54.9	34.7	48.9	60.3	47.0
55	55.1	60.1	56.3	33.1	43.7	63.3	47.2
56	47.2	58.4	52.3	27.5	38.9	61.1	45.6

Appendix Table 17 - continued.[a]

Hay[b,c]	OMI	OMD	DOM	DOMI	NDFI	NDFD	DNDF
57	46.7	57.9	51.2	27.0	38.8	61.4	45.0
66	62.9	60.4	56.2	38.1	50.7	63.9	48.2
68	61.5	56.6	53.3	34.8	51.5	59.6	46.7
70	61.6	59.2	55.9	36.5	50.9	61.9	48.4
71	51.6	59.7	56.1	30.9	40.9	63.5	47.6
73	50.1	61.4	57.4	30.9	40.3	65.2	49.0
75	52.2	51.6	48.8	27.5	42.9	55.4	43.2
76	52.6	61.1	57.9	32.0	43.8	64.3	50.7
77	49.7	58.2	55.0	28.8	38.2	58.9	43.0
84	52.5	62.4	57.2	32.8	43.7	64.2	48.7
85	52.2	56.1	52.2	29.3	42.6	57.4	43.3
86	64.1	56.6	53.6	36.3	53.2	56.5	44.4
87	47.4	54.0	50.3	25.6	39.3	54.4	41.9

[a]Abrams, 1980

[b]Refer to Table 1 and Appendix Table 14 for explanation of abbreviations and units of expression.

[c]Obtained from two field replications.

Appendix Table 18. Means of neutral detergent fiber by age and hay.

NDF	2	4	6	8
X46-2	71.7	81.7	77.9	77.8
X124-4	71.6	79.2	78.5	78.5
Paraguay	77.9	83.8	81.5	81.7
Argentine	79.2	84.5	80.6	81.6
Coastcross-1	79.8	81.4	81.8	83.2
NDF(H)				
X46-2	68.4	78.2	76.6	75.6
X124-4	68.5	75.7	76.3	75.8
Paraguay	74.8	79.8	79.3	78.0
Argentine	75.0	79.6	79.2	78.4
Coastcross-1	76.0	79.4	78.5	80.4

Appendix Table 19. Means of holocellulose by age and hay.

	2	4	6	8
X46-2	93.0	92.6	92.0	91.4
X124-4	92.1	92.6	93.5	91.3
Paraguay	93.3	93.2	92.4	92.2
Argentine	91.5	92.2	93.3	92.5
Coastcross-1	93.2	93.6	93.0	93.0

Appendix Table 20. Means of hemicellulose by age and hay.

HC	2	4	6	8
X46-2	53.1	51.9	49.9	49.4
X124-4	50.4	48.9	47.4	47.9
Paraguay	54.7	52.7	50.9	50.6
Argentine	52.5	52.4	51.6	50.4
Coastcross-1	56.6	52.2	57.1	55.1
HEMV				
X46-2	47.9	43.3	41.4	42.4
X124-4	44.6	42.1	39.6	42.1
Paraguay	51.6	46.3	44.2	44.2
Argentine	52.0	46.8	45.2	45.4
Coastcross-1	46.0	44.7	47.9	46.6

Appendix Table 21. Means of cellulose by age and hay.

CELL	2	4	6	8
X46-2	39.9	40.8	42.1	42.0
X124-4	41.7	43.2	46.1	43.4
Paraguay	38.6	40.5	41.3	41.6
Argentine	39.0	39.8	41.6	42.1
Coastcross-1	36.6	41.4	35.9	37.9
CELLV				
X46-2	41.9	44.8	47.0	45.8
X124-4	42.2	47.2	50.5	47.3
Paraguay	40.5	43.7	45.0	45.4
Argentine	40.7	44.0	44.9	44.9
Coastcross-1	35.8	38.7	39.1	40.1

Appendix Table 22. Means of lignin by age and hay.

LIG	2	4	6	8
X46-2	7.0	7.4	8.0	8.6
X124-4	7.9	7.4	6.5	8.7
Paraguay	6.7	6.8	7.6	7.8
Argentine	8.5	7.8	6,7	7.5
Coastcross-1	6.8	6.4	7.0	7.0
PLIG				
X46-2	6.2	9.8	9.6	9.8
X124-4	6.6	8.7	8.4	8.6
Paraguay	6.2	9.3	9.6	9.1
Argentine	5.8	8.4	8.7	8.4
Coastcross-1	9.3	9.0	9.0	10.6

Appendix Table 23. Analyses of variance of feces samples and *in vivo* digestibility.

		P Values								
	df	NDF[a]	HOLO	HC	CELL	LIG	NDFD	HOLOD	HCD	CELD
Age (A)	1	.0001	.4474	.0389	.1456	.4474	.0001	.0001	.0001	.0007
Hay (H)	2	.0001	.0079	.7763	.1159	.0079	.0213	.0167	.0527	.0033
A x H	2	.0027	.0183	.3172	.4527	.0183	.0462	.0651	.1034	.0890
C.V.		1.16	1.92	7.6	20.43	13.31	5.69	5.32	5.55	6.16
S.D.		.84	1.68	4.08	7.21	1.68	3.58	3.46	3.46	4.22
Mean		72.4	87.4	53.6	35.3	12.6	63.0	65.0	62.4	68.4

[a]Abbreviations may be found in Table 1.

Appendix Table 24. Least squares means of *in vivo* intake per unit body weight.[a] (g/kg body weight)

	Maturity (Weeks Regrowth)			
OMI	2	4	6	8
X46-2	26.2	22.2	21.6	22.1
X124-4	27.4	21.4	21.5	19.7
Argentine	22.9	23.1	23.7	22.2
Paraguay	23.6	23.3	21.4	21.9
Coastcross-1	21.3	17.3	20.0	21.5
NDFI				
X46-2	19.1	18.2	17.2	17.4
X124-4	19.4	17.1	17.3	15.7
Argentine	18.0	19.2	19.6	18.4
Paraguay	18.5	19.3	17.8	18.2
Coastcross-1	16.0	14.2	16.6	17.8

[a]Abrams (1980)

Appendix Table 25. Least squares means of *in vivo* digestibility data.[a]

	Maturity (Weeks Regrowth)			
OMD	2	4	6	8
X46-2	72.3	58.3	61.8	59.2
X124-4	70.4	65.2	68.5	60.6
Argentine	63.9	55.0	55.4	60.1
Paraguay	64.6	54.5	57.0	54.1
Coastcross-1	59.7	58.1	59.4	55.2
NDFD				
X46-2	77.3	63.2	65.6	61.2
X124-4	74.7	70.2	73.1	64.3
Argentine	67.9	57.7	58.0	62.9
Paraguay	69.1	57.3	59.9	57.5
Coastcross-1	60.9	61.1	61.1	55.4

[a]Abrams (1980)

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Sandra L. Russo was born November 24, 1948, at the Great Lakes Naval Training Base, Illinois. She graduated from La Porte High School, La Porte, Indiana, in June 1966. From September 1966 until June 1968 she attended Purdue University--North Central Campus, Westville, Indiana, on a part-time basis. From June 1968 until August 1971, she attended Purdue University, West Lafayette, Indiana. She worked in the Agronomy Department for Drs. Robert Barnes and Ray Bula from September 1968 to August 1971. She received the Bachelor of Science degree in animal science in August 1971.

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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.

Gerald O. Mott, Chairman
Professor of Agronomy

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