DIGESTIBILITY OF HAYS FROM IMPROVED SELECTIONS OF OLD WORLD BLUESTEMS

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OF OLD WORLD BLUESTEMS

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TABLE OF CONTENTS

LIST OF TABLES

CHAPTER I

INTRODUCTION

Development of improved types of grasses which are well adapted to the climatic and soil conditions of this area is important for the cattle industry. Old World bluestem grasses are a group of improved grasses developed in Oklahoma and surrounding states. Several varieties have been identified as productive pasture grasses in the semi-arid regions of the Southern Great Plains. With proper management, these can possibly replace a considerable portion of the present native pastures which are often characterized by low yield and quality.

Knowledge of the nutritive value of forages and feedstuffs is basic to animal nutrition. Determining this nutritive value by feed trials is laborious. Recently, use of internal and external markers to determine digestibility of feedstuffs has increased. Several markers have been suggested to evaluate digestibility of forages. Since the amount of pepsin insoluble nitrogen (PIN) in feces equals the amount fed (Zinn and Owens, 1981) it may be used as an internal marker.

Since little is known about the nutritional characteristics of Old World bluestems and probably nothing about PIN as a marker, the objectives of this study were: (a) to evaluate the in vivo digestibility of different nutrient components of four varieties of Old World bluestem have made after seeds had been harvested, and (b) compare digestibility values estimated by using PIN as an internal marker with values determined by total collection.

CHAPTER II

REVIEW OF LITERATURE

There are several factors to be considered in the introduction of new forage varieties. This review will investigate some of these factors: (a) characteristics of the Great Plains of the United States, including Oklahoma; (b) selection and development of Old World bluestems; (c) factors affecting diet quality and digestibility of forage; (d) digestibility of Old World bluestems; (e) the effect of supplementation of protein on low quality forages; and (f) use of internal markers to determine digestibility.

Characteristics Important in the Great Plains of the U.S.A.

The Great Plains of the U.S. consist of about 245 million acres, nearly 30% of which is the western grazing area (Heath et al., 1978). This acreage represents one of the largest and potentially most important areas of forage production in North America. It is characterized by variable climate conditions, but it is predominantly semi-arid (Savage, 1945; Rogler, 1945). Rainfall is the most limiting factor for crop production. Average annual precipitation varies from 10 inches to 45 inches (Savage, 1945; Harlan, 1955; Heath, 1978). The highest precipitation occurs during the warm or growing season, but high winds and hot weather decrease the effectiveness of the moisture. Droughts are irregular but persistent in recurrence (Savage, 1945). Temperature is also widely variable from time to time. During the summer the temperature often reaches over 100°F and in the winter it is sometimes below zero (Savage, 1945; Rogler, 1945; Harlan, 1955).

Most of the undisturbed soils are high in fertility and rarely respond to large inputs of fertilization, unless accompanied by irrigation. However, due to continuous cultivation and poor soil preservation, some soils have reduced fertility (Savage, 1945).

The elevation of the Great Plains varies from 650 to 4000 feet above sea level (Heath et al., 1978). The Northern Plains are dominated by "cool season" grasses and the Southern Plains by "warm season" grasses which start growth later in the spring and mature by the time that the fall season commences. This group is represented mainly by bluestems, Indiangrass, and switchgrass (Harlan, 1955).

Climate and Soil Conditions in Oklahoma

The climate in Oklahoma varies widely with season and geographic region. The western part is cooler and drier than the eastern region where rainfall is more frequent and abundant.

Across the state, summers are hot and drying winds occur causing rapid evaporation of soil moisture. About 75% of the annual rainfall occurs during the growing season (Gray and Galloway, 1969). Temperatures of 100°F or higher are common in the summer, dropping to zero during the winter. Summers are long and winters are relatively short and mild with some snow (Gray and Galloway, 1969). Rainfall, interacting with some other factors as fertility and soil texture, is considered to be the first limiting factor in native range production (Harlan, 1960).

Broad differences in soils types are found in Oklahoma. In the eastern part, leaching is intense due to humid conditions. Soils are light in color, low in nitrogen and phosphorus, and many do not have adequate amounts of potassium. Under cultivation, the nitrogen is rapidly depleted. They range from moderate to strongly acid (Gray and Galloway, 1969). The western prairies have redder soils, lighter colors and less leached than those in eastern section. Many are low in phosphorus and nitrogen. Low fertility is caused more often by erosion than by leaching or crop removal (Gray and Galloway, 1969).

Soils in the northwestern part, i.e., Panhandle soils, vary from neutral to alkaline. There is a moderate amount of calcium carbonate accumulation and nitrogen content is lower than in the eastern and western sections. Wind erosion is a problem in this area (Gray and Galloway, 1969).

In general, Oklahoma soils may be considered shallow, with uneven slopes, extremely poor internal drainage, light or reddish color with high clay content. However, some areas are covered by sandy deposits.

Horn and Taliaferro (1979) reported that the Old World bluestem grasses are well adapted to the adverse soil conditions of Oklahoma where many perennial grasses are not very persistent. Special consideration has been given to these grasses for purposes of soil conservation and production of livestock feed.

History of Selection and Development of Old World Bluestems for Release in Oklahoma

Neal (1893, p. 9), Director of the Oklahoma Agricultural Experimental Station said: "One of the most pressing needs of Oklahoma is a series of good grasses. . . ."

In the early 1900's attention to the development of superior forage for the Great Plains area was important. Several expeditions visited different parts of the world and new importations started. According to Harlan (1960) turkestan alfalfa, a new variety of alfalfa, was imported in 1893, and some others in 1906, 1908, and 1910; crested wheatgrass was introduced in 1906, and sudangrass was introduced to the Great Plains in 1909. The history and origin of some grasses is confusing or sometimes unknown. Celarier and Harlan (1955) reported that some of the Bothriochloa were introduced in 1917 into California from Arnoy, China. Archer and Bunch (1953) stated that some of the Old World bluestems were introduced from India in the early 1900's. Furthermore, Celarier and Harlan (1955) and Harlan (1958) reported that Bothriochloa intermedia was native in Africa, India, Pakistan, Ceylon, Malaya, China, Australia and most of the Pacific Islands. Somehow, seeds from this specie were introduced and hybridized in Oklahoma. Harlan and Celarier (1959) stated that Bothriochloa intermedia has received germ plasma from at least five species donors. Harlan (1952) said that regardless of the origin, Bothriochloa ischaemum was introduced in Stillwater from Maryland by 1935 and by 1949 was released fcr certification in Texas, under the name "Texas yellow beardgrass" (King Ranch).

Because of their resistance to adverse climatic conditions, researchers began investigating Old World bluestem grasses. In 1952, Celarier and co-workers studied some material available and in combination with researchers in Europe, Africa, Asia, Australia, and the East Indies, assembled in Stillwater the largest collection of Old World bluestems in the western hemisphere (Celarier and Harlan, 1955).

From a collection of over 750 accessions of Old World bluestems mainly by Harlan et al. (1958, 1959) several varieties have been developed which have potential as productive pasture grasses in the semi-arid regions of the southern Great Plains. Bluestems have been found to have most of the characteristics which are considered in selection of ecotypes for potential use, such as: adaptability to soil type, drought resistance, yield and quality of forage produced, winter hardiness, disease and pest resistance, aggressive seedling establishment (Ahring et al., 1973; Harlan et al., 1958; Dewald et al., (1981).

By 1961, over 50 accessions were being tested and some were discarded due to low winter hardiness (Harlan et al., 1961). Hybrid Bothriochloa intermedia x ischaemun was found to have more hardiness and drought resistance than the already released yellow beardgrass (Harlan, 1955).

Plains bluestem or Bothriochloa ischaemum var ischaemum was developed and released in the late 1960's by the Oklahoma Agricultural Experiment Station and the Plant Science Research Division, Agricultural Research Service, U.S. Department of Agriculture. The variety is derived from 30 selected seed sources of plants with uniform morphological characteristics, mainly from Pakistan, Iran, Iraq, India, Turkey, and Afghanistan (Taliaferro et al., 1972).

Recently, in 1973, five varieties of Old World bluestem were established at the Southwestern Livestock and Forage Research Station in El Reno, Oklahoma. Hay was collected in 1974 and 1975 and evaluated by Horn and Taliaferro (1979). During the last few years, information has been obtained indicating considerable potential for some of these selections and they are being considered for release to seed producers.

Factors Affecting Diet Quality and Digestibility of Forage

Quality of forages affect animal performance. Nutritive value of forages is determined, in part, by plant chemical composition. However, there are some variables which are not part of the forage that may affect the quality of the diet. Some of such variables are: unfavorable climatic conditions, e. g., high temperature decreased digestibility and type and composition of soils (Van Soest, 1982).

Maturity

Stage of maturity seems to be the most important factor affecting plant chemical composition and digestibility (Oelberg, 1956; Balch and Campling, 1962).

Van Soest (1967, 1982) stated that the digestibility of the cell content fraction declines as grasses mature. Also, the protein content decreases (Hopper and Nesbitt, 1930; Kamstra, 1973; Staples et al., 1951) and the fibrous fractions are increased (Kamstra et al., 1958; Kamstra et al., 1968). In general, the feeding value declines as the growing season advances.

Late cut prairie hay was found by Staples et al. (1951) to contain only 50 to 70% as much total protein as early-cut hay. The dry matter, ether extract, crude fiber and nitrogen-free extract contents were not affected by time of harvest. Decrease in digestibility due advanced stage of maturity is mainly due to an increase in lignin (Patten, 1943; Kamstra et al., 1955, 1958). Recent studies conducted by Horn and Taliaferro (1979) and Johnson et al. (1971) demonstrated a decrease in dry matter digestibility of selected varieties of Old World bluestem as maturity advanced.

Fiber

A high positive relationship between cell contents and overall digestibility should be expected since cell contents are almost completely digested (McCroskey, 1968). On the other hand, fiber components are inversely related to digestibility (Van Soest, 1963, 1982; Moir et al., 1975).

Intake of fiber mass varies inversely with the volume of indigestible matter in the digestive tract of ruminants (Balch and Campling, 1962). Van Soest (1965) reported that fiber mass becomes a limiting factor when the proportion of cell wall content increases to more than 55 to 60% of the forage dry matter.

Lignin-Cellulose

Acid detergent fiber is essentially composed of lignin, which is considered the least digestible part of the forage (Van Soest, 1965; Oelberg, 1956; Cramptom and Maynard, 1938), cellulose and insoluble minerals (Van Soest, 1968). Lignin may increase the rumen retention time (Rittenhouse, 1970) and it may have a direct influence reducing intake and digestion by ruminants. However, because there are some other factors affecting digestibility, Kamstra et al. (1958) noted that the most highly lignified material is not always the least indigestible. Van Dyne and Lofgreen (1964) found that lignin was inversely related to the most of the other chemical constituents of the plant.

Little bluestem was highly lignified at the end of the growing season (Kamstra, 1973). Cogswell and Kamstra (1976) observed increases in cellulose and lignin contents in four prairie grasses from June to September. Similarly, Horn and Taliaferro (1979) noted increases in acid detergent lignin in five varieties of Old World bluestem from July to September.

Voluntary Intake

Intake is highly correlated with dry matter digestibility (Blaxter et al., 1961, 1966; Van Soest, 1964). McCroskey (1968) reported that forage intake increases as digestibility increases up to about 60% and it decreases when cell wall constituents are more than 50 to 60% of the dry matter. Murdock (1967) reported that dry matter intake of hay with a digestibility of 72% was approximately twice that of hay with 55% digestibility.

Voluntary intake of long forages increases rapidly as the quality of forages increases, and tended to stabilize at an apparent digestibility of over 75% (Blaxter et al., 1961). They suggested that sheep would eat none of a food with an apparent digestibility lower than 30%.

Paladines et al. (1964) and Graham (1964) observed no appreciable change in digestibility of chopped hay when the intake was increased. Reid and Tyrrel (1964) reported that the effect of level of intake on digestibility in ruminants depends upon the chemical and physical nature of the diet. In general, the digestibility of long or chopped forages was not affected, while the digestibility of finely ground forages, usually decreased as intake was increased. However, Blaxter et al. (1961), Blaxter and Graham (1955, 1956) and Blaxter and Wainman (1964) observed that the digestibility of long and chopped forages decreased when the intake increased.

Plains bluestem forage was highly palatable for livestock. In grazing studies, it was preferred over Caucasian and found to be almost equal to King Ranch (Taliaferro et al., 1972). In a comparison of four new varieties of Old World bluestem and Caucasian by Dewald et al. (1981) voluntary consumption of Bothriochloa ischaemum var ischaemum was equal to or greater than that of other varieties being tested. Voluntary intake by calves of five high quality forages from different varieties of Old World bluestem, showed no differences among the different varieties at the same stage of maturity (Horn and Jackson, 1979).

Digestibility of Old World Bluestems

In vitro dry matter digestibility values for Plains bluestem was reported by Taliaferro and co-workers (1972). They found that the digestibility of Plains was higher than Caucasian (49.3 vs. 45.4%). The samples were collected once in May (highest digestibility value) and in June, and twice in August. The digestibility decreased as growing season advanced.

Horn and Taliaferro (1979) made an assessment of the seasonal changes in the nutritive value of five selected varieties of Old World bluestem, including "Plains" and Caucasian. There was a decreasing trend in in vitro dry matter digestibility as the season progressed, but it was not as great as is seen for most grasses. The lowest values were reached in August when crude protein also was lowest. In vivo dry matter digestibility of hays made from the same varieties was similar, although differences among cuts during the growing season were sizeable (Horn and Jackson, 1979). Values ranged from 61 to 63% for hays harvested in 1974 and 55 to 58% for those collected in 1975. Differences among varieties were not significant.

Dewald et al. (1981) reported that weight gains of steers grazing Bothriochloa ischaemum var ischaemum were equal to or higher than those of steers grazing other varieties being tested. Steers fed hay made from this variety had the highest average daily gain (1.44 lb/hd/day) compared to Bothriochloa ischaemum var sangarica and Bothriochloa intermedia var indica. Steers fed Bothriochloa ischaemum var ischaemum also had the most efficient feed conversion (7.6 lb hay/lb gain), indicating relatively high nutritive value of this hay. Hays were made from stands harvested for seed.

McLeod (1969) and Zalkow et al. (1980) found that Bothriochloa intermedia var indica contained a certain volatile acid (acorenone B) which may decrease palatability to ruminants. However, McLeod (1969) reported that this acid appears to have no adverse effect on the activity of rumen-microorganisms.

Protein Supplementation of Low Quality Forages

The literature does not reflect agreement among different researchers concerning the effect of protein on intake and/or digestibility of forages.

Early work by McCall (1940) showed an increase in intake of mature grasses by feeding a protein supplement. Crabtree and Williams (1971) reported a marked increase in hay consumption when soybean meal constituted 20% of the total dry matter in the ration. Elliot (1967a) obtained an increase in voluntary intake when protein was added to low quality roughages, and slight increase in digestibility was noted. Another evidence that addition of protein to low quality roughages increase intake was presented by Egan (1970). He added enough casein to increase nitrogen content of basal oaten chaff from .9 to 1.8% and observed an increase of 23% in mean dry matter intake. Apparent digestibility also was increased.

On the other hand, some researchers found that protein supplementation decreased intake. Murdock (1964) reported a marked reduction in hay intake when 454 g and 908 g of concentrate was fed daily to sheep and it was higher when a greater crude protein content was supplemented. Campling and Murdock (1966) reported that intake of hay was depressed from 7.92 to 7.50 kg with addition of 4 kg of concentrate in non-lactating cows. In a trial with lambs fed wheat straw and 200, 400, and 600 g of protein supplement, Horn et al. (1981) observed that the intake of wheat straw decreased with increased level of supplement.

Rittenhouse and co-workers (1970) and Clanton (1982) found that crude protein supplementation had a small influence on forage intake and dry matter digestibility. They concluded that factors other than protein may limit intake.

Use of Various Components of Plant Material as Internal Indicators to Determine Digestibility

Several markers, internal and external, are being used to determine nutritive nature of forages. A perfect marker is one that is completely recovered because it is neither digested nor absorbed (Block et al., 1981). Source of the components of plants used as internal markers are (a) Lignin (Connor et al., 1963; Cook and Harris, 1951; Crampton and Maynard, 1938: Elam et al., 1962; Ely et al., 1953; Forbes and Garrigus, 1948, 1950; Kane et al., 1953, Rittenhouse, 1970; Streeter, 1969; Sullivan, 1955, Thorney et al., 1979; Wallace and Van Dyne, 1970; Weston and Hogan, 1968); (b) Acid Insoluble Ash (AIA) (Block et al., 1981; Thorney et al., 1979; Van Keuten and Young, 1977); (c) Chromogens (Connor et al., 1963; Cook and Harris, 1951; Reid et al., 1950; Streeter, 1969); (d) Fecal Nitrogen (Fecal-N) (Scales et al., 1974; Streeter, 1969; Wallace and Van Dyne, 1970; Wilson et al., 1971); (e) Iron and Silica (Streeter, 1969).

Recently, Zinn and Owens (1981) stated that the amount of pepsin insoluble nitrogen (PIN) in feces was equal to amount of PIN fed.

Lignin

Lignin has been suggested as an internal marker in ruminal digestion trials. Its accuracy has been a point of discussion. Some reports have shown that lignin is completely undigested and others indicate that varying amounts of lignin may be digested.

In a series of seven trials with steers by Forbes and Garrigus (1948, 1950) average recovery of lignin ranged from 102 to 105%. Ellis et al. (1946) obtained 90.2%, and Weston and Hogan (1968) reported 96%. Due to the high recovery, they concluded that lignin may be a satisfactory marker for measuring digestibility. These results agree with those presented by Crampton and Maynard (1938) and Rittenhouse et al. (1970) who reported a lignin undigestibility of 93 to 108%. In contrast, Ely and co-workers (1953) obtained lignin digestion coefficients ranging from 3.8 to 16% in rations containing orchard gross hay cut at four stages of maturity. Similar results were presented by Sullivan (1955) relative to four different stages of maturity. Thorney et al. (1979)

obtained an average of 58% of lignin recovery from steers fed early and late cut hay, and 51% recovery was found by Lesperance et al. (1967).

Due to the divergences found among different studies, use of lignin as a digestibility marker, appears to merit further consideration.

Acid Insoluble Ash

AIA has been shown to be an acceptable natural marker for determining digestibility of feeds by livestock (Block et al., 1981). Van Keulen and Young (1977) found no significant difference in results using the total collection method compared with acid insoluble ash in their trial with brome grass pellets.

AIA recoveries of 102% were found by Thorney et al. (1977) who fed steers early and late cut hay. When hay was supplemented with 20, 40, 60 and 80% concentrate, recoveries ranged from 90 to 106%. Similar results were obtained by Block et al. (1981). They concluded that acid insoluble ash may be an adequate marker to determine digestibility when animals consume all the feed offered.

However, there are some limitations on using AIA as a marker. Block et al. (1981) concluded that when there are refusals some precautions have to be taken. Contamination of ingesta with soil or dust, or consumption of bedding material, were some limitations found by Van Keulen and Young (1977) which caused a bias when AIA was used. Also, they stated that this method is not appropriate for feedstuffs with low AIA content, such as alfalfa. In general, the authors said that additional research is needed to test the adequacy of AIA as a digestibility marker.

Chromogens

Chromogens as internal markers were proposed by Reid et al. (1950). They found an average of 100.5% of recovery of chromogenic substances from different forages in 36 trials. Greenhalgh and Corbett (1960) reported 95 to 154% recovery of chromogen. The chromogen content increased as season advanced. Chromogen gave satisfactory results when steers were fed summer range plants (Connor et al., 1963). Troelsen (1961) fed lambs with native hays, rough fescue and western wheatgrass. He obtained a chromogen recovery of 81 to 114%.

However, some disadvantages have been found to the chromogen technique. Use of chromogen as a marker is restricted to forages with high chlorophyll content or fresh green forage (Van Soest, 1981). Presence of certain oils may carry some chromogens through the intestinal wall and later to be excreted in the urine (Streeter, 1969; Cook and Harris, (1951). When the chromogen content of the feces was lower than 150 units per gram of dry matter, Richards et al. (1959) found serious limitations of using it as an internal marker.

Fecal-Nitrogen (Fecal-N)

Fecal-N has been extensively used as an internal marker to determine digestibility of grazing.forage. This method was developed by Lancaster in 1949 (Holechick et al., 1982). Generally, it is used to determine only dry matter and organic matter digestibility (Wallace and Van Dyne, 1970).

Similar digestibility values for grazed forages were found by Wallace and Van Dyne (1970) in their work with Fecal-N and conventional digestion trials. Scales et al. (1974) compared several techniques for determining digestibility. They concluded that fecal-N gave the best estimate of *in vivo* digestion. In contrast, fecal-N produced higher digestibility values when Wilson and co-workers (1971) compared this technique with the lignin ratio, two-stage *in vitro* rumen and *in vitro* cell wall techniques. Some objections have been made to the fecal-N technique. Fecal-N values have been found to vary with the season (Greenhalgh and Corbett, 1960; Langlands et al., 1963; Streeter, 1969), with application of nitrogenous fertilizers (Greenhalgh and Corbett, 1960) and with the different portions of the same plants (Streeter, 1969).

CHAPTER III

DIGESTIBILITY OF DIFFERENT COMPONENTS OF OLD WORLD BLUESTEM HAYS

Summary

Sixteen young lambs were used in a 4 X 4 Latin square experimental design to compare the digestibility of four varieties of Old World bluestem hay. Lambs were housed in individual digestion cages and fed mature hay grown at the Southern Plains Range Research Station at Woodward, Oklahoma. Each lamb was given 75 g of soybean meal (SBM) and 10 g of mineral mix per day as a supplement. Crude protein (CP) content of the hays ranged from 6.1 to 7.0% being relatively low because they were baled after seed had been harvested. Values for the other components were 70.8 to 74% neutral detergent fiber (NDF), 43.6 to 45.6% acid detergent fiber (ADF), 34 to 35.8% cellulose, and 5.6 to 6.1% lignin. Digestibility values ranged from 59 to 61.4% for dry matter (DM), 68.7 to 71.4% for CP, 58.4 to 61% for NDF, 50.9 to 54.6% for ADF, 60.7 to 64.1% for cellulose and 7.8 to 22.6% for lignin. In general, all four varieties of hay were relatively high in digestibility of the various nutrient components, considering the stage of maturity at harvest. High digestibility values may be attributed to the associative effects of feeding supplemental protein and also direct effect of supplemental protein. *Bothriochloa intermedia* var indica was lower in digestibility than the other three varieties compared in this study.

Introduction

Digestibility reflects the availability of nutrients of any feed given to animals. Moreover, dry matter digestibility (DMD) often is used as a measure of overall nutrient digestibility and is a good indicator of forage quality.

Digestibility of forages usually decreases as plant maturity increases. As plants become mature the lignin content increases and animal performance may decrease. Some researchers (Moir, 1971; Van Soest, 1965) have reported a negative relationship between digestion and increasing acid detergent fiber and lignin levels. Feed intake also is affected by plant maturity. Highly lignified forages have been shown to be poorly consumed due to increased rumen retention time (Rittenhouse, 1970). *In vitro* dry matter digestibility (IVDMD) of Old World bluestem declined as the season progressed, but not as great as is seen in most grasses (Horn and Taliaferro, 1979). Horn and Jackson (1979) concluded that crude protein (CP) content was adequate for growth and supplementation of grazing cattle would rarely be needed.

The purpose of this study was to determine *in vivo* digestibility of different nutrient components of selected varieties Old World bluestem hays.

Materials and Methods

Hay was made from four varieties of Old World bluestem after seed was harvested in July of 1980 at the Southern Plains Range Research Station at Woodward, Oklahoma. The varieties compared in a digestibility trial using young wethers were:

Code No.	Variety
WW-506	*Bothriochloa ischaemum* var sangarica
WW-573 (WW-SPAR)	*Bothriochloa ischaemum* var ischaemum
WW-477	*Bothriochloa ischaemum* var sangarica
WW-517	*Bothriochloa intermedia* var indica

Each hay was chopped to not less than 2.5 cm length and fed to each of 16 Finn X Dorset wethers, six months old, weighing 27.7 to 35 kg in sequences of a replicated 4 X 4 Latin square design, as shown in Appendix A, Table IX, to eliminate the effect of variations in forage intake among individual animals. Each hay was fed to four different lambs in each replication during respective periods of two weeks each. At the end of the four periods, each lamb had been fed all the hays being evaluated. The lambs were randomly allotted to digestion cages to avoid bias due to location in the building.

Hay was fed twice daily (8:30 in the morning and 4:30 in the afternoon) in sufficient quantity to allow 0 to 15% feed refusal in most instances. Since the hay used did not fulfill the CP requirement for the lambs, 75 g of SBM was fed each lamb daily to assure adequate protein intake for maintenance. A mineral supplement containing 13 to 15% calcium, 7% phosphorus, 30 to 36% salt, and undetermined amounts of other mineral elements, was fed at the rate of 10 g per day to provide adequate intake of minerals. In addition, a trace mineral supplement containing 96 to 99% salt and 1 to 4% trace minerals was available for *ad-libitum* consumption (Appendix A, Table X).

The collection period for feed refusals was six days, with feces collection starting at the third day of feed refusal collection and also lasting for six days. Each collection was made once daily. Feed refusal collection was done in the morning and feces collection at the time of the afternoon feeding.

Refused feed was weighed on each day of the six-day collection period and kept for future mixing. These orts were pooled and ground using a 1 mm screen in a Wiley mill. Also, the feces from each sheep were weighed individually and 10% of the total amount was dried in the oven (95°C) and kept for future composites. After six days of collection and drying the samples were pooled and ground through a 1 mm screen in a Wiley mill.

Samples of all feed, feed refusals and feces were analyzed, in duplicate, for crude protein (AOAC, 1975), dry matter and acid detergent fiber (Van Soest, 1963), lignin and cellulose by the permanganate oxidation procedure of Van Soest and Wine (1968), and neutral detergent fiber by the procedure of Van Soest and Wine (1967).

Statistical analyses were conducted using the Statistical Analysis System (SAS). An analysis of variance was performed. Since *Bothriochloa intermedia* var indica was the only variety used from a different species, it was compared to all other treatments by the orthogonal contrast procedure (Snedecor and Cochran, 1980).

Results and Discussion

Chemical Composition

Crude protein of the hays (Table I) were somewhat low because they were baled after seed had been harvested. In comparison, protein content of hays harvested three times during the growing season from five Old World bluestem plots at the Southwestern Livestock and Forage Research Station at El Reno, Oklahoma, were substantially higher (Horn and

TABLE I

CHEMICAL COMPOSITION OF OLD WORLD BLUESTEM HAYS[a]

Variety	Code No.	Crude protein	Acid detergent fiber	Neutral detergent fiber	Lignin	Cellulose
		---------------	---------------	%	---------------	---------------
Bothriochloa ischaemum var sangarica	WW-506	6.7	45.6	74.0	6.1	35.8
Bothriochloa ischaemum var ischaemum	WW-573[b]	6.6	44.0	72.8	5.8	34.3
Bothriochloa ischaemum var sangarica	WW-477	7.0	43.6	70.8	5.7	34.0
Bothriochloa intermedia var indica	WW-517	6.1	44.3	73.4	5.6	35.1

[a]Dry matter basis.
[b]Released for production as WW-Spar.

Jackson, 1979). However, the crude protein values obtained in the present study were higher than values obtained by Waller et al. (1972) and McMurphy et al. (1975) in work with native range grasses of Oklahoma.

ADF and NDF values were high because of high maturity stage (Table I). These values were similar to values obtained by Horn and Jackson (1979) and slightly higher than those reported by Horn and Taliaferro (1979) in work with selections of Old World bluestem. Values obtained in this study varied from 43.6 to 45.6% for ADF and 70.8 to 74% for NDF in Bothriochloa ischaemum var sangarica (WW-477) and Bothriochloa ischaemum var sangarica (WW-506), respectively.

Lignin content ranged from 5.6 to 6.1% being considered low taking into account the advanced maturity stage and also compared with values on bluestems obtained by Rao et al. (1973) and Coleman and Wyatt (1979). However, the values presented in this study were similar to those observed by Horn and Jackson (1979) and Horn and Taliaferro (1979).

Cellulose content varied from 34 to 35.8%. Values obtained were low relative to 58.4% found in bluestems by Coleman and Wyatt (1979).

Differences among varieties in composition were small and probably not of practical importance.

Voluntary Intake

Intake of the hays by the sheep was relatively low, about 20% less than needed to meet their energy requirements for maintenance (Table II). Using the formula of Garret et al. (1959) for digestible energy (DE), $DE = 138Wkg^{.75}(1 + 5.3 X gain)$, assuming no gain, the average DE requirement of the lambs for maintenance was 1785 kcal per day. The estimated amount consumed was 1410 kcal per day which gives a deficiency of 375

TABLE II

INTAKE BY SHEEP DURING DIGESTION TRIAL

Variety of hay	Code No.	Dry matter[a]	Crude protein[b]
		--------(g/day)--------	
Bothriochloa ischaemum var sangarica	WW-506	585	66.3
Bothriochloa ischaemum var ischaemum	WW-573	612	66.1
Bothriochloa ischaemum var sangarica	WW-477	601	68.4
Bothriochloa intermedia var indica	WW-517	600	63.5

[a]Values include 75 g per day of soybean meal and 10 g minerals.
[b]Values include about 34 g per day from soybean meal supplement.

kcal per day. It appeared that the ration provided 80% of the energy requirement for maintenance on the average, which was reflected in the average weight loss of .7 kg per lamb during the eight-week trial. The loss contributed an estimated 8316 kcal, using a 2.7 lb TDN per lb body weight as a conversion factor. The deficiency was 21,000 kcal during the eight-week trial. This difference may be explained by an overestimate of the requirement for the lambs, or use of an inaccurate conversion factor, i.e., 1 lb TDN not equivalent to 2000 kcal DE. Consumption of the hays ranged from 585 to 612 g per day for Bothriochloa ischaemum var sangarica (WW-506) and Bothriochloa ischaemum var ischaemum, respectively. Dry matter intake was similar for all the treatments, but CP intake by lambs consuming Bothriochloa intermedia var indica was significantly lower ($P<.0001$).

Intake of hay DM averaged only 1.75% of body weight, probably due to its advanced stage of maturity, which was reflected in the high amount of stems found in the refusals.

Because sufficient SBM was provided to complete protein requirements of the lambs, approximately one-half of the daily CP intake was from SBM. This must be taken into account in the interpretation of the values obtained since it has been demonstrated in other trials that supplemental protein may enhance both intake and digestibility of relative low-quality forage by ruminant animals.

Digestibility

Digestibility values for various components of the Old World bluestem hays are presented in Table III. DMD ranged from 59 to 61.4%, with Bothriochloa intermedia var indica being significantly lower ($P<.002$).

TABLE III

DIGESTIBILITY OF VARIOUS COMPONENTS OF OLD WORLD BLUESTEM HAYS

Variety	DM	CP	ADF	NDF	Lignin	Cellulose
	------------------------------(%)------------------------------					
Bothriochloa ischaemum var sangarica (WW-506)	61.1	70.2	54.6	61.0	22.6	64.1
Bothriochloa ischaemum var ischaemum (WW-573)	60.7	69.6	53.5	60.3	19.9	62.6
Bothriochloa ischaemum var sangarica (WW-477)	61.4	71.4	52.0	60.3	17.0	61.9
Bothriochloa intermedia var indica[a] (WW-517)	59.0	68.7	50.9	58.4	7.8	60.9

[a]Digestibility was significantly different from all other treatments ($P<.002$ for DM; $P<.01$ for protein; $P<.02$ for ADF, NDF and cellulose; and $P<.0001$ for lignin).

than the other three varieties. Horn and Jackson (1979) also observed lower values with Old World bluestem than those obtained in this study. Values obtained in this trial are higher than values reported for other forages, e.g., bermudagrass (Chapman et al., 1972), prairie hay (Staples et al., 1951), range grass hay (Coleman and Wyatt, 1979), bluestems (Rao et al., 1973) and "Plains" and native range (Taliaferro et al., 1975). Higher values obtained in this study may be attributed to the associative effect of supplemental protein.

The high digestibility of protein which varied from 68.7 to 71.4%, reflects the fact, mentioned above, that approximately one-half of the daily protein intake (34 g) was from SBM. By assuming a digestibility coefficient of 80% for SBM, it can be calculated that 27 g of the digestible protein in the daily ration was derived from this source. By difference, it is estimated that 19 g of the average amount of digestible protein is from the hay. Thus, digestibility of protein in the forage could be estimated to be approximately 59%. CP digestibility was positively related to CP content and DMD, a relationship also noted by Blaxter and Mitchel (1948) and McCroskey (1968).

ADF, NDF and cellulose digestibilities of Bothriochloa intermedia var indica were slightly and consistently lower ($P<.02$) than that of the other varieties. Lignin digestibility was also significantly lower ($P<.0001$) than for the other varieties. As expected, when cellulose and lignin digestibilities increased, the digestibility of ADF and NDF also increased. Values obtained in this study were similar to those reported by Robles et al. (1981) for four different orchargrass hays. However, Keys and Van Soest (1970) found higher values for orchargrass and bromegrass hays. Coleman and Wyatt (1979) obtained lower ADF and NDF digestibilities in range grass grown in El Reno, Oklahoma.

The largest difference among treatments was in regard to digestibility of lignin. The values obtained varied from 7.8 to 22.6%. The lowest value in Bothriochloa intermedia var indica may be attributed to uneven consumption of this hay during one of the periods which affected the calculations of digestibility. It resulted in negative digestibility values for some lambs in that specific period. Robles et al. (1981) reported higher lignin digestibility values for orchardgrass hay. In a study conducted by Ely et al. (1953) with orchardgrass hays at four different stages of maturity, there were large differences (3.8 to 16%) in digestibility of lignin. Similar results (-3.2 to 10.7%) were obtained by Sullivan (1955) in his trial with timothy hay at three different stages of maturity.

Due to digestibility values obtained for lignin (7.8 to 22.6%), it is suggested that this fraction is not an accurate indicator method to measure digestibility.

Cellulose digestibility were relatively high compared to values obtained by Kamstra et al. (1958) in orchardgrass and third-stage timothy, probably because of the low lignin content. It was demonstrated by Van Soest (1964) that cellulose digestibility is highly correlated with lignification. Values obtained were similar to those presented by Coleman and Wyatt (1979) for bluestems and Fannesbeck et al. (1981) for orchardgrass hay in early bloom.

Digestibility of only CP may have been affected directly by supplemental SBM which contributed only a small proportion of the other nutrient components in the diet. Its effect on overall digestibility of other components, if any, would probably be via an associative effect.

CHAPTER IV

DETERMINATION OF DIGESTIBILITY OF FORAGES USING PEPSIN INSOLUBLE NITROGEN AS AN INTERNAL MARKER

Summary

Digestibility of the different nutrient components of Old World bluestem hays were estimated by a conventional total collection trial and use of pepsin insoluble nitrogen (PIN) as an internal marker. The same samples were used for determination of digestibility by the two methods.

Average rates of recovery of PIN ranged from 72.1 to 86%. Using values unadjusted for recoveries of PIN digestibility was underestimated by PIN, the values were significantly lower (P<.0001) than those obtained by total collection. Results obtained by use of PIN as an internal marker in this study varied too much from results by the conventional method to suggest that PIN would be reliable for measuring digestibility of forages, without adjustment for PIN recovery. There was sufficient variation among varieties of hay in PIN recovery that adjustment of digestibility values for recovery would be of questionable value.

Introduction

Several markers are used to determine digestibility of forages. Kotbo and Luckey (1972) concluded that markers are cheap and convenient but they have some limitations.

Acid insoluble ash (AIA) is a natural marker used to determine digestibility of feeds by non-ruminants and ruminants. Block et al. (1981) conducted a trial with dairy cows and concluded that AIA is an adequate marker when there are no refusals from the feed offered to animals. Comparing digestibility values obtained by the AIA method and total collection method (TCM) with less than 10% of refusal. Block et al. (1981) obtained no difference between the two methods. However, when refusals were more than 10% and ort AIA content was ignored, digestibility values by AIA method were significantly different ($P<.05$) from those by TCM. When AIA in orts was taken into account, there was no difference between the methods. Under grazing conditions the use of AIA as an internal marker may have a restriction due to the contamination of ingesta with soil or dust; Van Keulen and Young (1977) recommended use of an appropriate correction factor for this contamination. Van Keulen and Young (1977) observed no differences in dry matter digestibility values using AIA method (four different concentrations of HCl) and TCM. AIA recoveries over 100% were obtained by Thonney et al. (1979) using a diet containing 80% of concentrate and 20% hay harvested in two different months; also, differences between AIA method and TCM were not significant.

Permanganate lignin was used as a marker by Thonney et al. (1979). Due to a low recovery (52.2%) they concluded that part of it was di gested. However, Elam et al. (1962) obtained 90.2% lignin recovery.

Three different internal markers were compared with TCM by Merrill et al. (1980). Acid detergent lignin, 2 N HCl acid soluble ash, and 72% H_2SO_4 acid insoluble ash were used to determine the digestibility value of straw-alfalfa plus alfalfa and millet straw using steers. Recovery of the markers used ranged from 96.4 to 96.8%. Digestibility values obtained with the first two markers mentioned, were significantly different ($P<.05$) compared with TCM; use of the third marker yielded similar values from those obtained with the conventional method. This latter result is in agreement with the conclusion by Van Soest (1982) who stated that as a marker sulfuric lignin yields more accurate than permanganate lignin.

Other internal markers that have been tested and some pertinent observations about these are as follows: Chromogens (Van Soest, 1982) are essentially limited to fresh green forages. Fecal nitrogen or indigestible protein is used in determining digestibility under grazing conditions. Holter and Reid (1952) reported that fecal nitrogen may be used as an internal marker in measuring dry matter digestibility of forages. However, Minson and Kemp (1965) observed some errors related with the equations in which digestibility is correlated with the nitrogen content of the forages or feces. Van Soest (1982) stated that several attempts have been made to improve the use of fecal nitrogen as a marker, but that it still has some limitations.

The purpose of this study was to evaluate pepsin-insoluble nitrogen (PIN) as an internal marker for determining the digestibility of the various nutrient components of hay.

Materials and Methods

Samples collected in a conventional digestibility trial were used to determine digestibility by the PIN method. Sixteen young lambs were randomly allocated in individual digestion cages and a 4X4 Latin square design was used. Animals were fed twice a day with hay made from four different varieties of Old World bluestem plus 75 g per day of SBM. In addition, 10 g per day of a mineral supplement was fed and a trace mineral supplement was available for ad-libitum consumption.

After an eight-day preliminary period, feed refusals and feces collection were made for six days. Feces samples were dried in the oven (95^{o}C) and next were ground through a 1 mm screen in a Wiley mill and kept for future composites. Samples of feed offered and refusals were stored without drying. The procedure for determining PIN was that of Goering and Van Soest (1970) (Appendix B).

Results and Discussion

Digestion coefficients for DM and CP are shown in Table IV. DM and CP digestibility values obtained using PIN were lower than those obtained by the conventional method. These results may be because of incomplete recovery of PIN, as shown in Table V. Recoveries ranged from 72.1 to 86% (SE ± .94) for Bothriochloa ischaemum var sangarica and Bothriochloa intermedia var indica, respectively. PIN content of the hay ranged from .45 to 54% of dry matter (Table VI). PIN content of the hay, as a percent of the total nitrogen, ranged from 45.8 to 50.5% for Bothriochloa intermedia var indica and Bothriochloa ischaemum var sangarica, respectively. PIN content of the SBM, fed as a protein supplement, was .66% of the dry matter which represented 10.2% of PIN as percent of the total nitrogen.

CP content was not related to apparent digestibility determined by PIN while it was positively related to values determined by TCM. Digestibility values for ADF, NDF, lignin and cellulose are presented in Table

TABLE IV

DIGESTIBILITY OF DRY MATTER AND CRUDE PROTEIN USING TCM AND PIN

Variety	Code No.	DM			CP		
		TCM	PIN	DIF[a]	TCM	PIN	DIF[a]
		------------%-----------			------------%------------		
Bothriochloa ischaemum var sangarica	WW-506	61.1	46.2	14.9	70.2	59.1	11.1
Bothriochloa ischaemum var ischaemum	WW-573	60.7	51.7	9.0	69.6	62.6	7.0
Bothriochloa ischaemum var sangarica	WW-477	61.4	48.6	12.8	71.4	61.9	9.5
Bothriochloa intermedia var indica	WW-517	59.0[d]	52.1[b]	6.9	68.7[c]	63.5[b]	5.2

[a]Differences between methods were significant, P<.0001.
[b,c,d]Digestibility was significantly different in comparison to other varieties: b) P<.01, c) P<.02, d) P<.002.

TABLE V

TOTAL INTAKE AND EXCRETION OF PEPSIN
INSOLUBLE NITROGEN

Variety	Code No.	PIN Intake	PIN in feces	Recovery[a]
		(g)	(g)	(%)
Bothriochloa ischaemum var sangarica	WW-506	20.1	14.5	72.1
Bothriochloa ischaemum var ischaemum	WW-573	18.4	15.0	81.5
Bothriochloa ischaemum var sangarica	WW-477	20.0	15.0	75.0
Bothriochloa intermedia var indica	WW-517	17.1	14.7	86.0[b]

[a]Pooled standard error for treatment mean is .94.
[b]Recovery was significantly higher ($P<.0001$) than all other varieties.

TABLE VI

PEPSIN INSOLUBLE NITROGEN
CONTENT OF HAY

Variety	Code No.	PIN Content	
		(% of DM)	(% of total N)
Bothriochloa ischaemum var sangarica	WW-506	.54	50.5
Bothriochloa ischaemum var ischaemum	WW-573	.48	46.5
Bothriochloa ischaemum var sangarica	WW-477	.54	47.9
Bothriochloa intermedia var indica	WW-517	.45	45.8

VII. Values obtained using PIN were lower than those obtained by TCM, with no adjustment made to account for incomplete recovery of PIN. Differences were significantly lower ($P<.0001$) by the PIN than by the TCM method (Table VIII). It was concluded that unadjusted values obtained by PIN, as mentioned above, provides evidence that part of the PIN was digested.

After the feces were collected, each fresh sample was dried overnight at 95°C. Refusals were not dried and were ground as they were collected. Since the procedure (Goering and Van Soest, 1970) indicated use of air dry samples (55°C), the requirement was not fulfilled with the feces. However, it was unlikely that this affected the digestibility values. The possible effect of digestibility may be illustrated by data obtained for lamb No. 1 in replication 1, period 1, treatment 2 and the formula:

$$\text{DMD} = 100 - \left\{100 \text{ X } \frac{\text{\% indicator in feed}}{\text{\% indicator in feces}}\right\}$$

We obtained 49.6% dry matter digestibility, with the feces dried at 95°C. Using a lower temperature (55°C), the value percentage indicator in feces possibly would be lower and the value of dry matter digestibility also would be lower. Thus, the use of a higher drying temperature does not explain the low values obtained by the PIN method. The effect of the higher drying temperature, if any, would have been to increase concentrations of PIN in the feces resulting in higher digestibility values.

The amount of PIN recovery in the feces was less than the amount consumed, as shown in Table V. It proves that some of the PIN was lost or was digested. It was possible that PIN was not digested but the analytical method used was incapable of accounting for all PIN in feed and/

TABLE VII

DIGESTIBILITY OF FIBROUS FRACTIONS OF HAYS BY TOTAL COLLECTION METHOD AND PEPSIN INSOLUBLE NITROGEN

Variety Code	Total Collection				Pepsin Insoluble Nitrogen			
	ADF	NDF	Lignin	Cellulose	ADF	NDF	Lignin	Cellulose
	%							
WW-506	54.6	61.0	22.6	64.1	37.1	46.2	-6.6	50.4
WW-573	53.5	60.3	19.9	62.6	42.7	51.2	1.3	54.0
WW-477	52.0	60.3	17.0	61.9	36.1	47.0	-10.2	49.2
WW-517	50.9[a]	58.4[a]	7.8[e]	60.7[a]	42.6[a]	51.3[b]	-8.1[d]	54.0[c]

[a,b,c,d,e]Digestibility was significantly different vs all other varieties: a) $P<.02$, b) $P<.04$, c) $P<.05$, d) $P<.4$, and e) $P<.0001$.

TABLE VIII

DIFFERENCE BETWEEN DIGESTIBILITY VALUES DETERMINED BY TOTAL COLLECTION AND BY USE OF PEPSIN INSOLUBLE NITROGEN AS AN INTERNAL MARKER[a]

Variety	Code No.	ADF	NDF	Lignin	Cellulose
		(%)			
Bothriochloa ischaemum var sangarica	WW-506	17.5	14.8	29.2	13.7
Bothriochloa ischaemum var ischaemum	WW-573	10.8	9.1	18.6	8.6
Bothriochloa ischaemum var sangarica	WW-477	15.9	13.3	27.2	12.7
Bothriochloa intermedia var indica	WW-517	8.3	7.1	15.9	6.7

[a]Differences between methods were significantly different, $P<.0001$.

or feces.

Since there were no other data available and no other analytical procedures which would improve the validity of results by the PIN method, additional research is necessary to test the adequacy of PIN as an internal marker. Adjustment of digestibility values based on the percentage of PIN recovery possibly has merit. However, the differences among varieties of hay in the percentage of recovery (Table V) are large enough that a different correction factor would be needed for each one. Then the question remains as to how many data are needed to establish a reliable correction factor for each treatment under conditions where PIN is to be used as a marker in lieu of making total collections for determination of digestibility.

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APPENDIXES

APPENDIX A

TABLES

TABLE IX

LATIN SQUARE DESIGN USED IN THE DIGESTION TRIAL

Period No.	Replication 1				Period No.	Replication 2			
	1	2	3	4		5	6	7	8
1	C	A	D	B	1	A	B	C	D
2	B	D	C	A	2	D	C	B	A
3	D	B	A	C	3	C	D	A	B
4	A	C	B	D	4	B	A	D	C

Period No.	Replication 3				Period No.	Replication 4			
	9	10	11	12		13	14	15	16
1	C	A	B	D	1	D	C	B	A
2	A	B	D	C	2	C	B	A	D
3	D	C	A	B	3	B	A	D	C
4	B	D	C	A	4	A	D	C	B

A = Bothriochloa ischaemum var. sangarica, WW-506
B = Bothriochloa intermedia var. indica, WW-517
C = Bothrochloa ischaemum var. ischaemum, WW-573
D = Bothrochloa ischaemum var. sangarica, WW-477

TABLE X

TRACE MINERAL MIXTURE

TRACE MINERAL SALT	Guaranteed Analysis:		
	Salt (NaCl)	Not more than	99.000%
	Salt (NaCl)	Not less than	96.000%
	Manganese (Mn)	Not less than	0.200%
	Iron (Fe)	Not less than	0.100%
	Magnesium (Mg)	Not less than	0.100%
	Sulfur (S)	Not less than	0.050%
	Copper (Cu)	Not less than	0.025%
	Cobalt (Co)	Not less than	0.010%
	Zinc (Zn)	Not less than	0.008%
	Iodine (I)	Not less than	0.007%
	Ingredients: Salt, Manganous oxide, Ferrous carbonate, magnesium oxide, calcium sulfate, copper oxide, cobalt carbonate, zinc oxide, calcium iodate, iron oxide. Color, natural and artificial flavors added. For animal feeding only.		

APPENDIX B

PROCEDURE

Pepsin-insoluble nitrogen

1. Weigh 1 g of air dry sample ground to pass 24 to 34 mesh (less than 1 mm) screen into 250 ml Erlenmeyer flask.

2. Add 1 g of pepsin and 100 ml of 0.1 N HCl to flask, stopper, and swirl. Place in oven or water bath at about 39°C for 20 hours.

3. Filter on 12.5 cm Whatman No. 4 filter paper. Leach thoroughly with distilled water. One way to ascertain if all soluble nitrogen is leached out is to determine if all traces of HCl has been removed from the filter paper. The taste test can be used for this.

4. Take filter paper containing pepsin insoluble residue and place in a Kjeldahl flask. Determine nitrogen according to standard Kjeldahl procedure.

5. Calculate pepsin-insoluble nitrogen:

   (g N/oven-dry sample)(100) which can be expressed as:
   (pepsin-insoluble N/total sample N)(100)

2

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