

# Forage quality of 10 Eastern gamagrass [*Tripsacum dactyloides* (L.) L.] genotypes

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## Abstract

Ten eastern gamagrass [*Tripsacum dactyloides* (L.) L.] entries from Kansas, Oklahoma, and Texas, were evaluated for in vitro dry matter digestibility (IVDMD), protein concentration, lignin concentration, and phenylalanine ammonia lyase (PAL) activity. Whole plants were harvested in Spring 1992 and 1995 from 4 replicates of each entry and divided into leaf and stem (plus sheath) components for analyses. Entry significantly affected all measurements except PAL; whereas the entry by year interaction significantly affected stem IVDMD, protein, and PAL. Across entries, stem IVDMD in 1992 (56.3% to 66.4%) and 1995 (55.9% to 64.9%) demonstrated greater variation than leaf IVDMD in 1992 (62.2% to 68.0%) and 1995 (66.7% to 71.0%). In 1992 and 1995, average leaf IVDMD and protein concentration were generally higher than average stem IVDMD and protein concentration. In 1992, lignin concentration was generally higher and varied more in stems (3.27% to 4.99%) than in leaves (3.54% to 4.11%). In 1995, lignin concentration was about the same and varied more in leaves compared with stems. Stem IVDMD was significantly correlated with lignin concentration in 1992 ( $r = -0.92$ ) and 1995 ( $r = -0.83$ ) as well as with protein concentration ( $r = 0.83$ ) in 1995. Activity of PAL in 1995 was correlated with stem IVDMD and protein. When all data were combined, IVDMD was significantly correlated with protein concentration ( $r = 0.82$ ). These results indicate that gamagrass entries demonstrate significant variation in forage quality, particularly in stems. Potential exists for development of new gamagrass cultivars with high IVDMD and protein.

**Key Words:** digestibility, gamagrass, forage, lignin, phenylalanine ammonia lyase, protein, *Tripsacum dactyloides*, quality

All programs and services of the U.S. Department of Agriculture are offered on a non-discriminatory basis without regard to race, color, national origin, religion, sex, age, marital status, or handicap.

Research funded in part by the University of Central Oklahoma Office of Faculty Research and the USDA-ARS Southern Plains Range Research Station. Authors wish to thank Jerry McLaughlin, ARS, for performing IVDMD, protein, and fiber analyses and extend appreciation to Carrie Barton and Travis Swink for assisting with enzyme analyses and data entry.

Manuscript accepted 23 Jan. 1999.

## Resumen

Se evaluó la calidad de forraje de 10 genotipos de "Eastern Gamagrass" (*Tripsacum dactyloides* (L.) L.) provenientes de Kansas, Oklahoma y Texas. Se determinó la digestibilidad in vitro de la materia seca (DIVMS), la concentración de proteína y lignina y la actividad de fenilalanina amonía liasa (PAL). En la primavera de 1992 y 1995 se cosecharon plantas enteras en 4 repeticiones de cada genotipo, para su análisis se separaron en hojas y tallos (mas la vaina). Hubo diferencias significativas por efecto de genotipo en todas las variables excepto el PAL, la interacción genotipo por año afectó significativamente la DIVMS de tallos, proteína y PAL. Entre genotipos, la DIVMS de tallos, en 1992 (56.3 % a 66.4% ) y 1995 (55.9% a 64.9%), mostró mayor variabilidad que la DIVMS de hojas (62.2% a 68.0% en 1992 y 66.7% a 71.0% en 1995). En 1992 y 1995, los promedios de la DIVMS y la concentración de proteína de las hojas fueron generalmente más altos que los promedios de los tallos. En 1992, la concentración de lignina fue generalmente más alta y más variable en tallos (3.27% a 4.99%) que en hojas (3.54% a 4.11%). En 1995, la concentración de lignina fue aproximadamente la misma y varió más en hojas que en tallos. La DIVMS de tallos se correlaciono significativamente con la concentración de lignina ( $r = -0.92$  en 1992 y  $r = -0.83$  en 1995) y con la concentración de proteína ( $r = 0.83$  en 1995). En 1995, La actividad de PAL se correlaciono con la DIVMS y la concentración de proteína de los tallos. Cuando se combinaron todos los datos, la DIVMS se correlaciono significativamente con la concentración de proteína ( $r = 0.82$ ). Estos resultados indican que los genotipos de "Gamagrass" tienen una variación significativa en cuanto a calidad de forraje, particularmente los tallos. Existe el potencial para desarrollar nuevos cultivares de "Gamagrass" con altos contenidos de proteína y alta DIVMS.

The new world grass genera, *Tripsacum* and *Zea* are phylogenetically allied with the subtribe *Tripsacinae* of the *Andropogoneae* tribe of the family Poaceae (Clayton 1973, de Wet and Harlan 1978). Brink and de Wet (1983) recognized 16 species of *Tripsacum*, with eastern gamagrass [*Tripsacum dactyloides* (L.) L.] being the most widespread and morphologically variable species. Eastern gamagrass can be found from Massachusetts to Nebraska in the USA (42° N Lat.) and southward sporadically through Meso America and into upper

South America (24° S Lat.). The western escarpment of central Mexico is cited as the center of *Tripsacum* diversity (Wilkes 1972).

Eastern gamagrass has been recognized as a highly productive and palatable forage of the eastern prairies (Rechenthin 1951). Previous work has demonstrated the productive superiority of eastern gamagrass compared with other grasses of the central and south central USA (Faix et al. 1980, Sims 1985, Vogel et al. 1985), as well as its unique morphological and reproductive traits (Dewald and Louthan 1979, Dewald et al. 1987). Evaluation of gamagrass germplasm showed a considerable range in yield, forage quality, and seed production (Wright et al. 1983, Kenna 1984). There is limited information on genotype and plant part variation in eastern gamagrass forage quality and metabolism. Knowledge of genotypic differences in forage quality is required for plant breeders to develop improved cultivars.

Ten eastern gamagrass entries from Oklahoma, Texas, and Kansas, and their progeny from specific crosses, were investigated as an observational study, to determine variation in *in vitro* dry matter digestibility (IVDMD) and protein concentrations. Lignin was studied because of its reported correlation with digestibility (Harkin 1973, Jung and Deety 1993) and the activity of phenylalanine ammonia lyase (PAL) was determined because of its relationship with lignin biosynthesis (Bidlack et al. 1995, Bidlack et al. 1997). The objectives were to determine: 1) if the variation in digestibility among different entries of eastern gamagrass was sufficient for the development of cultivars with improved quality, and 2) whether or not genetic variation in IVDMD is related to differences in protein and lignin concentration and PAL activity.

## Materials and Methods

Eastern gamagrass entries were selected for visual diversity, persistence, and uniformity among replicates from a field nursery established vegetatively in April 1991 at Woodward, Okla. Plants were arranged in a randomized complete block design with 4 replicates and irrigated and fertilized with nitrogen as necessary. The selected entries included

4 parents: 1023, from Nowata, Okla.; 1218 from Flush, Kan.; 1379 from Texarkana, Tex.; and 1582 from Ottawa County, Kan. A plant of unknown origin (OP) and the 4 parents were used to produce 4 progeny: 1673 (1023 X OP), 1748 (1582 X 1218), 1759 (1582 X 1379), and 1760 (1582 X 1,379). Plants from the F<sub>1</sub> generation were used to produce the 2 remaining entries, 1792 (1673 X 1760) and 1793 (from another 1673 X 1760). Each plant crown originally contained 30 to 200 vegetative shoots which remained vegetative or became reproductive tillers in the spring of subsequent years.

Reproductive tillers from each crown were sampled on 14 May 1992 and 1 June 1995. Stage of plant development at harvest, according to Simon and Park (1983), ranged from 52 (25% of inflorescence emerged) to 56 (75% of inflorescence emerged) in 1992 and from 56 to 62 (beginning of anthesis) in 1995. Fifty to 100 g of fresh weight (FW) tillers were removed by cutting to a 5.0 cm stubble. Tissue was transported, within 15 minutes of cutting, and divided into leaf, and stem plus sheath components. Since sheaths were included with stem samples, the word "stem" should hereafter be interpreted as including both stem and sheath.

A subsample of the fresh leaf and stem tissue was used for PAL assays. The remaining material was weighed, placed in a paper bag, dried at 55°C for 48 hours, ground in a mill to pass a 1 mm screen, and used for analyses.

## Digestibility, Protein, and Lignin Determination

*In vitro* dry matter digestibility was determined by the 2-stage direct acidification method described by Marten and Barnes (1980). Rumen fluid was collected from a fistulated steer fed on a native grass diet supplemented with an alfalfa-cotton seed mix. Duplicate samples from each experimental unit were hydrated in buffer for at least 1 hour before inoculation. Calculation of IVDMD was based on dry matter disappearance after 48 hours of fermentation followed by acid pepsin digestion for 24 hours.

Kjeldahl nitrogen concentration (Bremner and Breitenbeck 1983) was multiplied by 6.25 to estimate crude protein. Sequential fiber analysis out-

lined by Van Soest and Robertson (1980) was used to determine levels of lignin and other cell wall (CW) components in the ground stem samples. Lignin was calculated as the acid detergent lignin residue isolated from a 72% H<sub>2</sub>SO<sub>4</sub> extraction minus ash weight.

## Enzyme Extraction and Assay

Acetone powders of plant material were prepared to avoid enol tautomer-borate complexes associated with direct aqueous extracts (Erez 1973). Approximately 3.0 g of fresh plant material was homogenized in acetone (-78 °C, 200 ml) with a blender for 2 min. An acetone powder was collected from the homogenate by Büchner filtration through Whatman<sup>1</sup> No. 54 filter paper and rinsed with 50 ml of cold acetone. After air-drying for 1 hour, the powder was stored in an ultra-low freezer at -70 °C.

Complete replicates were removed from the freezer and 25 mg of acetone powder from individual samples was mixed with 25 ml of 100 mM borate buffer, pH 8.7, at 0 to 2°C. Suspensions were shaken at least once every 5 min for 1 hour and then filtered through Whatman No. 54 filter paper to give a clear, crude enzyme extract. These extracts contained PAL associated with the endoplasmic reticulum, which is known to be involved with lignin biosynthesis (Wagner and Hrazdina 1984). Duplicate measurements of protein were determined for these extracts (Bradford 1976) to enable expression of PAL on a per unit protein basis.

The PAL enzyme was assayed spectrophotometrically from a mixture of 1.5 ml of extract and 4.5 ml of 6.68 mM phenylalanine in borate buffer (pH = 8.7) over a period of 1 hour at 30°C in duplicate. Concentration of phenylalanine in the assay mixture was 5.01 mM. Enzyme activity (Bidlack et al. 1995) was obtained by measuring production of cinnamic acid at 290 nm using a molar extinction coefficient of 9000 cm<sup>-1</sup> (Saunders and McClure 1974). One unit of activity was expressed as the amount of cinnamic acid produced in micromoles per hour.

<sup>1</sup>Mention of a trademark, proprietary product, or vendor does not constitute a guarantee or warranty of the product by the USDA or University of Central Oklahoma and does not imply its approval to the exclusion of other products or vendors that may be suitable.



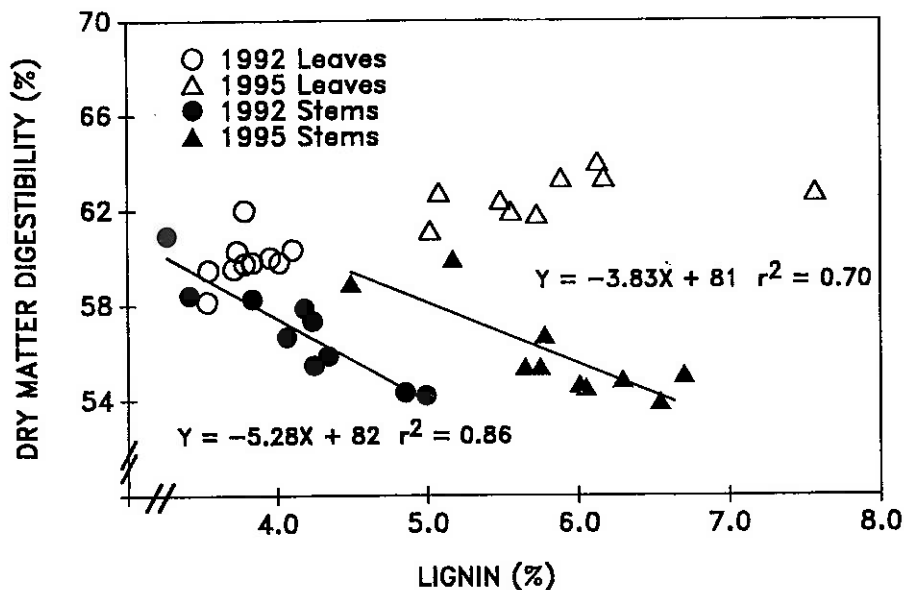


Fig. 1. Relationship between in vitro dry matter digestibility and lignin concentration of 10 lines of eastern gamagrass grown in the field at Woodward, Okla. Plants were established vegetatively in Spring 1991 and harvested in Spring 1992 and 1995. Each point represents the mean of 4 values

64.9%) demonstrated greater variation than leaf IVDMD in 1992 (62.2% to 68.0%) and 1995 (66.7% to 71.0%) (Table 2). Previous studies have shown that there is greater genetic variation in digestibility of stems compared with leaves of grasses (Hides et al. 1983, Ugherughe 1986, Buxton and Marten 1989). In 1992 and 1995, average leaf IVDMD (64.8% and 68.8%) and protein concentration (19.0% and 18.6%) were generally higher than average stem IVDMD (60.4% and 58.9%) and protein concentration (8.2% and 7.7%). These results concur with previous research and indicate that eastern gamagrass demonstrates wider variation in stem IVDMD than in leaves.

Differences in stem lignin concentration for entry (Table 1) suggested that lignin concentration may have affected digestibility of gamagrass genotypes. In 1992, lignin concentration was generally higher and varied more in stems (3.27% to 4.99%) than in leaves (3.54% to 4.11%) (Table 2). In 1995, lignin concentration was about the same between plant parts and varied more in leaves (5.08% to 7.57%) compared with stems (5.17% to 6.70%).

Relative ranking of gamagrass entries generally exhibited consistency in IVDMD, protein, and lignin concentrations between years and plant parts

(Table 2). Entries 1793, 1759, and 1792 were among the most digestible throughout the experiment; whereas entries 1218 and 1748 were consistently among the least digestible. Similarities among entries 1793 and 1792 were expected since these entries were derived from parents of similar lineage. Entry 1760, which was one of the parents of both 1793 and 1792, demonstrated inconsistent IVDMD between years and plant parts. Although the 1760 entry exhibited average leaf IVDMD in 1992, stem IVDMD of 1760 was very low in 1992 and 1995 while its leaf IVDMD was among the highest of entries in 1995. Perhaps hybridization, performed to generate entries 1793 and 1792, resulted in expression of traits different from one of the parent entries, 1760 (Poehlman 1979). As in the case of entries 1793 and 1792, expression of traits different from the parents resulted in plants with desirable characteristics for improving forage quality of eastern gamagrass.

Crude protein and lignin values followed expected trends in that, entries with highest IVDMD had high protein and low lignin concentrations. However, these trends were not always consistent. For instance, leaf IVDMD in 1759 was not as high as 1793 or 1792 in 1992 but leaf and stem IVDMD of 1759 was just

as high as that of 1793 and 1792 in 1995. This may have resulted from natural variation in the field. For most entries, consistencies among IVDMD, protein and lignin concentrations were reliable in assessing forage quality even though occasional variation was encountered.

The relationship between lignin and IVDMD was negative and significant ( $r = -0.92$ , 1992 and  $r = -0.83$ , 1995;  $P < 0.01$ ) for stems, but not for leaves. Stem IVDMD was reduced 5.3 ( $r^2 = 0.86$ ) and 3.8 ( $r^2 = 0.70$ ) percent for each percentage increase in stem lignin concentration in 1992 and 1995, respectively (Fig. 1). Natural variation in lignin concentration and phenolic composition of plants (Grand et al. 1982, Bidlack and Buxton 1992) has provided incentive to breed for decreased lignin concentration and increased digestibility (Kephart et al. 1990). Other investigations have exploited large variation in measurements of forage stems for digestibility (Buxton and Russell 1988) and cell wall deposition (Bidlack and Buxton 1992) studies. High variation in IVDMD of gamagrass stems and strong negative correlations between IVDMD and lignin suggest that the best approach for improvement of eastern gamagrass forage quality is through selection for high IVDMD and low lignin genotypes.

In 1995, stem IVDMD was positively correlated with protein ( $r = 0.83$ ,  $P < 0.01$ ). Regression analysis showed a 3.3 percentage unit increase in IVDMD for each unit increase in protein. Activity of PAL in 1995 was correlated with stem IVDMD ( $r = 0.81$ ,  $P < 0.01$ ) and stem protein ( $r = 0.66$ ,  $P < 0.05$ ). The combined data set demonstrated a positive correlation between IVDMD and protein ( $r = 0.82$ ,  $P < 0.01$ ). These results indicate that eastern gamagrass entries with higher protein concentrations were generally more digestible than gamagrass entries with lower protein concentrations. The positive correlation between PAL activity and stem protein was not surprising since the PAL enzyme is a protein. However, the anticipated relationship between PAL and lignin concentration was not revealed in this experiment; perhaps because it is the amount, as well as the activity of the PAL enzyme, that determines the extent of lignification (Bidlack et al. 1995, Bidlack et al. 1997). Other investigations suggest that, while PAL may initiate the lignification process,

cinnamyl alcohol:NADPH dehydrogenase activity (Morrison and Buxton 1993) and syringaldazine oxidase activity (Bidlack et al. 1997) are more directly correlated with lignin concentration in stem tissues.

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