

## RELATIONSHIPS BETWEEN LIGNIN CONTENT AND FERMENTABILITY OF INTACT AND CHEMICALLY TREATED BIG BLUESTEM FIBER

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### Summary

An accurate assessment of forage quality is required to allow prediction of animal performance. One of the most commonly used methods of forage evaluation is to measure lignin content, with more heavily lignified materials being considered less digestible. Two measures of lignin, acid detergent lignin (ADL) and acetyl bromide lignin (ABL), were assessed with regard to their ability to predict forage digestibility. Big bluestem forage samples were collected from three ungrazed, annually burned pastures at 38, 58, and 97 days postburn. These times were selected to represent a broad range of forage quality. Cell wall material was treated chemically by: 1) partial delignification (chlorite), 2) isolation of  $\alpha$ -cellulose, or 3) NaOH extraction.

Control and treated cell-wall material was analyzed for ABL and ADL and 24 and 72 hr in vitro dry matter disappearance (IVDMD). ABL increased with advancing maturity for intact fibers, whereas ADL was highest in the most mature forage but lowest for the intermediate maturity. Fermentability of the intact fiber decreased with maturity and was correlated highly to ABL content. ABL was a better indicator of forage degradability for intact bluestem fiber than was ADL, but neither ABL nor ADL was adequate for evaluating fermentability of treated residues.

(Key Words: Big Bluestem, Forage Quality, Lignin.)

### Introduction

An accurate assessment of forage quality often is required to allow prediction of animal performance. One of the most commonly used methods of forage evaluation is to measure lignin content, with more heavily lignified materials being considered less digestible. Lignin is a large polyphenolic compound found in plants that not only is indigestible itself, but also reduces the digestibility of other forage fractions.

Unfortunately, lignin is not a uniform entity, and, therefore, the laboratory techniques that are used to assess its concentration are problematic. One of the most common techniques for measuring lignin is the acid detergent lignin (ADL) procedure that uses strong sulfuric acid to degrade all of the nonlignin structures in the plant; lignin then is calculated as the residue that remains. This technique typically underestimates the true lignin content because some of the lignin is solubilized by the strong acid. Another technique for measuring lignin content is the acetyl bromide lignin (ABL) procedure that solubilizes lignin and subsequently measures it spectrophotometrically. The biggest drawback to this procedure is the difficulty in finding an appropriate standard to compare to samples.

The objective of this experiment was to identify changes in the lignin composition of big bluestem forage as it matures and to relate changes in lignin concentration to depressions in digestibility. We also subjected the forage to various chemical treatments in order to identify structures in bluestem that could limit digestibility.

## Experimental Procedures

Big bluestem was selected as representative of warm-season grasses found in the native range of Kansas. Samples of big bluestem were collected from three ungrazed, annually burned pastures on the Konza Prairie at 38, 58, and 97 days after the April 24, 1993 burning. These times were selected to represent a broad range of forage quality. Although the initial clipping was performed on June 1, the cold spring temperatures and slower than normal growth rate of the big bluestem caused this sample to be quite immature.

Entire plants were clipped 1 cm above the ground with clipping sites being marked to avoid collection of regrowth. Forage material was dried (50 °C) and ground, and cell wall material was isolated by extracting with hot (70 °C) water for 1 hour.

Cell wall material was treated chemically by: 1) partial delignification (chlorite), 2) isolation of  $\alpha$ -cellulose (treatment of delignified material with 2N KOH for 24 hr), or 3) sodium hydroxide extraction to solubilize alkali-labile components and remove some of the core lignin (1N NaOH for 24 hr). Control and treated cell-wall material was analyzed for ABL, ADL, and 24 and 72 hr in vitro dry matter disappearance (IVDMD).

## Results and Discussion

The lignin concentrations of bluestem as measured by the ADL and ABL procedures were quite different (Table 1); ABL yielded much higher estimates than did ADL. This is probably due to an underestimation of lignin by the ADL procedure, but could be partly due to the use of an inappropriate standard for the ABL procedure.

For the hot water extracted forage, ABL concentration increased with increasing maturity and was highly correlated to the depression in 72 h IVDMD that was observed as the bluestem aged. ADL content of the bluestem was more variable; although it was highest for the most mature sample, it was lower for the intermediate maturity than for the first harvest date. The relationship between ADL content and depressions of IVDMD was not as strong as that for ABL.

For the chlorite-delignified and NaOH-treated residues, ABL content increased with increasing maturity, mimicking the values for the untreated residues. However, ADL concentration in the chlorite-treated samples decreased with increasing maturity. Across maturities, IVDMD of chlorite-delignified and NaOH-treated samples were greater than that of the untreated material, indicating that the phenolic constituents that were solubilized played a significant role in the maturity-related decline in digestibility.

The cellulose residues should represent the largest single fraction within the plant cell wall. Typically, we believe that cellulose, when not encrusted by other cell wall materials like lignin, is highly digestible. With the ABL procedure, no lignin was found in the cellulose residues, whereas the ADL procedure indicated that some lignin remained.

As plants matured, the digestibility of the cellulose residue decreased, indicating that perhaps the size or crystallinity of the cellulose itself may limit digestion.

In conclusion, ABL was a better indicator of forage degradability for intact bluestem fiber than was ADL, but neither ABL nor ADL was adequate for evaluating fermentability of treated residues.

**Table 1. Lignin Content and Digestibility of Big Bluestem and Chemically Treated Residues**

Date/Treatment	ABL	ADL	24h IVDMD	72h IVDMD
<u>June 1, 1993</u>	----- % of dry matter -----			
Water	15.12	4.63	15.5	63.6
Chlorite	7.33	2.68	38.9	67.8
Cellulose	nd	2.73	34.6	84.5
NaOH	5.32	4.09	26.2	73.9
<u>June 21, 1993</u>				
Water	16.89	4.23	16.5	57.6
Chlorite	8.35	2.22	28.2	64.2
Cellulose	nd	2.30	21.1	77.6
NaOH	7.30	3.80	24.2	74.0
<u>July 30, 1993</u>				
Water	21.04	5.23	11.6	47.8
Chlorite	10.95	1.61	26.2	73.9
Cellulose	nd	1.36	26.8	72.5
NaOH	9.86	4.39	20.8	72.0
SEM	.19	.25	3.0	2.4

ABL = Acetyl bromide lignin, ADL = Acid detergent lignin, IVDMD = In vitro dry matter disappearance.

Water = Forage soaked in 70 °C water for 1 hour. Chlorite = Delignification with sodium chlorite. Cellulose = Cellulose isolated from chlorite delignified residue by soaking in 2N potassium hydroxide for 24 hours. NaOH = Forage soaked in 1 N sodium hydroxide for 24 hours.

nd = Not detectable.