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Alkali-Labile Cell-Wall Phenolics and Forage Quality in Switchgrasses Selected for Differing Digestibility

B. C. Gabrielsen, K. P. Vogel,* B. E. Anderson, and J. K. Ward

ABSTRACT

Alkali-labile cell-wall phenolics have been implicated in previous research as factors that affect forage digestibility by ruminants. Alkali-labile cell-wall phenolics, in vitro dry matter digestibility (IVDMD), neutral-detergent fiber (NDF), acid-detergent fiber (ADF), lignin (permanganate-oxidation), and crude protein (CP) were determined in three switchgrass (Panicum virgatum L.) strains differing genetically for IVDMD to determine relationships between these quality parameters and IVDMD during the grazing season. Grazed (upper 1/3 of grazed plants) and ungrazed (whole plants in caged exclosures) forage was collected weekly from replicated 0.4-ha pastures of ‘Trailblazer’ (high IVDMD), ‘Pathfinder’, and a low-IVDMD strain during three grazing seasons from 1983 to 1985. The principal alkali-labile phenolics (g kg⁻¹ NDF) detected were p-coumaric acid (PCA) and ferulic acid (FA). Increased PCA concentration due to increased maturity averaged >70% during each grazing season and corresponded with increased NDF, ADF, and lignin and decreased IVDMD, CP, and FA/PCA ratio. Ferulic acid concentration either declined slightly or remained unchanged. Averaged across 3 yr, Trailblazer had higher (P < 0.06) IVDMD, lower (P < 0.09) PCA and higher (P < 0.10) FA/PCA ratio than a divergently selected low-IVDMD strain. Differences between strains in detergent-fiber constituents, FA, and CP were either not apparent or inconsistent with strain differences in IVDMD. Results were consistent with both grazed and ungrazed switchgrass and indicate that alkali-labile cell-wall phenolic composition in switchgrass is heritable and genetically correlated to IVDMD.

Lignification is associated with reduced forage digestibility (Moore and Mott, 1973; Cowling, 1975; Jung and Vogel, 1986) and poor animal performance (Duble et al., 1971), although the precise mechanism by which this control is exerted is unclear. Some studies (Van Soest, 1973; Cowling, 1975) suggest that lignin functions to physically prevent accessibility of digestive enzymes. Other evidence (Hartley, 1972; Morrison, 1974; Gaillard and Richards, 1975) indicates that lignin is chemically linked to cell-wall carbohydrates, which also limit digestion.

In grasses, lignin may be partitioned into core and noncore fractions (Hartley, 1972; Gordon, 1975). Core lignin arises from three phenylpropanoid monomers (p-coumaryl, coniferyl, and sinapyl alcohols), which are interconnected in varying proportions and random sequences, primarily through C–C bonds and ether linkages (Harkin, 1973). Noncore lignin consists mainly of ester-linked PCA and FA (Hartley, 1972).

Information pertaining to changes in noncore lignin components during forage maturation and their relationship to cell-wall fiber constituents and forage digestibility is limited. Most studies have examined legumes and cool-season grasses (Hartley and Jones, 1977; Burritt et al., 1984; Scalbert et al., 1985); however comparable research involving temperate warm-season forage grasses, such as switchgrass, has not been reported.

Vogel et al. (1981) described the progress achieved in improving switchgrass IVDMD following one cycle of divergent selection. Although the high- and low-IVDMD strains resulting from this work were similar in maturity and forage yield, differences in IVDMD were significant. Subsequent research (Vogel et al.,
closures was clipped to a 7.5-cm stubble height and com-

On each sampling date, a forage subsample within the ex-

clipping schedule as above. Four exclosures, measuring 1.2

respectively. In 1984 and 1985, ungrazed forage samples also

terminated as grazed were randomly clipped at ~ 1-wk intervals

ance data were not compiled.

(Ward et al., 1989). In that study, grazing was initiated

lings and mature, crossbred, esophageal-fistulated steers

established in 12 0.4-ha pastures in a randomized complete-

Ward et al., 1989), the animals selectively grazed the top

are associated with differences in alkali-labile lignin

switchgrass strains described previously for IVDMD

and (ii) determine if the genetic differences among the

and crude protein in grazed and ungrazed switchgrass

These studies indicate that differences among switch-

bucking sheep to estimate IVDMD. Sheep were Ceiling

The study was conducted during three grazing seasons

MATERIAL AND METHODS

The objectives of this study were to (i) determine

Results and Discussion

Private et al. (1988) for herbage collected during grazing trials with

The animals used for this study were Herefords, Angus, and cross-

The mature plant was defined as the date when 20% of the panicles

Therefore, days of the year were used to quantify maturity.

3392A integrator (Hewlett-Packard Co., Sunnyvale, CA).

isocratically using a Bio-Rad C~s ODS-5S reversed-phase

acid solution. Phenolic compounds were separated by high-

2 mL methanol and diluted to 6 mL with a 0.87-M acetic

washed thoroughly with distilled water. The filtrate was im-

residues (0.5 g) in 1 M NaOH (20 mL) (Hartley, 1972).

initially determined as described by Van Soest and Robertson

lignin (permanganate-oxidation) concentration were sequen-

procedure (AOAC, 1975). Neutral-detergent fiber, ADF, and

lignum (Zea mays L.) cobs. Crude pro-

(1988) for herbage collected during grazing trials with

fications (HgCl2 and NazCO3 were not added after the first

An ANOVA was performed on individual quality param-

the regression analysis, the untransformed values are used for

For example, regression analysis of the alfalfa data indicated a

Alkali-labile phenolics detected in the switchgrass

Switchgrass is a strongly determinate plant in which mat-

determined as described by Van Soest and Robertson

relationships.
GABRIELSEN ET AL.: ALKALI-LABILE CELL-WALL PHENOLICS

... droxybenzoic acid, (iii) p-hydroxybenzaldehyde, (iv) vanillin, (v) p-coumaric acid (PCA), and (vi) ferulic acid (FA). However, the first four were detected only in trace amounts relative to PCA and FA and varied little throughout the grazing season. Therefore, only the results for PCA and FA are reported. As expected, forage quality was higher in the grazed forage (Table 1) than in the ungrazed forage (Table 2) throughout each season, presumably due to a relatively higher proportion of leaves in these samples. As the switchgrass matured and leafiness declined, differences in forage composition between the grazed and ungrazed forage were less pronounced. The effect of maturity on all quality parameters was significant (P < 0.01) in both forage treatments each year except for FA concentration, which exhibited an inconsistent response (Fig. 1). Increases in PCA were substantial, particularly during 1983 and 1984, and corresponded to declines in the FA/PCA ratio. The phenolic values at the later stages of maturity are similar to those reported...
by Cherney et al. (1988) for switchgrass harvested in a late stage of maturity. As expected, NDF, ADF, and lignin concentrations were higher in switchgrass. Burdtt et al. (1984) observed that grass samples with high ratios of FA/PCA. In addition, increases in PCA concentration also contained high levels of PCA and lignin. Researchers (Hartley, 1972; Burritt et al., 1984) examined alkali-labile lignin phenolics in various species of maturing cool-season grasses. Jones (1976) suggested that FA exists, in part, as a dimer (i.e., diferulic acid) that is esterified between cell-wall carbohydrates and core lignin but remains resistant to alkaline hydrolysis. In work with Italian ryegrass (Triticum aestivum L.), Hartley and colleagues (1972) reported that alkali-labile phenolics extracted from isolated cell-wall constituents with a concomitant decrease in forage tissue degradation. In the present study, the average PCA concentration during growth were larger and more consistent than for FA. Accordingly, Akin (1982) found that PCA monomers were markedly more toxic than FA monomers to forage-degrading rumen microorganisms. Our results with switchgrass appear to support this observation.

### Table 1: Comparison of Forage Quality Parameters between Grazed and Ungrazed Forage

<table>
<thead>
<tr>
<th>Year</th>
<th>FA (kg kg⁻¹)</th>
<th>PCA (kg kg⁻¹)</th>
<th>FA/PCA</th>
<th>IVDMD (%)</th>
<th>CP (%)</th>
<th>NDF (kg kg⁻¹)</th>
<th>ADF (kg kg⁻¹)</th>
<th>Lignin (kg kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1983</td>
<td>2.72</td>
<td>1.57</td>
<td>0.93</td>
<td>1.19</td>
<td>0.50</td>
<td>1.91</td>
<td>2.13</td>
<td>0.53</td>
</tr>
<tr>
<td>1984</td>
<td>3.52</td>
<td>2.03</td>
<td>1.75</td>
<td>1.95</td>
<td>0.46</td>
<td>2.03</td>
<td>2.26</td>
<td>0.52</td>
</tr>
<tr>
<td>1985</td>
<td>3.09</td>
<td>1.71</td>
<td>1.91</td>
<td>2.13</td>
<td>0.53</td>
<td>2.13</td>
<td>2.26</td>
<td>0.53</td>
</tr>
</tbody>
</table>

Regression analysis of each forage-quality parameter was performed, and the regression coefficients were determined using the mean values of three switchgrass strains. Weekly sampling coincided with early vegetative growth stage of panicle emergence each year.

Whole plants collected from caged exclosures. Forage clipped from the upper one-third of the panicle is harvested for each quality parameter with maturity were used to compare responses between years (Table 3). The regression analysis revealed that the response of a few quality parameters indicated some variation across years, particularly within the grazed treatments. A comparable response of FA, which was generally not well correlated with forage digestibility. In vitro dry matter digestibility and the FA/PCA ratio tended to be quadratically related. This relationship was observed in the ungrazed forage. FA/PCA (not shown) indicated that forage digestibility was generally lower than in previous years (Tables 1 and 2).
These observations, and the inconsistent relationship between FA concentration and maturity observed in the present study, suggest that extraction methods based on alkaline hydrolysis may not accurately assess the role of FA in fiber degradation processes. However, our results indicate that PCA

Table 4. Coefficients of determination for cell-wall constituents with in vitro dry matter digestibility in grazed and ungrazed switchgrass during three grazing seasons at Mead, NE.

<table>
<thead>
<tr>
<th></th>
<th>Grazed</th>
<th>Ungrazed</th>
</tr>
</thead>
<tbody>
<tr>
<td>NDF</td>
<td>0.64**, NS</td>
<td>0.69**, NS</td>
</tr>
<tr>
<td>ADF</td>
<td>0.67**, 0.74*</td>
<td>0.86**, NS</td>
</tr>
<tr>
<td>Lignin</td>
<td>0.74**, NS</td>
<td>0.66**, 0.86**</td>
</tr>
<tr>
<td>FA</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>PCA</td>
<td>0.81**, NS</td>
<td>0.71**, NS</td>
</tr>
<tr>
<td>FA/PCA</td>
<td>0.76**, 0.88**</td>
<td>0.74**, 0.85**</td>
</tr>
</tbody>
</table>

Correlations were determined using the mean values of three switchgrass strains. NDF = neutral detergent fiber, ADF = acid detergent fiber, FA = ferulic acid, PCA = p-coumaric acid, FA/PCA = ratio of ferulic acid to p-coumaric acid, and n = sample size.

Genetic Effects
Performance of cattle grazing these pastures and the IVDMD of the strains over the grazing season for 1983 and 1985 have been previously reported (Anderson et al., 1988). The objective of this portion of the study was to determine if the previously reported genetic differences among the switchgrass strains for IVDMD is associated with genetic difference in FA and PCA concentration of the forages. The three strains in this study were all developed from the same germplasm source and consistently flower on the same date in eastern Nebraska (Vogel et al., 1981; Vogel et al, 1984). Hence, the three strains are similar in phenological development throughout the growing season, so any differences among strains for quality traits on a sample are not due to maturity differences.

In vitro dry matter digestibility was consistently higher in Trailblazer than in Pathfinder and the low-IVDMD strain as previously reported by Anderson et al. (1988) (Tables 5 and 6). Lower PCA concentrations and high FA/PCA ratios were associated with high forage digestibility. This was particularly evident in both forage sampling treatments when these parameters were considered across all grazing seasons; however, differences in PCA concentration were less apparent when switchgrass strains were compared within grazing seasons. Some variation in FA concentration also occurred; however, responses among

Table 5. Forage quality constituents in three grazed switchgrass strains during three grazing seasons at Mead, NE.

<table>
<thead>
<tr>
<th>Strain</th>
<th>IVDMD</th>
<th>CP</th>
<th>NDF</th>
<th>ADF</th>
<th>Lignin</th>
<th>FA</th>
<th>PCA</th>
<th>FA/PCA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trailblazer</td>
<td>669</td>
<td>127</td>
<td>662</td>
<td>338</td>
<td>41</td>
<td>3.5</td>
<td>3.7</td>
<td>1.04</td>
</tr>
<tr>
<td>Pathfinder</td>
<td>633</td>
<td>119</td>
<td>672</td>
<td>347</td>
<td>43</td>
<td>3.4</td>
<td>4.0</td>
<td>0.94</td>
</tr>
<tr>
<td>Low-IVDMD</td>
<td>636</td>
<td>125</td>
<td>664</td>
<td>338</td>
<td>40</td>
<td>3.2</td>
<td>4.0</td>
<td>0.87</td>
</tr>
<tr>
<td>LSD</td>
<td></td>
<td></td>
<td>17</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.3</td>
</tr>
<tr>
<td>Signif. level</td>
<td>0.01</td>
<td>0.06</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>0.01</td>
<td>NS</td>
<td>0.02</td>
</tr>
<tr>
<td>Trailblazer</td>
<td>649</td>
<td>117</td>
<td>727</td>
<td>368</td>
<td>40</td>
<td>3.8</td>
<td>4.9</td>
<td>0.88</td>
</tr>
<tr>
<td>Pathfinder</td>
<td>627</td>
<td>119</td>
<td>738</td>
<td>371</td>
<td>44</td>
<td>3.4</td>
<td>5.0</td>
<td>0.76</td>
</tr>
<tr>
<td>Low-IVDMD</td>
<td>602</td>
<td>116</td>
<td>734</td>
<td>378</td>
<td>49</td>
<td>3.8</td>
<td>5.6</td>
<td>0.75</td>
</tr>
<tr>
<td>LSD</td>
<td></td>
<td></td>
<td></td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td>0.6</td>
</tr>
<tr>
<td>Signif. level</td>
<td>NS</td>
<td>0.10</td>
<td>NS</td>
<td>NS</td>
<td>0.10</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Trailblazer</td>
<td>591</td>
<td>105</td>
<td>728</td>
<td>370</td>
<td>42</td>
<td>6.0</td>
<td>7.2</td>
<td>0.85</td>
</tr>
<tr>
<td>Pathfinder</td>
<td>581</td>
<td>106</td>
<td>725</td>
<td>370</td>
<td>43</td>
<td>5.5</td>
<td>7.5</td>
<td>0.76</td>
</tr>
<tr>
<td>Low-IVDMD</td>
<td>546</td>
<td>99</td>
<td>727</td>
<td>377</td>
<td>46</td>
<td>5.4</td>
<td>7.8</td>
<td>0.71</td>
</tr>
<tr>
<td>LSD</td>
<td></td>
<td>33</td>
<td></td>
<td></td>
<td>3</td>
<td></td>
<td></td>
<td>0.01</td>
</tr>
<tr>
<td>Signif. level</td>
<td>0.07</td>
<td>0.05</td>
<td>NS</td>
<td>NS</td>
<td>0.05</td>
<td>NS</td>
<td>NS</td>
<td>0.08</td>
</tr>
</tbody>
</table>

Forage clipped from the upper one-third of grazed plants.
IVDMD = in vitro dry matter digestibility, CP = crude protein, NDF = neutral detergent fiber, ADF = acid detergent fiber, FA = ferulic acid, PCA = p-coumaric acid, and FA/PCA = ratio of ferulic acid to p-coumaric acid.
content was highest in Trailblazer when averaged.

Lignin concentration in the grazed forage was significantly (P < 0.05) lower in Trailblazer than in the low-IVDMD grasses. Evidence for a similar effect of lignin concentration on IVDMD among the divergent populations of switchgrass strains was inconsistent between grazed and ungrazed forages.

Researchers evaluating alkali-labile phenolics in smooth bromegrass genotypes selected for increased IVDMD; however, lignin concentration was a major limiting factor to IVDMD. Evidence for a similar effect of phenolic monomers on orchardgrass plants collected from caged exclosures.

Researchers evaluating alkali-labile phenolics in switchgrass strains in our study was indicated only in the grazed forage (Table 5). During the grazing season for each of the 3 yr of this study, significant strain differences for these quality parameters were not attributable to measured responses at only one or two sampling dates. Strain × date interactions were not detected for the FA/PCA ratio. Except for a few dates, linear trend throughout each grazing season, whereas FA/PCA ratios exhibited a curvilinear response, decreasing more rapidly early in the season (Figures 1, 2, 3). A significant (P < 0.05) strain × date interaction for IVDMD occurred among the grazed forages during 1983 and 1985; however, this appeared to be confounded in a way detected (Tables 5 and 6). Ferulic acid concentrations did not appear to be related to strain differences in IVDMD, suggesting a less important role of this cell-wall constituent in fiber digestion processes.

Researchers evaluating alkali-labile phenolics in smooth bromegrass genotypes selected for increased IVDMD; however, lignin concentration was a major limiting factor to IVDMD. Evidence for a similar effect of phenolic monomers on orchardgrass plants collected from caged exclosures.

Researchers evaluating alkali-labile phenolics in smooth bromegrass genotypes selected for increased IVDMD; however, lignin concentration was a major limiting factor to IVDMD. Evidence for a similar effect of phenolic monomers on orchardgrass plants collected from caged exclosures. During grass growth, increases in both PCA and lignin concentrations occur which may result in increased digestibility. The responses observed in our study seem to support these findings, although strain differences for these quality parameters were not attributable to measured responses at only one or two sampling dates. Strain × date interactions were not detected for the FA/PCA ratio. Except for a few dates, linear trend throughout each grazing season, whereas FA/PCA ratios exhibited a curvilinear response, decreasing more rapidly early in the season (Figures 1, 2, 3). A significant (P < 0.05) strain × date interaction for IVDMD occurred among the grazed forages during 1983 and 1985; however, this appeared to be confounded in a way detected (Tables 5 and 6). Ferulic acid concentrations did not appear to be related to strain differences in IVDMD, suggesting a less important role of this cell-wall constituent in fiber digestion processes.
acids. This response is probably due to a physical inhibition that restricts cellulase accessibility into the cell-wall matrix. In addition, free cinnamic acids such as PCA, which are released from the cell wall during digestion, may subsequently exert toxic effects on forage-degrading microorganisms (Akin, 1982; Akin and Rigsby, 1985; Varel and Jung, 1986).

Trailblazer and the low-IVDMD strain were divergently selected from the same population for differences in IVDMD. The results reported here indicate that correlated responses for PCA content and the FA/PCA ratio occurred as a result of this selection, indicating that these traits are heritable in switchgrass and that they are genetically correlated to IVDMD. A possible explanation for this correlated response is that the variation in PCA may have been responsible, in part, for the observed differences in IVDMD among the switchgrass strains.