Interspecies Variation of Forage Nutritive Value and Nonstructural Carbohydrates in Perennial Cool-Season Grasses

Duli Zhao,* Charles T. MacKown, Patrick J. Starks, and Bryan K. Kindiger

ABSTRACT

Knowledge of grass genotypes nonstructural carbohydrate (NSC) variation is one component to be considered when developing a successful forage and livestock management program. The objective of this study was to determine variations in concentrations of NSC, crude protein (CP), neutral detergent fiber (NDF), and acid detergent fiber (ADF) among perennial cool-season grasses grown in the southern Great Plains at the preheading stage. Grasses evaluated included four tall fescue (Festuca arundinacea L.), two Festulolium (Lolium. multiflorum Lam. × Festuca arundinacea), one tall wheatgrass [Thinopyrum ponticum (Podp.) Z.-W. Liu & R.-C. Wang], two intermediate wheatgrasses [T. intermedium (Host) Barkworth & D.R. Dewey], and four smooth bromegrasses (Bromus inermis Leyss). Grass species influenced forage concentrations of NSC, CP, NDF, and ADF. Averaged across years, concentrations of forage total NSC, CP, NDF, and ADF ranged from 70 to 112, 81 to 138, 548 to 614, and 288 to 321 g kg⁻¹ dry weight (DW), respectively, among the 13 entries. Differences in the relative contribution of each NSC component to total NSC were small among the five species. Averaged across the entries, glucose, fructose, sucrose, fructans, and starch accounted for 14, 10, 15, 38, and 23% of total NSC, respectively. Producers seeking to improve the provision of energy to livestock would be served best by using smooth bromegrass with superior NSC concentration rather than a tall fescue entry with low NSC concentration.

In traditional winter wheat (Triticum aestivum L.) grazing systems of the southern Great Plains, there are major forage gaps in late fall and spring (Reuter et al., 1999). Unpredictable precipitation in autumn often delays planting of winter wheat, resulting in a lack of high quality forage for cattle to graze (Malinowski et al., 2005). Development of perennial cool-season grasses and their utilization as complementary or alternative sources of forage could improve net economic returns of forage production and livestock management systems in the southern Great Plains by reducing risks due to unpredictable precipitation and the annual expenses of establishing winter wheat pasture (Redmon 1997, 1999; Malinowski et al., 2005). Reuter and Horn (2002) found that perennial cool-season grasses in Oklahoma provided sufficient fall and late spring forage that complements wheat and perennial warm-season grass pastures, but in late winter and early spring available forage was insufficient for cattle pulled from wheat pasture at first hollow stem in a dual-purpose wheat system (graze + grain). However, few improved indigenous or introduced perennial cool-season grass forages are known to be productive or persistent in the southern Great Plains (Malinowski et al., 2005). Consequently, the selection and development of agronomically superior cultivars of perennial cool-season grass forages will be an important contribution to the regional agricultural and livestock production systems.

Concentrations of NSC, CP, NDF, and ADF are major factors affecting forage nutritive value (Ball et al., 2001). The efficiency of forage CP conversion into meat and milk is currently low and poor N conversion by cattle is primarily associated with forage carbohydrate (energy) limitation (Cairns, 2002). Excretion of unutilized N is inefficient in terms of productivity and could have adverse environmental consequences on surface and ground water (Powell et al., 1995). For high-producing cows, the demand for high energy intake requires an abundant concentration of forage carbohydrates. The microbes in the rumen also require readily available carbohydrates for growth and N accumulation. Thus, increasing NSC concentration in forage has the potential to increase cattle performance.

Knowledge of plant NSC metabolism and composition as affected by forage genotypes and environment may assist in improving the provision of nutritional energy for animals. Nonstructural carbohydrates are the major source of energy in the diet of dairy cattle. Readily fermentable carbohydrates in forages provide energy to animals and may be one of the cues grazing ruminants utilize when selecting which forage plants to graze (Fisher et al., 1999; Mayland et al., 2000). Hungry animals display a rapid response to energy-dense test diets and total NSC is readily fermentable, serving as an energy resource to ruminants (Provenza, 1995; Baumont, 1996). Thus, animal grazing behavior might be conditioned by NSC levels in forage. Total NSC concentration in forages has been identified as one of the most important parameters requiring the attention of forage producers.


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Abbreviations: ADF, acid detergent fiber; CP, crude protein; DW, dry weight; NDF, neutral detergent fiber; NIRS, near infrared reflectance spectroscopy; NSC, nonstructural carbohydrate; SEC, standard error of the calibration; SECV, standard error of the cross-validation.
of forage breeders (Wheeler and Corbett, 1989) and should be a primary goal of plant breeding programs and forage harvest management strategies for ruminants (Shewmaker et al., 2006). Horses fed forage with high concentration of NSC have an increased risk of developing laminitis (Longland and Byrd, 2006). Therefore, a broader understanding of NSC variation among grass species, as well as cultivars within a species, is a critical element to be considered when developing a successful forage and livestock management program.

To find appropriate perennial cool-season grasses for the southern Great Plains, 117 cultivars and experimental lines representing 16 species were evaluated in environmentally adverse field conditions (Kindiger and Conley, 2002; Kindiger et al., 2004). Thirteen entries (cultivars and experimental lines), representing five species, grew and performed well enough to warrant additional evaluations. The specific objective of this study was to determine the variation of forage quality traits of NSC, CP, NDF, and ADF concentrations among the 13 perennial cool-season grass entries when grown in the southern Great Plains area of the United States and harvested as a hay crop.

MATERIALS AND METHODS

Experimental Design and Plant Culture

The experiment was conducted at the USDA-ARS Grazinglands Research Laboratory, El Reno, OK (35°32’ N, 98°02’ W) in 2004 and 2005. Based on results of previous field screening of perennial cool-season grasses (Kindiger and Conley, 2002; Kindiger et al., 2004), 13 superior performing grass entries from five species were evaluated for forage NSC and other major nutritive components. These entries represented four cultivars of tall fescue (endophyte-free ‘Carmine’, ‘Dovey’, and ‘Maximize’; endophyte-infected ‘Kentucky 31’), two festuloliums (‘Felina’ and ‘Hykor’), four smooth brome grasses (‘Lincoln’, ‘Lincoln YD’, ‘NE B1-2-C0’, and ‘NE B1-2-C2’), one tall wheatgrass (‘Jose’), and two intermediate wheatgrasses (‘Luna’ and ‘Manska’). Entry maturity, source of seed and entry status are provided in Table 1. Grasses were established late September 1999 on a Brewer silty clay soil (fine, mixed, superactive, thermic Undertic Argiustoll). The experimental design was a randomized complete block design with three replications. Plot size was 1.5 × 10 m. After the forages were well established, granular N fertilizer was applied at a rate of 43 kg N ha⁻¹ by a calibrated broadcast spreader in October each year. To simulate rotational grazing, plots were mechanically clipped to a 7.5-cm height in November, March, and May of each year from 2000 to 2005. Consequently, the effects of naturally occurring environmental stress (i.e., drought, heat, etc.) were used to evaluate tolerance, persistence, and adaptability of the entries.

Sample Collection

To minimize the effect of environmental conditions on forage NSC and other nutritive values, all cultivars were harvested on a sunny day (16 Apr. 2004 and 27 Apr. 2005) when Dovey, the earlier maturing entry, started to head. Most other entries head 9 to 14 d after Dovey, except for Jose, which heads about 21 d after Dovey. Thus all entries, except Dovey, were harvested at the preheading stage. This stage was selected to reflect an early spring hay harvest in the southern Great Plains. Specifically, forage vegetation samples were clipped from a 0.25-m² quadrant within 1 cm of the ground surface using a pair of handheld clippers from all plots between 1100 and 1200 h CST on 16 Apr. 2004 and 27 Apr. 2005. The samples were transported to a laboratory and immediately dried at 60°C for 48 h in a forced-draft oven (Heberer et al., 1985). Dried plant tissues were ground to pass a 2-mm screen in a Wiley mill (Arthur H. Thomas Co., Philadelphia, PA) for subsequent measurements of forage CP, NDF, and ADF concentrations. A subsample of the 2-mm ground tissue was ground through a 1-mm screen in a cyclone mill (Udy Corporation, Fort Collins, CO) for measurements of forage NSC concentration.

Nonstructural Carbohydrates

The modified methods of Hendrix (1993), Zhao and Oosterhuis (1998), and Velterop and Vos (2001) were used to extract NSC from ground forage samples and to quantify the concentrations of NSC components. Briefly, soluble sugars, including glucose, fructose, sucrose, and fructans, were extracted by heating in an 80°C water bath for 15 min in a mixture of 70 mg of ground tissue and 2 mL of 80% (v/v) ethanol. Each sample was extracted two more times and the three supernatants were combined in a test tube and brought to 6 mL with 80% ethanol. Then 60 mg of finely ground activated charcoal was added to each sample tube with the 6-mL extract before the tubes were covered and shaken by hand. After 5 min, the tubes were centrifuged at 3000 × g for 15 min to obtain

Table 1. Cool-season forage grasses utilized in the study, relative flowering maturity at El Reno, OK, and the source of each entry.

<table>
<thead>
<tr>
<th>Species</th>
<th>No.</th>
<th>Entry</th>
<th>Maturity†</th>
<th>Material status</th>
<th>Seed resource</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tall fescue</td>
<td>1</td>
<td>‘Carmine’</td>
<td>Medium</td>
<td>Variety</td>
<td>DLF-Jenks, Albany, OR</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>‘Dovey’</td>
<td>Early</td>
<td>Variety</td>
<td>Barenbrug USA, Tangent, OR</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>‘Kentucky 31’</td>
<td>Medium</td>
<td>Variety</td>
<td>Ross Seed Co., El Reno, OK</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>‘Maximize’</td>
<td>Medium</td>
<td>Variety</td>
<td>Pure Seed Testing Inc., Hubbard, OR</td>
</tr>
<tr>
<td>Festulolium</td>
<td>5</td>
<td>‘Felina’</td>
<td>Medium</td>
<td>Variety</td>
<td>DLF-Jenks, Albany, OR</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>‘Hykor’</td>
<td>Medium</td>
<td>Variety</td>
<td>DLF-Jenks, Albany, OR</td>
</tr>
<tr>
<td>Smooth brome grass</td>
<td>7</td>
<td>‘Lincoln’</td>
<td>Medium</td>
<td>Variety</td>
<td>USDA-ARS, Univ. of Nebraska, Lincoln</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>‘Lincoln YD’</td>
<td>Medium</td>
<td>Breeder-line</td>
<td>USDA-ARS, Univ. of Nebraska, Lincoln</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>‘NE B1-2-C0’</td>
<td>Medium</td>
<td>Breeder-line</td>
<td>USDA-ARS, Univ. of Nebraska, Lincoln</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>‘NE B1-2-C2’</td>
<td>Medium</td>
<td>Breeder-line</td>
<td>USDA-ARS, Univ. of Nebraska, Lincoln</td>
</tr>
<tr>
<td>Tall wheatgrass</td>
<td>11</td>
<td>‘Jose’</td>
<td>Very late</td>
<td>Variety</td>
<td>USDA-ARS, Forage Range Res. Lab., Logan, UT</td>
</tr>
<tr>
<td>Intermediate wheatgrass</td>
<td>12</td>
<td>‘Luna’</td>
<td>Late</td>
<td>Variety</td>
<td>USDA-ARS, Forage Range Res. Lab., Logan, UT</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>‘Manska’</td>
<td>Late</td>
<td>Variety</td>
<td>USDA-ARS, Northern Great Plains Res. Lab., Mandan, ND</td>
</tr>
</tbody>
</table>

† Number of days between the earliest (Dovey) and latest (Jose) maturing entries is about 21 d; all other entries had relative flowering maturities within a 5-d period starting 9 d after Dovey.
a clear extract. Three 20-μL aliquots from each sample were added to separate wells of a 96-well microtitration plate, except for three columns on the left side of the plate that were reserved for glucose standards. Plates were placed in an oven at 50°C for 30 min to remove the ethanol.

As standards, eight sets of three wells received 20 μL of a solution containing 0, 5, 13, 25, 50, 125, 250 or 500 mg L⁻¹ glucose. To each sample well, 20 μL of deionized water was added, and then a 100-μL mixture of adenosine tri-phosphate, oxidized nicotinamide adenine dinucleotide, hexokinase, and glucose-6P dehydrogenase [glucose (HK) assay kit, GAHK-20; Sigma Chemical Company, St Louis, MO] was added to all the wells on a plate. Plates were covered and incubated at 30°C for 15 min and then uncovered and the absorbances measured at 340 nm (Velterop and Vos, 2001) with a Spectramax Plus microplate reader (Molecular Devices Corporation, Sunnyvale, CA). A glucose standard curve with the linear equation was obtained for each plate based on the standard glucose concentrations and mean absorbance. Glucose concentration in plant tissue samples was calculated according to the standard-curve equation, sample solution absorbance, extract volume, and tissue sample mass. Subsequently, 10 μL of phosphoglucone isomerase (PGI enzyme, 0.25 units, Sigma P-9544) was added to each well and covered plates were incubated at 30°C for 15 min and absorbances of uncovered plates were measured at 340 nm to obtain the sum of glucose and fructose concentrations. Finally, 10 μL of invertase (83 units, Sigma I-4504) was added to each well and covered plates were re-incubated at 30°C for 60 min. The absorbances of uncovered plates were determined at 340 nm to obtain a combination concentration of glucose + fructose + sucrose. Concentrations of fructose and sucrose were further calculated based on these combination concentrations.

Fructans, dominant ethanol-soluble polysaccharides in cool-season grasses, were quantified by boiling a subsample of the clear sugar extract with 0.2 M acetic acid in a water bath for 1.5 h. The acid was then neutralized with 1 M NaOH (Skinner et al., 1999). This procedure breaks down the polysaccharides to fructose, which was quantified by the procedures described above, providing an estimate of total soluble sugars. Glucose, fructose, and sucrose levels that had been determined separately were then subtracted from the total ethanol soluble sugars to provide an estimate of fructans (Skinner et al., 1999).

After extraction of the soluble sugars, 1 mL of 0.1 M KOH was added to the test tube containing the tissue residue and the tubes were placed for 1 h in a boiling water bath. Tubes were then removed from the water bath and allowed to cool to room temperature. Both α-amylase (Sigma A-3403) and amyloglucosidase (Sigma A-3042) preparations were used to hydrolyze starch to glucose as described by Hendrix (1993). After hydrolysis, the tubes were brought to 6 mL with deionized water. The covered tubes were shaken and centrifuged. The supernatants were collected and the glucose concentrations measured as described above. Starch concentration in the sample was calculated according to the glucose concentration in the tissue residue multiplied by 0.9 to account for water loss when glucose units are linked to form starch. The sum of glucose, fructose, sucrose, fructans, and starch in a forage sample was defined as total NSC.

**Nutritive Value**

Forage N was analyzed by a flash combustion instrument (varioMacro, Elementar Americas, Inc., Mt. Laurel, NJ) and concentrations of NDF and ADF were determined using a bench-top near infrared reflectance spectroscopy (NIRS) analyzer (Model 6500, FOSS-NIRSystems, Inc., Silver Spring, MD). Ground samples were scanned from 400 to 2500 nm with the NIRS system. During scanning, computer software automatically measures reflectance from an internal reference standard. Regression equations were developed by modified partial least squares as reflectance data were regressed against standard laboratory chemically derived data from the subsamples of these tested entries and other cool-season grass samples from our forage breeding and germplasm research project at the Grazinglands Research Laboratory (n = 103). Optimum regression equations were selected on the basis of $r^2$, the standard error of the calibration (SEC), and the standard error of the cross-validation (SECV) (Shenk and Westerhaus, 1991). The $r^2$ values were 0.89 and 0.92, SEC values were 19.4 and 10.4 g kg⁻¹ DW, and SECV values were 23.8 and 13.5 g kg⁻¹ DW, respectively for forage NDF and ADF. Chemical analyses of forage NDF and ADF were performed using an ANKOM Fiber Analyzer (ANKOM Technology, Fairport, NY) and manufacture recommended protocols (ANKOM Technology, 2003). Concentration of herbage CP was calculated by multiplying the N concentration by 6.25 (Pearson and Ison, 1987).

**Data Analysis**

Because the experiment was a randomized complete block design with 2-yr repeated measurements, the repeated-measures ANOVA was selected for all data using PROC MIXED (SAS Institute, 1997). Blocks were considered random, years were considered repeated, and entries were considered fixed factors to determine the main and interactive effects of year and plant entries on forage NSC, CP, NDF, and ADF concentrations. If the hypothesis of equal means for all entries was rejected by the ANOVA test, trait means were compared with Fisher LSD values at $P = 0.05$ that were calculated with the difference of means SE values generated in PROC MIXED. Correlation coefficients ($r$) among forage CP, NDF, ADF, and NSC components were also determined.

Additionally, the cluster analysis routine PROC CLUSTER (SAS Institute, 1997) was used to determine the forage similarity in NSC concentrations and to group the 13 grass entries based on NSC components. In cluster analysis, the PROC ACECLUS was first used to transform the data to provide a spherical within-cluster covariance matrix. The procedure obtains approximate estimates of the pooled within-cluster covariance matrix and then computes canonical variables to be used in subsequent cluster analyses and TREE procedures (SAS Institute, 1997).

**RESULTS AND DISCUSSION**

**Nonstructural Carbohydrates**

Grass entry affected forage concentrations of glucose, fructose, fructans, starch, and total NSC significantly ($P < 0.01$), but did not affect sucrose concentration (Table 2). Except for glucose, sucrose and fructans, year significantly affected concentrations of other NSC components and total NSC.
An entry × year interaction was detected for starch (P < 0.001) and total NSC (P < 0.05) concentrations. The variation in responses of starch and total NSC concentrations to year was complex among entries. Most entries had significantly greater starch and total NSC concentrations in 2004 than in 2005, but starch concentration of Carmine tall fescue, Felina and Hykor festulolium, and Jose tall wheatgrass was not different (P > 0.05) between years. In contrast to other entries, total NSC of Kentucky 31 tall fescue, Jose tall wheatgrass, and Luna and Manska intermediate wheatgrass in 2004 did not differ (P > 0.05) from their 2005 levels (Fig. 1). Overall, concentrations of starch and total NSC in 2004 were significantly greater than those in 2005 (Fig. 1). Averaged across grass entries, starch and total NSC concentrations were 23.1 and 97.3 g kg⁻¹ DW, respectively in 2004; and 15.4 and 82.8 g kg⁻¹ DW, respectively in 2005. The differences in starch and total NSC concentrations between years in the present study may partially be associated with climatic conditions (Housley and Volenec, 1988), because both drought (Norris and Thomas, 1982; Spollen and Nelson, 1994) and temperature (Chatterton et al., 1989; Ball et al., 2001) have a profound effect on NSC concentration of cool-season grasses. In 2004, total precipitation for March and April was 122 mm, but only 21 mm for the same time period in 2005. Additionally, the daily mean temperature for March and April in 2004 (13.3°C) was 1.1°C greater than in 2005 (12.2°C). More precipitation and warmer daily mean temperature in March and April of 2004 (data not shown) might have stimulated photosynthesis of grasses (Shewmaker et al., 2006), resulting in greater NSC concentrations in the cool-season grass forage as compared with 2005. Earlier studies indicated that concentrations of fructans and other water-soluble carbohydrates decrease in both perennial ryegrass and tall fescue basal tissue with water deficit conditions (Norris and Thomas, 1982; Spollen and Nelson, 1994).

Fructans and starch were two dominant components of NSC and accounted for 39% (range: 34 to 45%) and 21% (range: 17 to 23%) of total NSC concentration, respectively, when averaged across grass entries. Chatterton et al. (1989) suggested that fructans were an ancillary form of carbohydrate storage and accumulation in the vacuole allowed photosynthesis to continue at cool temperature with the saturation of other storage pools. Among the 13 grass entries of the five species tested (i.e., tall fescue, festulolium, smooth brome-grass, tall wheatgrass, and intermediate wheatgrass), total NSC

![Fig. 1. Variation of nonstructural carbohydrate concentrations among 13 entries of perennial cool-season grasses at the preheading stage. Grass entries from 1 to 13 are ‘Carmine’, ‘Dovey’, ‘Kentucky 31’, and ‘Maximize’ tall fescue; ‘Felina’ and ‘Hykor’ festulolium; ‘Lincoln’, ‘Lincoln YD’, ‘NE B1-2-C0’, and ‘NE B1-4-C2’ smooth brome-grass; ‘Jose’ tall wheatgrass; and ‘Luna’ and ‘Manska’ intermediate wheatgrass, respectively.

Table 2. Significance of each source of variation for the concentrations of glucose, fructose, sucrose, fructans, starch, total nonstructural carbohydrates (NSC), crude protein (CP), neutral detergent fiber (NDF), and acid detergent fiber (ADF) in forage at preheading of 13 cool-season grass entries in 2004 and 2005 using SAS MIXED model.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Glucose</th>
<th>Fructose</th>
<th>Sucrose</th>
<th>Fructan</th>
<th>Starch</th>
<th>NSC</th>
<th>CP</th>
<th>NDF</th>
<th>ADF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Year (Y)</td>
<td>1</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>Entry (E)</td>
<td>12</td>
<td>***</td>
<td>***</td>
<td>NS</td>
<td>**</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>Y × E</td>
<td>12</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>***</td>
<td>*</td>
<td>NS</td>
<td>NS</td>
<td>***</td>
<td>***</td>
</tr>
</tbody>
</table>

* Significant at the P < 0.05 level.
** Significant at the P < 0.01 level.
*** Significant at the P < 0.001 level.
concentration of the four smooth bromegrass entries was greatest in both years (Fig. 1). Except for smooth bromegrass, the other four plant species had comparable concentrations of glucose and starch. In contrast, for smooth bromegrass the primary components of the total NSC were glucose, fructans, and starch, rather than fructose or sucrose.

Differences in fructose and sucrose concentrations among grass entries were less than those in the other carbohydrate components (Fig. 1). Furthermore, variation of NSC concentrations among entries within a species was relatively small. Shewmaker et al. (2006) investigated daily changes in NSC concentrations of eight tall fescue cultivars and found that time of sampling is critical to accurately assess NSC. Differences in total NSC concentrations in forage grasses are the result of complex interactions of day-length, solar radiation, temperature, soil moisture and fertility, leaf:stem ratio, and time of sampling (Shewmaker et al., 2006). Our results of differences in NSC concentrations between years can be explained partially by differences in temperature and precipitation. We collected all forage samples on the same day within a year and between 1100 and 1200 h of sunny days to minimize daily environmental effects on levels of NSC. Consequently, differences in NSC concentrations among the entries primarily reflect genotype variability. Jung et al. (1974) investigated seasonal fluctuations of NSC concentration in the forage of eight cool-season grass species and found that smooth bromegrass produced the most total NSC in spring among the tested grasses. Our total NSC results for the four smooth bromegrass entries we evaluated was consistent with the report by Jung et al. (1974). Therefore, smooth bromegrass represents a cool-season grass species with high NSC concentration.

Total NSC concentration in forages has been identified as one of the most important characteristics requiring the attention of forage breeders (Wheeler and Corbett, 1989) because it is closely correlated with preference and production in ruminants (Shewmaker et al., 2006). Cattle grazing preferences among several tall fescue cultivars were related to the concentrations of total NSC (Mayland et al., 2000), indicating the importance of NSC concentrations in forage and grazing systems. For example, according to Mayland et al. (2000), an increase in forage total NSC from 105 to 163 g kg$^{-1}$ structural DW corresponds to an increase in the preference score from 4 to 8, where a score of 0 represents no evidence of grazing and a score of 10 indicates all available forage is eaten. On the other hand, avoidance of grass forage with abundant NSC is important in horse management (Watts, 2004; Longland and Byrd, 2006). Longland and Byrd (2006) suggested intake of 3.5 kg of fructans d$^{-1}$ could activate laminitis in a 500-kg horse. To achieve this intake, a fructan concentration of 350 g kg$^{-1}$ of dried hay would be required for a 500-kg horse consuming daily 2.0 kg hay per 100 kg body weight. While this fructan concentration greatly exceeds the concentrations we measured, horses receiving supplemental feed high in energy (cereal grain starch) plus the starch and fructans contained in the hay could increase the level of hindgut dysfunction sufficiently to induce laminitis (Longland and Byrd, 2006; Milinovich et al., 2006).

We used cluster analysis to explore the similarities and differences in concentrations and components of forage NSC among the 13 entries of the five grass species (Fig. 2). Because cluster analysis by year produced similar results (data not shown), we used data averaged across the 2 yr. Cluster analysis using the averaged data revealed that the 13 grass entries were clustered conservatively into three groups; the first three clusters were sufficient to explain nearly 80% of the variation ($R^2 = 0.79$). Entries from the smooth bromegrass species formed a cluster (Group 1) with high NSC concentrations. Entries of Kentucky 31 and Maximize (Group 2) among the tall fescues also stood out from other entries because both the entries had intermediate concentrations of fructan and/or sucrose compared to the other grass entries (Fig. 1). The remaining seven entries were clustered into one group (Group 3) exhibiting low concentrations of NSC (Fig. 2).

These results of cluster analysis further indicate that differences in forage NSC composition and concentration at preheading of the 13 cool-season grass entries evaluated can be separated primarily by species. However, some differences in NSC concentrations among individual entries within a species, especially Kentucky 31 and Maximize among the tall fescues, were also detected (Fig. 1 and 2). Therefore, there is a potential...
for screening and selection of a suitable cool-season grass to fit the specific forage needs of livestock and forage production systems in the southern Great Plains of the United States.

Forage CP, NDF, and ADF

Significant year and grass entry effects were found for forage concentrations of CP, NDF, and ADF ($P < 0.001$; Table 2). Averaged across the 13 entries, the 2005 forage concentration of CP was 21% less, NDF 4% greater, and ADF 27% greater than the 2004 averages (Fig. 3). Among the 13 grass entries, forage CP concentration ranged from 120 to 163 g kg$^{-1}$ DW in 2004 and from 88 to 145 g kg$^{-1}$ DW in 2005 (Fig. 3). In general, forage CP concentration of tall fescue and festulolium (111 g kg$^{-1}$ DW) was less than that of intermediate wheatgrass (135 g kg$^{-1}$ DW) and smooth bromegrass (147 g kg$^{-1}$ DW), averaged across years. Our CP results are consistent with the report of Hedtcke et al. (2002) who found that smooth bromegrass ranked highest in CP concentration and tall fescue ranked lowest among seven cool-season grasses. Differences ($P < 0.001$) among entries in NDF and ADF were not as consistent as differences in CP concentration. Forage NDF and ADF (the 2-yr means) among grass entries ranged from 548 to 614 and from 288 to 321 g kg$^{-1}$ DW, respectively.

There was no interaction of year × grass entry for CP and NDF, but the interaction of year × entry for ADF was significant ($P < 0.01$; Table 2). The ADF concentrations of all entries were greater in 2005 than 2004 (Fig. 3), but compared to 2004 levels, increases in ADF ranged from 19% (Lincoln smooth bromegrass) to more than 32% (Maximize tall fescue and Felina festulolium). Forage nutritive value depends not only on plant genotype and development stage, but also on management practice and growth environment (Ball et al., 2001). The differences in concentrations of forage CP, NDF, and ADF between years were probably due to climatic conditions in March and April, especially temperature and precipitation as mentioned above. While water deficit stress generally increased forage CP and NDF concentrations in perennial cool-season grasses grown across an irrigation gradient (Jensen et al., 2003), we found that forage CP concentrations at preheading were greater with more favorable precipitation in 2004 than 2005. Additionally, the daily mean temperature for March and April in 2004 was greater than in 2005. The more favorable temperature in the 2004 spring might have partially contributed to better plant growth and the measured forage nutritive values. Because the ADF interaction of year × grass entry effect was significant, expression of genotype differences in ADF nutritive values was not consistent and indicates that the use of this trait to select the best genotypes for livestock and forage production systems in the southern Great Plains will be challenging.

Correlation among Forage Nutritive Values

To determine relationships among tested forage quality variables, forage CP, NDF, ADF, and NSC data were pooled over plant species, entries, and years. Correlation coefficients ($r$) between variables were calculated (Table 3). Forage CP concentration was negatively correlated with NDF and ADF ($P < 0.001$), but positively correlated with concentrations of fructans ($P < 0.01$), glucose, starch, and total NSC ($P < 0.001$). Both forage NDF and ADF were negatively correlated with concentrations of most NSC components. Bahrani et al. (1983) reported that N concentration of tall fescue (Kentucky 31) tiller bases 10 d following clipping (around early October in Athens, GA) was linearly and negatively correlated with water-soluble carbohydrate concentration. In our study, forage CP content was
positively correlated with total NSC concentration across the 13 cool-season grass entries of five species at preheading. Our results indicate that when perennial cool-season grasses are used for hay, forage concentrations of CP and NSC may not be contradictory quality parameters.

Among the five NSC components tested, glucose was positively correlated with concentrations of fructans and starch \( (P < 0.001) \), negatively correlated with fructose \( (P < 0.05) \), and not correlated with sucrose. Smith (1973) reported that sucrose was often negatively related to glucose and fructose. In contrast to Smith (1973), results of the present study indicated that sucrose concentration did not correlate with either glucose or fructose (Table 3). Neither fructose nor sucrose was correlated with fructans or starch concentration. Fructans and starch (both storage forms of NSC) were positively correlated \( (P < 0.001) \). In addition, change in the forage total NSC level was most closely associated with fructan concentration, the primary component of total NSC among the 13 entries (Table 3). Our results of correlations between NSC components were in agreement with the findings of Chatterton et al. (1989) who reported that among a large number of cool-season species, leaf fructan concentration did not correlate with sucrose. Therefore, accumulation of fructans in cool-season grasses was not at the expense of any other form of NSC (Chatterton et al., 1989), because fructans were not negatively correlated with any other NSC components in the present study (Table 3).

### CONCLUSIONS

Results of this study indicate that forage total NSC concentration of perennial cool-season grasses at preheading is positively correlated with forage CP concentration and negatively correlated with NDF and ADF. Fructans are a dominant NSC of cool-season grass forages, while fructose is the lowest among the five NSC components measured. High concentration of fructans in perennial cool-season grass forages at preheading apparently did not cause a deficit in other forms of NSC. Although great variation for total NSC concentrations across the grass species was detected, changes in the fraction of each NSC component in total NSC across the 13 grass entries were relatively small. Differences in total NSC concentrations among the species of perennial cool-season grasses tested are much greater than that among entries within a species. Smooth bromegrass exhibited the highest NSC level among the five species; therefore, smooth bromegrass may be a better choice for cattle, but less so for horses.

### ACKNOWLEDGMENTS

Dale Purdue and Jeffrey Weik provided excellent technical assistance with sample collection and laboratory quantification of nonstructural carbohydrates.

### Table 3. Correlation coefficients among concentrations of forage crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), nonstructural carbohydrate components, and total nonstructural carbohydrate (NSC) of perennial cool-season grasses. Data are pooled across grass entries, replicates, and years \( (n = 78) \).

<table>
<thead>
<tr>
<th></th>
<th>NDF</th>
<th>ADF</th>
<th>Glucose</th>
<th>Fructose</th>
<th>Sucrose</th>
<th>Fructan</th>
<th>Starch</th>
<th>Total NSC</th>
</tr>
</thead>
<tbody>
<tr>
<td>CP</td>
<td>-0.603***</td>
<td>-0.679***</td>
<td>0.675***</td>
<td>-0.080</td>
<td>0.071</td>
<td>0.329***</td>
<td>0.657***</td>
<td>0.584***</td>
</tr>
<tr>
<td>NDF</td>
<td>0.548***</td>
<td>-0.584***</td>
<td>-0.123</td>
<td>-0.325**</td>
<td>-0.684***</td>
<td>-0.460***</td>
<td>-0.779***</td>
<td></td>
</tr>
<tr>
<td>ADF</td>
<td>-0.248*</td>
<td>-0.402***</td>
<td>0.015</td>
<td>-0.355***</td>
<td>-0.625***</td>
<td>-0.539***</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>-0.264*</td>
<td>0.162</td>
<td>0.551***</td>
<td>0.499***</td>
<td>0.728***</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fructose</td>
<td>0.112</td>
<td>0.019</td>
<td>0.081</td>
<td>0.135</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sucrose</td>
<td>0.082</td>
<td>-0.210</td>
<td>0.211</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fructan</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.437***</td>
<td>0.892***</td>
</tr>
</tbody>
</table>

\* Significant at the \( P < 0.05 \) level.

\** Significant at the \( P < 0.01 \) level.

\*** Significant at the \( P < 0.001 \) level.

### REFERENCES


