Nonstructural Carbohydrates in Tall Fescue Cultivars: Relationship to Animal Preference

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ABSTRACT

Grazing animals prefer some plants to others. These choices are likely related to physical and chemical factors such as energy-dense carbohydrates contained in plants. This study quantified the nonstructural carbohydrate fractions in each of eight vegetatively growing, endophyte-free, tall fescue cultivars (*Festuca arundinacea* Schreb.) and relates their sugar concentrations to cattle grazing preferences. The experimental area consisted of eight cultivar plots replicated three times in each of three pastures. Within each pasture, forage was sampled between 0830 and 1000 h mountain daylight time (MDT) during each of four seasons and 2 yr. Freeze-dried forage samples were extracted with hot water and an amylase (Clarase) solution. Sugars were quantified colorimetrically using potassium-ferricyanide and glucose-oxidase methods. Cattle grazing preferences among these tall fescue cultivars were related to the concentrations of total nonstructural carbohydrates (TNC) ($r^2 = 0.49$, $P < 0.05$). Other sugar fractions were not significantly related to grazing preference in this study. The nonstructural carbohydrate concentrations averaged over the entire study were glucose, 14; fructose, 5; sucrose, 40; fructan, 23; insoluble starch, 24; and TNC, 129 g kg^{-1}. A forage selection criterion should include measures of the TNC because of their close relationship to animal grazing preference.

Readily fermentable carbohydrates in forages provide energy to grazing animals and may be one of the cues used when selecting which forage plants to eat (Fisher et al., 1999). For example, Tava et al. (1995) reported that three tall fescue varieties having 133 g kg^{-1} water-soluble carbohydrates (WSC) were considered more palatable to cattle than three others having only 108 g kg^{-1}. The TNC, which include WSC, are readily fermentable and serve as energy sources to ruminants. Hungry animals display a rapid response to energy-dense test diets (Provenza, 1995; Baumont, 1996). Thus, animal grazing behavior might be conditioned by the TNC concentrations in forage. However, no published data are known in which TNC have been quantitatively linked with animal grazing preference. In this study, we report the concentrations of TNC and other sugar fractions in eight endophyte-free tall fescues and relate these to previously reported quantitative data on grazing-cattle preferences among the same tall fescues (Shewmaker et al., 1997).

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MATERIALS AND METHODS

Cultivars and Experimental Design

The eight endophyte-free tall fescue cultivars included in this study were 'Barcel', 'Kenhy', 'Kentucky-31' (KY-31), 'Missouri-96' (MO-96), 'Mozark', 'Stargrazer', and the experimental selections C-1 and HiMag (Mayland and Sleper, 1993). On 20 Sept. 1991, these grasses were seeded in rows into an irrigated, Portneuf silt loam loess soil (Durinodic Xeric Haplocalcid) near Kimberly, in south-central Idaho (42°30′ N, 114°08′ W, elevation 1200 m). The experimental design was a randomized complete block with three pastures (blocks), three replications nested within blocks, eight entries (main plots), and six rows per main plot. Rows were 0.56 m apart and main plots were 3.35 by 6.7 m.

Harvesting and Sample Preparation

Vegetatively growing forage was clipped at a stubble height of 8 cm from randomly located 0.6-m sections of Rows 3 and 4 in each plot. The forage in Block 1 was clipped on d 131, 165, 221, and 256 of 1993 and d 129, 164, 220, and 262 of 1994. The forage samples in Blocks 2 and 3 were clipped 1 and 3 d later, respectively. Samples were clipped between 0800 and 0930 MDT, cooled to about 5°C, weighed, composited within plots, frozen within 3 h of cutting, freeze-dried, and sequentially ground to pass through 1-mm screens in a Wiley shear mill and Cyclotec abrasion mill.

After sampling and grazing, the pastures were flail-mowed to an 8-cm stubble height, fertilized with 56 kg ha^{-1} N, and furrow-irrigated as needed until the next sampling period. Other soil nutrients were adequate for good plant growth. Meteorological information was obtained hourly (CR7, Campbell Scientific, Logan, UT) at a site next to the plots (J.L. Wright, personal communication, 1996).

Grazing Preference

This soluble carbohydrate study was part of a larger study in which animal grazing preference was related to various chemical and physical characteristics of eight tall fescue cultivars. The experimental area was grazed by cattle for 48 h at a stocking rate of 11 animal units ha^{-1}, which removed about 50% of the available forage. Detailed results of the grazing-preference study were previously reported (Shewmaker et al., 1997) but are summarized here for the convenience of the reader (Table 1). Preferencing entailed a subjective scoring of forage usage after a 48-h grazing period by cattle. Four trained observers independently scored each row on a scale ranging from 0 (no use) to 10 (100% consumption of available forage). The subjective scoring had about one-fourth of the experimental error as clipping measurements (Shewmaker et al., 1997).

Abbreviations: MDT, mountain daylight time; SAS, statistical analytical systems; SDM, structural dry mass; TNC, total nonstructural carbohydrates; WSC, water-soluble carbohydrates.

Table 1. Cattle preference scores for tall fescues grazed in each of four seasons and 2 yr, where 0 shows no evidence of grazing and 10 indicates all available forage is eaten (adapted from Shewmaker et al., 1997).

	Preference							
	1993				1994			
Cultivar	May	June	Aug.	Sept.	May	June	Aug.	Sept.
Kenhy	7.1	6.4	8.6	7.7	6.8	8.8	8.6	8.2
Kentucky-31	5.3	3.9	7.1	5.2	3.9	7.3	6.9	6.6
HiMag	4.5	4.0	7.4	4.4	3.8	6.6	6.6	6.5
C-1	6.6	3.9	5.6	5.3	4.0	5.9	5.9	5.0
Stargrazer	4.0	3.7	6.8	4.2	3.7	6.4	6.5	6.8
Barcel	4.5	3.3	6.4	4.0	2.9	6.5	6.4	6.8
Missouri-96	4.6	3.1	6.5	3.4	2.9	5.8	5.7	5.4
Mozark	3.9	2.4	6.1	2.9	1.9	6.3	6.4	6.8

Carbohydrate Analyses

Nonstructural carbohydrates were quantified as insoluble starch, fructan, sucrose, glucose, fructose, and TNC, which included additional but not individually quantified soluble sugars (Chatterton et al., 1987). One 50-mg sample was extracted with a commercial amylase preparation (2 g L^{-1} Clarase 40,000) for 24 h at 40°C, and another 50-mg sample was extracted using boiling deionized water. The tissue that was digested with Clarase was first boiled in a small volume of water to stop any endogenous enzyme activity.

The Clarase enzyme mixture contained amylase, invertase, and maltase activities, so insoluble starch and sucrose were hydrolyzed by Clarase while fructan remained essentially intact. Any reducing sugars other than glucose would be included in the final value reported for fructose. Analyses using potassium ferricyanide and glucose oxidase methods were automated using a Technicon Autoanalyzer II (Chatterton et al., 1987). Sugar extracts were passed through a high-performance anion exchange using a Dionex Ion Chromatograph equipped with a Carbo-Pac PA-100 column, and sugars were measured by a Pulsed Amperometric detector with a 3.2-mm gold electrode. Nonstructural carbohydrate concentrations (from the Autoanalyzer data) were computed as

$$\text{nonstructural carbohydrate mass}/(\text{dry mass} - \text{TNC mass})$$

and reported on a structural dry mass basis (g kg^{-1} SDM) to avoid the confusion associated with simultaneous changes in carbohydrate content and dry weight (Moser et al., 1982). The SDM was computed as

$$\text{mass of given sugar}/(\text{dry matter mass} - \text{TNC mass})$$

Statistical Analyses

Sucrose, insoluble starch, and the $\log_{10}$ transformations of TNC, fructose, and fructan met the requirements for an analysis of variance (Schlotzhauer and Littell, 1987) and were analyzed by least squares to fit general linear models (PROC GLM, SAS Inst., 1990). Comparisons among treatments were tested at $P < 0.05$ by Duncan's multiple range test. The GLM model assumed that year (Y), cultivar (C), and harvest (H) were fixed effects and that pasture (P) and replicates (R) were random. Tests for differences among the main effects used the following error terms: Y tested by P × Y, C tested by P × C, P × C tested by C × R(P), C × Y tested with C × P × Y, H tested by P × H, which was tested with R × H(P), and P tested by R(P). The transformed data were then back-transformed for presentation. The preferences were visually scored 0 if grass was not eaten, up to and including 10 if all available forage was eaten (Shewmaker et al., 1997). The preference scores for each cultivar were regressed against arithmetic carbohydrate data using the SAS selection techniques PROC REG and MAXR for stepwise multiple-regression analysis (Cody and Smith, 1991).

RESULTS AND DISCUSSION

The total nonstructural carbohydrates in cultivars across harvest periods ranged from 91 to 162 g kg^{-1} during 1993 and from 68 to 192 g kg^{-1} during 1994 (Table 2). No forage cultivar was ever significantly greater in TNC than Kenhy nor significantly lower than Mozark. This relative ranking of cultivars based on TNC values was recognized by the animals who showed the greatest preference for Kenhy and the least for Mozark. Overall, the TNC levels were positively related to the animal grazing preference scores summarized in Table 1.

The smallest TNC values were measured in August for both years (Table 2). The warmer air temperature in August contributed to the aging of cellular tissue accompanied by a reduction in TNC (Smith, 1973). Variation in air temperature, solar radiation, and pan-evaporation data for the 1-, 2-, or 3-d period before forage sampling (only means shown for meteorological data) did not explain the variation in either the TNC or the sum of monosaccharides plus disaccharides (Table 3). Lacking evapotranspiration data, the dry matter concentration of the tissue at clipping time was the best available indicator of water stress in plants. This factor did not explain the variability in either the TNC or simple sugars. Nevertheless, the August-harvested forage grew slower than forage harvested in other months, requiring 56 d vs. 34 to 42 d for comparable regrowth. Thus, August-harvested leaves were chronologically older, perhaps explaining the reduction in the TNC. Jung et al. (1976) noted that the TNC concentrations were reduced as plants matured physiologically but an effect of increasing chronological age was not found.

Carbohydrate fractions other than the TNC were not

Table 2. Total nonstructural carbohydrate (TNC) concentrations [g kg^{-1} SDM (structural dry mass)]† by cultivar, harvest month, and year.

	1993					1994					
Cultivar	May	June	Aug.	Sept.	$\bar{X}$ 1993	May	June	Aug.	Sept.	$\bar{X}$ 1994	$\bar{X}$ 1993–1994
Barcel	160	114	106a	129	127a	122	131b	79cd	156abc	122ab	125
C-1	141	122	112a	129	126a	105	154a	86bc	155abc	125ab	126
HiMag	154	133	114a	130	133a	119	160a	81c	162abc	131ab	132
Kenhy	143	130	108a	141	131a	112	168a	103a	192a	144a	138
KY-31	162	125	115a	124	132a	126	168a	87bc	151bc	133ab	133
MO-96	153	117	105a	121	124ab	116	125b	75cd	135cd	113bc	118
Mozark	140	108	91b	116	114b	113	113b	68d	114d	102c	108
Stargrazer	154	129	109a	136	132a	129	165a	101ab	177ab	143a	138

† Means in a column followed by letters in common are not different by Duncan's multiple range test ($P < 0.05$).

Table 3. Mean TNC, monosaccharides + disaccharides, dry matter in tall fescue at clipping time, min.–max. air temperature, daily radiation levels, and pan evaporation during each harvest period.†

Year/month†	TNC‡	Monosaccharides + Disaccharides‡	Forage dry matter‡	Min.–max. air temperature	Daily radiation	Pan evaporation
	g kg^{-1} SDM		g kg^{-1}	°C	MJ m^{-2}	mm
1993						
May	152a	73a	234b	6.8–25.0	27.0	10.9
June	124b	69ab	215c	5.8–21.4	29.0	7.7
Aug.	109c	63c	248a	10.6–25.9	21.7	5.7
Sept.	130b	67b	236b	3.8–22.0	19.9	6.2
1994						
May	119b	45c	185c	7.9–24.4	24.9	7.5
June	155a	61a	244b	8.9–24.4	26.1	6.8
Aug.	86c	35d	257a	12.7–31.3	24.3	7.6
Sept.	159a	53b	254a	10.2–26.9	20.2	6.0

† Sampling periods: 10–14 May, 13–17 June, 8–12 August, 12–16 September 1993, and 8–13 May, 12–16 June, 7–11 August, and 18–22 September 1994.
‡ Column means within years and across cultivars followed by letters in common are not different by Duncan's multiple range test ($P < 0.05$).

different among cultivars, harvest dates, or years. Therefore, we report only the grand means for each constituent. The concentrations on an SDM basis were glucose, 14 (CV = 16%); fructose, 5 (CV = 56%); sucrose, 40 (CV = 19%); fructan, 23 (CV = 50%); and insoluble starch, 24 g kg^{-1} (CV = 25%). The grand mean for the TNC was 129 g kg^{-1} (CV = 16%). When expressed on a dry matter basis, these average values were glucose, 12.6; fructose, 3.6; sucrose, 34.8; fructan, 20.9; insoluble starch, 20.9; and TNC, 112 g kg^{-1}. When expressed on a TNC-free basis, the data are free of the confusion of simultaneously changing TNC mass and dry matter mass (Chatterton et al., 1987).

The relationships among concentrations of various sugar fractions are shown in Table 4. The TNC data were positively correlated with concentrations of other measured sugars. This may seem obvious, but there were other soluble sugars that were not measured in this study, and the entire sugar profile may not have changed in synchrony. The positive relationship between the two monosaccharides, glucose and fructose, is attributed to their early accumulation via the C-3 photosynthetic pathway. Sucrose is the primary component of the TNC and is often negatively related to glucose and fructose (Smith, 1973).

About one-half of the variation in the preference score was explained by a similar variation in the TNC ($r = 0.71$, $P < 0.05$). The regression equation was

$$\text{preference score} = -3.26 + 0.069 \text{ g kg}^{-1} \text{ TNC}$$

The sums of monosaccharide and disaccharide sugars (Table 3) made up about 45% of the TNC and ranged from 33 to 58% across the different grazing periods. The grazing preference was positively related to the sum of monosaccharides and disaccharides ($r = 0.67$, $P < 0.05$).

Table 4. Pearson correlation coefficients among sugar components of eight tall fescue cultivars.

	Sucrose	Fructose	Glucose	Fructan	Insoluble starch
TNC	0.52***	0.23***	0.29***	0.73***	0.14**
Sucrose		−0.14**	−0.15***	0.16***	−0.09**
Fructose			0.71***	−0.01	−0.04
Glucose				−0.11**	0.24***
Fructan					−0.04

** Significant at the 0.01 level.
*** Significant at the 0.001 level.

The concentrations of these sugar fractions increase during the day and decrease during the night (Lechtenburg et al., 1972). Shewmaker and Mayland (1999) showed that it was important to clip a set of plots within a 1-h period to keep the experimental error within ±5% for comparable means. Some changes in the TNC concentrations probably occurred throughout the day in this experiment. It is, however, assumed that clipping between 0830 and 1000 h MDT had a minimal effect on sugar levels among the eight cultivars. However, our experimental error was likely due, in part, to not immediately freezing the samples in liquid N (not available) or placing them with dry ice, which led to a several hour delay until the actual freezing of the samples.

Grasses, even at the morning sampling, contained more than the 80 g kg^{-1} TNC required for proper fermentation of grass silage (Jung et al., 1976). The TNC influence the amount and ratio of volatile fatty acids produced in the rumen (Bowden et al., 1968), which affects the efficiency of forage utilization. Reid et al. (1966) observed that the palatability of grasses increased with increasing WSC concentrations. This relationship was also noted by Tava et al. (1995). However, the palatability was only qualitatively estimated.

Taste may be the primary sense used by ruminants when discriminating among forages (Krueger et al., 1974). Large animals can discriminate among the various primary tastes (sweet, sour, salty, or bitter), preferring sweet flavors (Nombekela et al., 1994). Dairy cows prefer sweet flavors to others and are able to distinguish between two diets—control and control plus 1.25% sucrose. The presence of sugars in the diet, including the TNC or an individual sugar like sucrose, may provide cues to either monogastrics or ruminants. Hungry animals prefer energy-dense diets and will identify such feeds within minutes after initial ingestion (Provenza, 1995). Increasing the dry matter intake and energy utilization is an intermediary goal in most ruminant feeding operations.

The TNC concentrations in forages have been identified as the third most important characteristic requiring the attention of forage breeders (Wheeler and Corbett, 1989). We have shown that animal preferences among tall fescue cultivars are related to the TNC concentrations, further emphasizing their importance in forage systems. Plant breeding programs and harvest management strategies ought to be directed toward increasing the TNC in forages.

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Chicory and English Plantain Seedling Emergence at Different Planting Depths

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ABSTRACT

Chicory (*Cichorium intybus* L.) and English plantain (*Plantago lanceolata* L.) have been introduced in the USA as perennial herbs for pastures. A knowledge of seedling emergence and the structure of these species under different planting conditions is necessary for developing planting recommendations. We conducted controlled environment and field studies to compare the emergence and morphology of chicory and plantain seedlings from three planting depths. 'Grasslands Puna', 'La Certa', and 'Forage Feast' chicory, and 'Ceres Tonic' and 'Grasslands Lancelot' plantain were sown at 1, 3, and 6 cm depths in the growth chamber and greenhouse. The seedlings were destructively sampled 14 d after emergence, and the number and mass of leaves and roots (primary, lateral, basal, and adventitious) were recorded. The same cultivars were sown in field plots in July and September 1998 to determine seedling size and emergence from 1-, 3-, or 6-cm planting depths. Controlled environment studies showed that deeper planting reduced the root weight, length, and number more in chicory than in plantain. Planting at 3 and 6 cm in the field reduced seedling emergence by 34 and 60% (avg. of cultivars), respectively, compared with the 1-cm planting depth. Differences in seedling size among cultivars within species were mainly related to differences in seed mass. Plantain had better seedling emergence in the field than chicory (54 vs. 27% in July and 60 vs. 17% in Sept.), and these differences were not related to differences in seed mass. Both chicory and plantain should be planted no deeper than 1 cm for rapid establishment.

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Producers in the northeastern USA would like pasture species that are productive during the *summer slump* period of July, August, and early September. Chicory (cv. Grasslands Puna) has been investigated as an alternative pasture herb because of its reported drought tolerance and good production in summer (Belesky et al., 1999; Kunelius and Macrae, 1999). Data on the proper planting depth for chicory are limited. Moloney and Milne (1993) recommended 1 cm as the optimum planting depth for Grasslands Puna.

English plantain (narrow-leaf plantain, buckhorn plantain, ribwort, and ribgrass) commonly occurs as an occasional weed in temperate-region pastures (Grime et al., 1990). It is palatable to livestock (Foster, 1988; Ivins, 1952) and is described as deep rooting and drought resistant (Sagar and Harper, 1964). Recently, domesticated cultivars of plantain have been selected for pasture production in New Zealand (Rumball et al., 1997; Stewart, 1996). Data on the planting depth for these plantain cultivars are lacking; however, some ecological research has shown that plantain establishes best on uncompacted soils (Blom, 1978). Both chicory and plantain have epigeal seedling emergence (Sanderson and Elwinger, 2000).

Rapid development of the leaf area and the establishment of a critical number and mass of roots are neces-

Abbreviations: DAP, days after planting.