Daily carbohydrate accumulation in eight tall fescue cultivars

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Abstract

Eight cultivars of tall fescue (*Lolium arundinaceum* Schreb., S.J. Darbyshire = *Festuca arundinacea* Schreb.), Barcel, Kenhy, Kentucky-31, Missouri-96, Mozark, Stargrazer, C-1 (an experimental selection), and HiMag, were sampled at 2-h intervals during daylight on four cutting dates. Cultivars varied in concentrations of carbohydrate fractions but accumulation rates were not different. Daily mean total non-structural carbohydrate (TNC) concentrations for cutting dates in May, July, August and September declined from 239 to 231, 143 and 120 g TNC kg^{-1} adjusted dry weight (ADW) respectively. Concentrations of fructans were highest in July but sucrose, glucose and starch concentrations were highest in May. Sucrose was the largest contributor proportionately to TNC daily means across accessions in May (0·33), August (0·30) and September (0·38). Glucose composed an equivalent proportion of TNC in the August harvest. Starch concentration was highest in May at 53 g kg^{-1} ADW and lowest in August at 23 g kg^{-1} ADW. The TNC concentration increased by 22·4 (May), 16·8 (July), 21·0 (August) and 30·8 g kg^{-1} ADW (September) from dawn to dusk. Forage samples taken to estimate preference by ruminants or for TNC analyses should be cut and preserved within 1 h to control the diurnal variation of TNC proportionately within 0·05. Tall fescue should generally be cut between noon and sunset for TNC concentrations to be greater than the daily mean.

Keywords: glucose, NIRS, sucrose, total non-structural carbohydrates

Introduction

Total non-structural carbohydrates (TNC) accumulate during daylight because photosynthesis produces more TNC than are metabolized for plant growth and maintenance. The net balance of leaf photosynthesis, respiration and carbohydrate export produces a diurnal variation in TNC such that minimum concentrations occur in early morning and maximum in late afternoon (Bowden *et al.*, 1968; Lechtenberg *et al.*, 1972). The concentration of TNC in the green tissues of C_3 cool-season grasses is also influenced by the ratio of stem to leaf tissue. Concentration of TNC appears to increase with advancing maturity and is largely due to an increase in the proportion of fructans. Sicher *et al.* (1984) reported a rapid synthesis of TNC during the first 15 min of light followed by a slower rate of accumulation. This increase in TNC is likely to be accounted for by sucrose, which is the major photosynthetic product synthesized during the initial hours of the light period. Sucrose in barley (*Hordeum vulgare* L. subsp. *vulgare*) leaves increased three-fold during the first 12 h of light (Sicher *et al.*, 1984). Net accumulation of TNC may continue to increase in the plant because of temporary storage in conductive tissue.

Non-structural carbohydrates are sources of readily available energy for continued plant growth and survival and, when ingested by ruminants, provide readily available energy for rumen microbial activity thus improving forage utilization. Ruminants prefer afternoon-cut forage compared with morning-cut forage (Burns *et al.*, 2005). Dairy cows allocated fresh pasture

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in the afternoon grazed longer during their evening meal than cows allocated fresh pasture in the morning (Orr *et al.*, 2001). Simple changes in management can take advantage of the diurnal TNC cycle.

Plant breeders are selecting plant materials based on non-structural or water-soluble carbohydrates concentrations (Humphreys, 1989) with the goal of increasing energy content and ultimately to improve livestock production and nutrient-use efficiency. Knowledge of the range of TNC concentrations and rates of accumulation among cultivars will be useful to breeders and producers.

The objectives were to determine variability of TNC concentrations among cultivars, and to investigate daily rates of carbohydrate accumulation in tall fescue (*Lolium arundinaceum* (Schreb.) S.J. Darbyshire = *Festuca arundinacea* Schreb.).

Materials and methods

Plant materials and site description

The eight cultivars of tall fescue tested were Barcel, Kenhy (*L. perenne* × *L. arundinaceum*), Kentucky-31, Missouri-96, Mozark, Stargrazer, C-1 (an experimental selection) and HiMag (Sleper *et al.*, 2002). Cultivars were free of the fungal endophyte [(*Neotyphodium coenophialum* Morgan-Jones and Gams) Glen, Bacon and Hanlin comb. nov.]. Seeds (4·7 kg ha^{-1}) of each cultivar were sown on day of year (DOY) 263 in 1991 in plots of six rows spaced at 0·56 m. Plots were 3·35 m wide by 6·7 m long. The soil was a surface-irrigated Portneuf silt loam (coarse-silty, mixed, mesic, Durinodic Xeric Haplocalcid) near Kimberly, ID, USA (42°32′N and 114°20′W, elevation 1200 m). The experimental design was a randomized complete block having six replications of eight cultivars. Plots were grazed or cut for hay for 3 years prior to this experiment. In October 1994, plants were flail-mowed to a height of 8 cm. The following April (DOY = 91), 45 kg N ha^{-1} was applied as broadcast urea.

Sampling procedure

Sampling periods, maximum/minimum air temperatures and solar irradiance are shown in Table 1. Herbage was sampled by clipping portions at random locations from each of six rows, at 8-cm stubble height, bulked within plots and immediately placed on dry ice on each of the four harvest dates. Six replications were sampled at eight times with seven equal time intervals (approximately 2 h) between sunrise and sunset in May, July, August and September 1995 (Table 1). Samples were stored at −5°C, freeze-dried, ground to pass a 1-mm screen in a Wiley shear-mill, and secondly through an Udy cyclone sample mill (Udy Corporation, Fort Collins, CO, USA). Following sampling, all plots were clipped to an 8-cm stubble height and forage was removed. Each harvest was followed by application of 45 kg N ha^{-1} and furrow irrigation.

Laboratory analyses

Samples were analysed by near-infrared (NIR) spectroscopy for TNC, fructans, starch, sucrose, glucose and fructose. Samples were scanned from 1100 to 2500 nm using a Pacific Scientific[1] 6500 spectrophotometer (Foss NIRSystems, Silver Spring, MD, USA) and commercial software from Infrasoft International (Port Matilda, PA, USA). Regression equations were developed by modified partial least squares as reflectance data were regressed against chemically derived data. Samples with similar spectra (*H*-distance $\leqslant$0·5) were eliminated which resulted in 168 samples identified for chemical analyses. Optimum regression equations were selected on the basis of squared correlation coefficients calculated in calibration, variance ratios calculated in cross-validation, and s.e. calculated in calibration and cross-validation (Shenk and Westerhaus, 1991).

Chemical analyses of non-structural carbohydrates were characterized according to six constituent categories (Chatterton *et al.*, 1986, 1987): TNC, insoluble starch, fructans, sucrose, glucose and fructose. The first of two 50-mg samples was extracted using a commercial amylase preparation (0·1% Clarase 40 000) for 24 h at 40°C and the second using boiling deionized water. Tissue digested with Clarase was first boiled in a small volume of water to stop any endogenous enzyme activity. Clarase enzyme mixture contained amylase, invertase and maltase activities. Insoluble starch and sucrose were hydrolyzed by Clarase, but fructans remained essentially intact. Reducing sugars other than glucose were included in the final value reported for fructose. Analyses employing the Potassium ferricyanide and glucose oxidase methods were automated using a Technicon Autoanalyzer II (Technicon Instruments, Ardsley, NJ, USA). Sugar extracts were verified by HPAEC-PAD using a Dionex Ion Chromatograph equipped with a Carbo-Pac-100 column and a Pulsed Amperometric detector with a 3·2 mm gold electrode (Dionex Corporation, Sunnyvale, CA, USA). Non-structural carbohydrate concentrations were expressed on an adjusted dry weight (g kg^{-1} ADW) basis (dry weight minus TNC) to avoid errors associated with

[1]Mention of a trade name does not imply endorsement by the University of Idaho or USDA-ARS of the products named, nor criticism of similar ones not named.

Table 1 Eight scheduled sampling times (seven intervals between sunrise and sunset) and meteorological data for each of four harvest dates in 1995. Meteorological data are minimum–maximum temperatures for day of sampling, sum of 10-min radiation values measured for day (data from J. L. Wright, ARS, Kimberly, ID, USA) and relative radiation for day of sampling. Coordinates are 114°20′W, 42°32′N.

	Date and day of year			
Sampling period	**17 May**	**6 July**	**8 August**	**13 September**
	137	187	220	256
Mid-time of 40 clippings (h and min)				
1	05:14	05:07	05:37	06:13
2	07:20	07:17	07:39	08:01
3	09:26	09:27	09:41	09:50
4	11:32	11:38	11:43	11:38
5	13:38	13:48	13:45	13:26
6	15:44	15:59	15:47	15:16
7	17:50	18:09	17:49	17:03
8	19:56	20:20	19:51	18:53
Daily meteorological data				
Min-max air temperature (°C)	8–20	13–31	8–20	8–27
Σ MW m^{-2} by 10-min intervals	40·7	44·3	46·9	35·0
Actual/potential radiation × 100 (%)	83	88	100	99·4

simultaneous changes in carbohydrate concentration and dry weight content (Moser *et al.*, 1982).

Statistical analyses

Analysis of variance techniques were used to initially assess the significance of month, time of day and cultivar on carbohydrate fractions. Given the significance of the time of day effect, a more detailed examination of the relationship between time of day and the responses was carried out by estimating the following linear model:

$$y_i = B_0 + B_1 * x_i$$

where y_i is the observed response at time x_i, B_0 is an intercept term measuring initial concentration and B_1 is a slope term measuring the rate of change in concentration. Models were fitted to the linear model for specific time frames when the response was essentially linear. Within each month, individual cultivars were modelled and assessed separately. Following model estimation, parameter estimates (intercept and slope terms) for each cultivar model were contrasted within a month using dummy variable techniques (Littell *et al.*, 1991). The SAS procedures GLM and REG were used for all statistical computations (SAS, 2004).

Results and discussion

The NIR calibration equations (Table 2) for TNC, fructans, sucrose, glucose and total starch concentrations were acceptable; however, calibrations for fructose and insoluble starch concentrations were not, and were omitted from further consideration. Cultivar, month, time of day and the cultivar–month interaction were significant ($P < 0{\cdot}01$) for TNC, fructans, sucrose, glucose and total starch concentrations. Thus, further analyses were carried out by month.

Seasonal and defoliation effects

Carbohydrate concentrations varied with month of sampling (ANOVA not shown) probably because of different radiance levels (Table 1), temperature and evapo-transpiration interactions, day-length and physiological status of the grass. Concentrations of TNC declined with each successive cutting during the season (Table 3). Concentrations of fructans were highest in July, but sucrose, glucose and starch concentrations were highest in May. Monthly TNC concentrations in an earlier study with four cuttings also varied seasonally (Mayland *et al.*, 2000).

Razmjoo *et al.* (1997) also reported seasonal variation in carbohydrate concentrations in tall fescue managed as turf. They reported that glucose concentrations increased from spring to summer, did not change from summer to autumn, but decreased from autumn to winter; whereas fructose concentrations were constant from spring to summer, increased sharply from summer to autumn and decreased from autumn to winter (Razmjoo *et al.*, 1997). In contrast to the results reported here, they showed that sucrose concentrations increased from spring to summer, decreased from

Table 2 Regression statistics for near-infrared spectroscopic determination of concentrations of carbohydrate fractions [total non-structural carbohydrates (TNC), fructans, sucrose, glucose and total starch] in tall fescue leaves.

Variable	*n*	Mean (g kg^{-1} DM)	SE_C (g kg^{-1} DM)	R^2	SE_{CV} (g kg^{-1} DM)	1-VR*
TNC	162	178·0	8·0	0·983	9·4	0·978
Fructans	162	66·8	5·2	0·987	7·0	0·977
Sucrose	151	41·9	6·5	0·909	7·4	0·880
Glucose	150	31·6	3·6	0·939	4·3	0·917
Total starch	155	34·4	5·7	0·901	7·1	0·847

*1 − VR = 1 minus the variance ratio (VR) calculated in cross validation using modified partial least squares regression.
SE_C, standard error of calibration; SE_{CV}, standard error of cross-validation.

Table 3 Means of carbohydrate concentrations (n = 384, g kg^{-1} DM) across eight cultivars of tall fescue as affected by hour of daylight (eight sampling periods between 05.00 and 20.00 h).

Concentration	17 May	6 July	8 August	13 September
TNC	238	232	141	121
Fructans	64	116	35	27
Sucrose	79	55	42	46
Glucose	46	22	41	27
Total starch	53	32	28	23

All linear functions for each carbohydrate are significant ($P < 0·05$) except for glucose concentration on 17 May and fructans concentration on 8 August, when the quadratic function was significant.
TNC, total non-structural carbohydrates.

summer to autumn and sharply increased from autumn to winter. In this study, mean total starch concentrations were 53 g kg^{-1} DM in May, decreased to 32 g kg^{-1} DM in July, 28 g kg^{-1} DM in August and 23 g kg^{-1} DM in September. Razmjoo *et al.* (1997) reported that starch concentrations increased from spring to summer and from summer to autumn, but decreased from autumn to winter. These differences in results may be due to different environments or possible differences in cutting frequencies. The tall fescue in Razmjoo *et al.* (1997) was managed as turf, implying increased number of cuttings although they did not report cutting frequency or time of sampling, which complicates direct comparisons.

Concentration of water-soluble carbohydrates in perennial ryegrass (*Lolium perenne* L.) decreased from a range of 250–350 g kg^{-1} DM in May to about 0·50 of those concentrations in regrowths after defoliation in June and August (Miller *et al.*, 2001). The results reported here, as well as those mentioned above, should serve as a warning that TNC concentrations are dynamic because of timing of defoliation, climate and plant interactions. Additional research is needed before these results can be extrapolated to other environments.

Cultivar effects

Initial analyses of variance indicated significant time-of-day effects (data not shown). Thus, regression analyses were used to further explore time-of-day effects on the various cultivars. In all cultivars and months, the period of time from 07:00 to 18:00 h was essentially linear and was, therefore, deemed best for modelling the time-of-day effect. While the resultant models may not provide a complete picture of the response over the full time course of the experiment, the period of time chosen does provide cultivar-specific measures of change for purposes of cultivar comparisons. Hence, within this period of time, each cultivar was fitted to the linear regression model. In all cases, parameter estimates for the intercept terms (B_0) were significant while for five cases, the slope terms or rate of change parameter, B_1, was not significant (Table 4). For example, on 17 May in the model for the cultivar Mozark, the initial TNC concentration was estimated at 208 g kg^{-1} DM and this changed at a rate of 3·4 g kg^{-1} DM h^{-1} (Table 5). In comparison, the cultivar Stargrazer on the same date, gave the highest estimate of initial TNC concentration of 260 g kg^{-1} DM, although the rate estimate of 2·0 g kg^{-1} DM h^{-1} was not significant indicating that there was no measurable change in TNC concentration during the period from 7.00 to 18.00 h. Further comparisons of model estimates are given in Table 5.

Differences in TNC concentrations are the result of complex interactions of day-length, solar radiation, temperature, soil water status, soil fertility, leaf to stem ratio and time of sampling. These factors affect photosynthesis and metabolism in plants. Apparently several of these cultivars have the genetic ability to cope with these various factors and were comparatively richer in TNC than other cultivars (Table 5). Figure 1 illustrates the contrast (except May) in intercepts between the cultivars Kenhy and Mozark, while the accumulation

Table 4 Intercepts (g kg^{-1} SDW) and slopes (g kg^{-1} SDW h^{-1}) of linear regression coefficients of carbohydrate concentrations (*y*) as a function of time of day within sampling periods 2–7 (s.e. in parentheses) for four dates. The values of *x* start from sampling period 2, which is after the morning inflection point, or from 07.20 to 08.00 h (x = hour 0) to sampling period 7 (x = hour 10). Carbohydrate concentrations are the means for eight tall fescue cultivars and are expressed on a structural dry weight (g kg^{-1} SDW) (dry weight minus TNC) basis.

	Date			
	17 May	**6 July**	**8 August**	**13 September**
Intercept				
TNC	222 (3)	219 (2)	127 (3)	98 (4)
Fructans	61 (2)	119 (3)	33 (2)	22 (2)
Sucrose	65 (1)	45 (1)	36 (2)	38 (2)
Glucose	47 (1)	18 (1)	38 (1)	18 (1)
Total starch	52 (1)	30 (1)	25 (1)	21 (1)
Slope				
TNC	3·20 (0·50)	2·40 (0·70)	3·00 (0·40)	4·40 (0·40)
Fructans	0·60 (0·30)	NS	NS	0·81 (0·25)
Sucrose	3·20 (0·18)	2·20 (0·15)	1·60 (0·27)	3·10 (0·29)
Glucose	NS	0·52 (0·13)	0·81 (0·17)	0·71 (0·21)
Total starch	NS	0·42 (0·13)	0·56 (0·10)	NS

NS indicates coefficient not significantly different from zero ($P > 0·05$).
TNC, total non-structural carbohydrates.

Table 5 Coefficients of intercept (g kg^{-1} SDW) and slope (g kg^{-1} h^{-1}) of linear regression for total non-structural carbohydrate concentrations (TNC; *y*) as a function of time of day within sampling periods 2 through 7. The *x* values start from sampling period 2, which is after the morning inflection point, or from 07.20 to 08.00 hours (x = hour 0) to sampling period 7 (x = hour 10). TNC in eight tall fescue cultivars are expressed on a structural dry weight (g kg^{-1} SDW) basis (dry weight minus TNC). The accession x hour interaction (slope) was not significant ($P > 0·05$) for any month.

	Date			
Cultivar	**17 May**	**6 July**	**8 August**	**13 September**
Intercept†				
Mozark	208 a	178 a	96 a	71 a
Barcel	203 a	184 a	112 b	82 b
MO96	205 a	197 b	120 c	92 b
C1	224 b	222 c	122 c	98 c
Kenhy	210 a	233 d	141 d	122 d
HiMag	236 b	244 d	143 d	104 c
Stargrazer	260 c	247 d	139 d	119 d
KY31	233 b	249 d	146 d	97 c
Slope				
Mozark	3·4**	3·0*	3·6**	4·1**
Barcel	4·6**	0·9	2·4*	5·0**
MO96	3·0**	3·7**	3·8**	3·4**
C1	3·8**	2·1	3·5**	4·1**
Kenhy	3·4**	3·4**	3·9**	4·6**
HiMag	3·1**	2·5	1·7	4·6**
Stargrazer	2·0	2·1	3·2**	3·8**
KY31	2·6**	1·0	1·9	5·6**

†Intercepts with the same postscript letter are not different ($P > 0·05$).
*,** indicate slopes are significant from 0 at $P < 0·05$ and $P > 0·01$, respectively.

rates (slopes) are the same. Except for May, cultivar Kenhy contained among the highest and cultivar Mozark the lowest concentration of TNC (Table 4). Cultivars Kenhy and Mozark were selected to represent the contrast in TNC concentration because they were the most and least preferred cultivars in a previous study (Shewmaker *et al.*, 1997). These cultivars have similar flowering dates and thus maturity does not explain these differences. The ranking of cultivars for TNC concentration was similar to those reported by Mayland *et al.* (2000). Concentrations of TNC in tall fescue may be inversely related to DM yield when compared with two previous year's production (Shewmaker *et al.*, 1997). Others have found that selection of perennial ryegrass (Humphreys, 1989; Smith *et al.*, 1998) and Italian ryegrass (*Lolium multiflorum*) (Hopkins

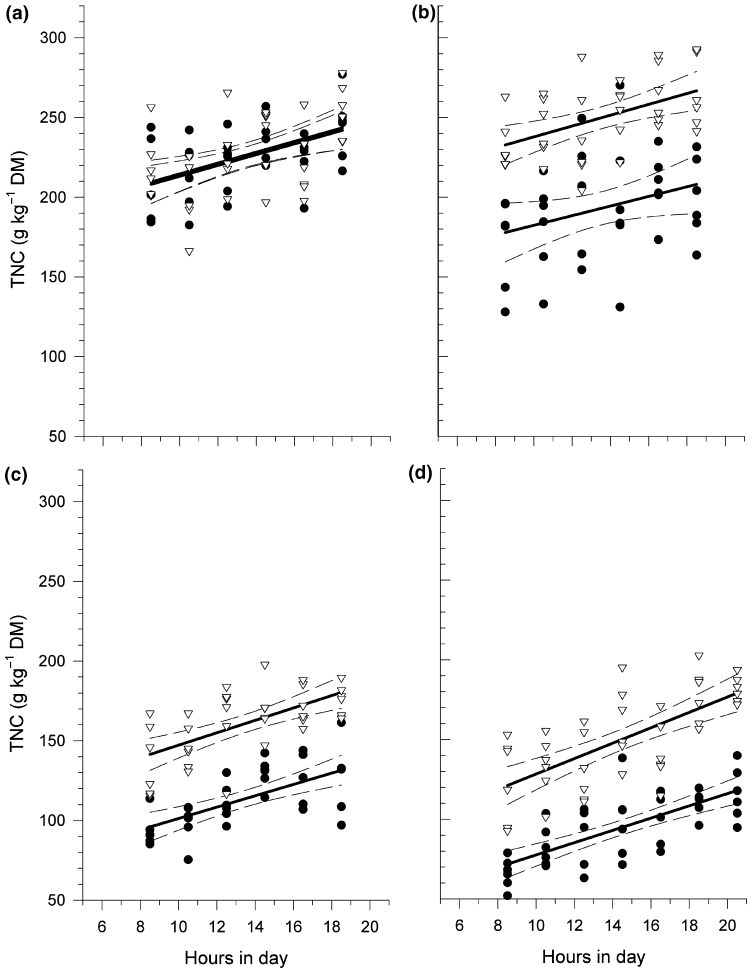

Figure 1 Relationship between concentration of total non-structural carbohydrates and time of day for cultivars Mozark (●) and Kenhy (▽) on (a) 17 May, (b) 6 July, (c) 8 August and (d) 13 September. The linear regression lines are in bold and the 95% confidence limits are the dashed lines.

et al., 2002) for increased water-soluble carbohydrate concentrations was often associated with reduction in DM yield.

Total non-structural carbohydrates concentrations

Vegetative tissue contained, on average across cultivars and time of day, 238, 232, 141 and 121 g TNC kg^{-1} ADW in May, July, August and September respectively. Monthly TNC concentrations in an earlier study with four cuttings also varied seasonally (Mayland *et al.*, 2000). As the carbohydrates in this paper are expressed on an ADW basis, values are higher than if computed on total DM basis. The lowest mean TNC concentration was 43 g kg^{-1} ADW, occurring on 13 September and the highest mean TNC concentration was 335 g kg^{-1} ADW occurring on 17 May.

The TNC concentrations were generally above 80 g kg^{-1} DM required for proper fermentation of grass silage (Jung *et al.*, 1976) although higher values should benefit fermentation and nutrient retention in silage.

Glucose and sucrose concentrations

Glucose and sucrose concentrations varied among cultivars in each of the 4 months except glucose in August (analysis not shown). Sucrose composed 0·33, 0·24, 0·30 and 0·38 of TNC for May, July, August and September harvests respectively. Sucrose was the largest contributor to TNC daily means across cultivars in May, August and September harvests. Glucose composed 0·19, 0·09, 0·29 and 0·22 of TNC for May, July, August and September harvests respectively. Glucose and sucrose composed equivalent proportions of TNC in the harvest on 8 August. Sucrose, not glucose, is the major carbohydrate available for cell growth and glucose oxidation fuels plant respiration (Bryce and Thornton, 1996). Sicher *et al.* (1984) reported that sucrose was the major reserve carbohydrate in barley

(*Hordeum vulgare* L.) and that it was mobilized in the dark to a greater extent than starch. They also noted diurnal changes in the total glucose and fructose concentration of barley leaves were only about one-third that of sucrose.

Concentrations of fructans

Sucrosyl-oligosaccharides other than fructan are included in the total fructan value. In this study, concentrations of fructans (intercepts) on the morning of 6 July were twice those measured on 17 May, four times the concentrations on 8 August and about six times the 13 September concentrations (Table 4). Fructans comprised 0·50 of TNC across cultivars and time-of-day in the July harvest, and about 0·25 of TNC for the May, August and September harvests. Fructans are the major form of stored carbohydrate in many temperate C_3 grasses but may not always be associated with high sucrose concentrations (Chatterton *et al.*, 1989). They noted that accumulation of fructans during cool temperatures supports the hypothesis that fructans may accumulate in plants when carbon fixation exceeds plant translocation and utilization. Chatterton *et al.* (1989) suggested that there was a threshold value of about 150 g TNC kg^{-1} DM before significant increases in fructans would occur. This study suggests that the threshold on 13 September may be lower because the mean across cultivars was 121 g TNC kg^{-1}DM, yet the accumulation rate (Table 4) was 0·81 g fructan kg^{-1} DM h^{-1} ($P < 0·05$).

Air temperature was higher on 13 September than on 8 August (Table 1) but concentrations of fructans were lower in September. These results are in contrast to those of Housley and Volenec (1988) who found that low temperature and high photon flux density result in accumulation of fructans in tall fescue. Chatterton *et al.* (1988) proposed that biosynthesis of fructans in leaf vacuoles of 'Hycrest' crested wheatgrass (*Agropyron cristatum* × *A. desertorum*) provided a sink for photosynthates when temperatures were too cold for translocation and growth. The stored fructans then provided a readily available substrate for sucrose synthesis and plant growth. No diurnal accumulation was evident in leaf fructans (Sicher *et al.*, 1984).

Starch concentrations

Generally, starch concentrations are low in grasses of temperate origin (Chatterton *et al.*, 1987). Daily means for starch across the tall fescue cultivars were highest in May at 53 g kg^{-1} DM and lowest in September at 23 g kg^{-1} DM (Table 3). These results on vegetative stage tissue agree with findings that starch concentrations ranged from 20 to 90 g kg^{-1} DM in crested wheatgrass (Chatterton *et al.*, 1988) although starch concentrations in crested wheatgrass were lowest at physiological maturity. Chatterton *et al.* (1987) found starch to be even lower when either fructan or sucrose was present in significant amounts.

Carbohydrate accumulation rates

Sucrose and TNC concentrations followed a cubic (sigmoid) function for time of daylight when all eight sampling times were included in the model (data not shown). However, concentrations increased linearly through the six sampling times from 07:30 through 17:30 h (Table 4). The TNC and sucrose concentrations peaked between 16:00 and 18:00 hours, similar to what Lechtenberg *et al.* (1972) reported for tall fescue. Linear regression coefficients for carbohydrate concentrations as a function of time of day between 07:30 and 17:30 h are shown in Table 4. The intercepts for TNC concentrations on 17 May and 6 July were about twice those on 8 August and 13 September, but the rate of TNC accumulation (slope) was highest on 13 September. Intercepts for fructan concentrations were highest on 6 July and lowest on 13 September whereas accumulation rates (slopes) were low in May and September and not different from zero in July and August. Intercepts for sucrose, glucose and total starch concentrations were higher on 17 May than for the other months. The accumulation rates (slopes) for sucrose were much higher than glucose or total starch.

The TNC concentrations increased by 22·4, 16·8, 21·0 and 30·8 g kg^{-1} ADW from dawn to dusk on 17 May, 6 July, 8 August and 13 September respectively. Fisher *et al.* (1999) reported diurnal differences in TNC concentrations in HiMag tall fescue hay of 14·8 (20–22 August) and 25·0 g kg^{-1} ADW (20–21 September). Dry matter intake for cattle, sheep and goats was positively correlated with TNC concentration in HiMag tall fescue (Fisher *et al.*, 1999). Thus, there is evidence to expect responses in the productivity of ruminants to the diurnal TNC concentrations measured in this study.

Conclusions

The accumulation rates did not generally differ among cultivars but concentrations and morning low points (linear intercepts) may differ depending on variety. Time of sampling is critical to accurate assessment of forage quality because of the diurnal variation in carbohydrate concentrations in forage. Carbohydrate concentrations are dynamic because of cultivar, time of day and defoliation or season of harvest. Comparisons of TNC concentrations should only be made at the same time of day within 1 h, under the same environmental conditions, and for plants at the same physiological

state. Division of the TNC accumulation rate by the intercept estimates that forage samples taken for carbohydrate analyses should be taken within 1 h to control the diurnal variation of TNC proportionately within 0·02 in May or 0·05 in September. Alternatively, the proper use of experimental designs such as the use of time as blocks or as a covariate in analyses may account for the effects of differences of time. Tall fescue should be cut between noon and sundown for TNC concentration to be greater than the daily mean. Forage breeders should consider TNC as another selection criterion because of its apparent relationship to preference and production in ruminants.

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