Grassland at the heart of circular and sustainable food systems

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Changes in sugar concentration over the day and the season in green forages

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Abstract

The aim was to investigate the variation in sugar concentration over the day in green forages in two seasons. Plots with perennial ryegrass, timothy, tall fescue, meadow fescue, orchard grass and lucerne were established in spring 2020, at Foulum, Denmark. First regrowth was sampled in August and the second regrowth was sampled in October, three times over the day (morning, noon and evening) on three days. All samples (n=126) were analysed for glucose, fructose, sucrose and fructans, and the sum were set as total water soluble carbohydrates (WSC). Across species, WSC concentration was lower in October than in August (74.3 vs 120 g kg^{-1} of dry matter (DM)). Generally, lucerne and orchard grass had lowest WSC concentrations, whereas perennial ryegrass had the highest. Increase in sucrose concentration was the main driver for increase in aboveground plant WSC concentration over the day.

Keywords: grass, lucerne, water soluble carbohydrates, diurnal variation, animal nutrition

Introduction

All plants produce simple sugars during photosynthesis, a process activated by solar radiation. The photosynthetic cells use a part of the sugar for respiration, but sugar in surplus is transported to parts of the plants with high energy demand or for storage for later use. The transport of sugar in the plant is primarily in the form of sucrose, which is not a reducing sugar. In many plant species, the excess energy is stored as starch, but in cool-season C_3 grasses, energy is stored as fructans. As part of growth and maturation of the plants, the sugars are also incorporated into structural carbohydrates, a process which is accelerated by increased temperature. Therefore, many factors such as temperature, radiation, maturation, day length and species affect the composition of carbohydrates in plants and will contribute to variation over day and season. Sufficient sugar is essential for a good preservation when making silage for cows, but within equine nutrition, sugars, especially fructans, in grass have been associated with an increased incidence of laminitis (Van Eps and Pollitt, 2006). The aim of this study was therefore to investigate the variation in water soluble carbohydrate (WSC) concentration in different green forages over the day in two seasons under Danish conditions.

Materials and methods

As part of a larger study, 60 plots (effective plot size: 27 m^2) with different grass and legume species were established on 22 April 2020 at AU Foulum (56°29'N, 9°35'E), Tjele, Denmark. Seven of these plots were selected for the current study, including perennial ryegrass (*Lolium perenne* L.) both a diploid (var. 'Bovini', DPR) and a tetraploid (var. 'Sherlock', TPR) variety, timothy (*Phleum pratense* L., var. 'Radde', TIM), tall fescue (*Festuca arundinacea* Schreb., var. 'Ferguson', TF), meadow fescue (*Festuca pratensis* Huds., var. 'Schwetra', MF), orchard grass (*Dactylis glomerata* L., var. 'Kobako', OG) and lucerne (*Medicago sativa* L., var. 'Cigale', LUC). The primary growth, except for LUC, was harvested and removed on 19 June. Due to a poor and uneven establishment, the plots with TIM, MF and OG were reseeded on 24 June. In August, at three days (6, 10 and 13 Aug.), samples (~500 g) from the plots were collected manually with hand-held scissors to a stubble height of 7 cm at 06:00, 12:00 and 18:00 hour each day. After the last sampling day in August, all plots were fully harvested, and the regrowth were sampled at three days in October (1, 5 and 8 Oct.) at 08:00, 12:00 and 16:00 hour each day. Immediately after aboveground plant sample collection, the samples were dried (60 °C, 48 h) after removal of any weed.

Dried samples (n=126) were milled to 0.5 mm and analysed for glucose, fructose, sucrose and fructans using a sequential enzymatic colorimetric method after extraction with a 0.1 M acetate buffer (Larsson and Bengtsson, 1983). The sum of the four analytes were considered as total WSC. Statistical analyses were performed in R (version 4.1.1) using a linear mixed model (*lmer*) including species (n=7), period (n=2), day (n=3) and time (n=3) as main effects as well as their two- and three-way interaction, except species × period × day. The three samples taken at the same plot within day were considered as repeated measurements with a compound symmetry covariance structure. *P*-values are presented in Table 1.

Results and discussion

Across species, WSC was lower in October than in August (Table 2; 74.3 vs 120 g kg^{-1} of DM across species). In both periods, TPR had highest and LUC had lowest WSC concentration. For LUC, WSC concentration did not differ between periods, whereas MF was the species that differed the most between periods (149 and 62.2 g WSC kg^{-1} of DM in August and October, respectively). LUC did not contain any fructans because legumes store excess energy as starch rather than fructans as is the case for the grasses. For the grasses, OG had a lower concentration of fructans, both absolute and relative to WSC (11% of WSC) compared to the others (>30% of WSC). In all species, sucrose concentration increased as expected (Pelletier *et al.*, 2010) from morning to evening (Table 3) with highest increase in absolute amount in TF (+24.1 g kg^{-1} of DM) and lowest increase in OG (+10.6 g kg^{-1} of DM). For TF, MF and LUC, there was only a numeric increase in glucose concentration over day, but also only a minor increase was observed for the other species. Even though the time between morning and evening was shorter in October than in August (-4 h due to reduced day length), the increase in glucose, fructose and sucrose over the day was higher in October than August (Table 4); no increase was observed for fructose in August. In both periods, sucrose concentration was the main driver for increase in aboveground plant WSC concentration over the day, as sucrose concentration increased by 58.7% in August (from 31.5 to 50.0 g kg^{-1} of DM) and by 90.6% in October (19.2 to 36.6 g kg^{-1} of DM). Additionally, concentration of fructans decreased over the day in August, but stayed stable over the day in October. In both periods, sampling day affected the increase in sucrose over the day ($P_{P\times T\times D}$ <0.01, Table 1) due to differences in solar radiation on the sampling days (Jørgensen, 2021).

Conclusions

The concentration of sugars in green forage depended on species and time of harvest, and was generally higher in August than in October. Sucrose was the main sugar component driving the increase in WSC concentration over the day, and sucrose increased more in October than in August from morning to evening.

Table 1. *P*-values for the effect of the variables species, period, day and time and their interactions from the model analysing concentrations of glucose, fructose, sucrose, fructans and total WSC[1] in green forages.

	P-values[2]										
	S	P	D	T	S×P	S×D	S×T	P×D	P×T	D×T	P×D×T
Glucose	< 0.01	< 0.01	0.04	< 0.01	< 0.01	0.59	0.10	0.89	0.07	< 0.01	0.23
Fructose	< 0.01	< 0.01	0.29	< 0.01	0.35	0.86	0.39	0.88	< 0.01	< 0.01	0.27
Sucrose	< 0.01	< 0.01	0.06	< 0.01	< 0.01	0.50	< 0.01	0.02	0.05	< 0.01	< 0.01
Fructans	< 0.01	< 0.01	0.03	0.38	< 0.01	0.13	0.37	0.03	0.03	0.03	0.07
Total WSC	< 0.01	< 0.01	0.16	< 0.01	< 0.01	0.21	0.57	0.27	0.02	< 0.01	0.01

[1] WSC = sum of glucose, fructose, sucrose and fructans.

[2] S = species, P = period, D = day, T = time. The remaining 3-way interactions were not significant.

Table 2. Concentrations of glucose, sucrose, fructans and total WSC (g kg^{-1} of dry matter) in regrowth above 7 cm of different species in August and October across sampling day and sampling time.[1,2]

		Species							
Analyte	**Period**	**DPR**	**TPR**	**TIM**	**TF**	**MF**	**OG**	**LUC**	**SEM**
Glucose	August	17.4$^{c, x}$	18.5$^{c, x}$	27.4$^{d, x}$	14.6$^{bc, x}$	15.1$^{bc, x}$	12.6$^{b, x}$	5.66$^{a, x}$	0.95
	October	12.3$^{b, y}$	11.5$^{b, y}$	9.22$^{b, y}$	9.24$^{b, y}$	8.00$^{ab, y}$	8.00$^{ab, y}$	4.19$^{a, x}$	
Sucrose	August	43.3$^{bc, x}$	54.7$^{d, x}$	35.6$^{ab, x}$	49.9$^{cd, x}$	44.6$^{bc, x}$	25.8$^{a, x}$	28.1$^{a, x}$	1.99
	October	24.9$^{ab, y}$	28.5$^{bc, y}$	25.9$^{abc, y}$	35.7$^{c, y}$	25.4$^{ab, y}$	17.4$^{a, y}$	27.7$^{bc, x}$	
Fructans	August	71.5$^{c, x}$	73.5$^{c, x}$	65.2$^{c, x}$	44.8$^{b, x}$	73.2$^{c, x}$	7.14$^{a, x}$	-2.38$^{a, x}$	3.08
	October	52.6$^{cd, y}$	56.7$^{d, y}$	19.1$^{b, y}$	40.2$^{c, x}$	18.1$^{b, y}$	4.73$^{ab, x}$	-2.31$^{a, x}$	
Total WSC	August	153$^{cd, x}$	170$^{d, x}$	141$^{bc, x}$	126$^{b, x}$	149$^{bcd, x}$	61.3$^{a, x}$	39.7$^{a, x}$	4.82
	October	106$^{c, y}$	114$^{c, y}$	62.4$^{b, y}$	98.2$^{c, y}$	62.2$^{b, y}$	41.1$^{ab, y}$	36.0$^{a, x}$	

[1] WSC = sum of glucose, fructose, sucrose and fructans; DPR = diploid perennial ryegrass; TPR = tetraploid perennial ryegrass, TIM = timothy, TF =tall fescue, MF = meadow fescue; OG = orchard grass; LUC = lucerne; SEM = standard error of the mean.

[2] Values within each row with different subscript letters (a-d) differ ($P<0.05$). Values within each column for each analyte with different subscript letters (x,y) differ ($P<0.05$).

Table 3. Concentrations of glucose and sucrose (g kg^{-1} of dry matter) in regrowth above 7 cm of different species at different time points during the day across sampling period and sampling day.

		Species							
Analyte	**Time**	**DPR**	**TPR**	**TIM**	**TF**	**MF**	**OG**	**LUC**	**SEM**
Glucose	Morning	13.0$^{cde, x}$	13.5$^{de, x}$	16.0$^{e, x}$	11.1$^{bcd, x}$	8.97$^{b, x}$	9.15$^{bc, x}$	3.87$^{a, x}$	0.85
	Noon	15.2$^{cd, xy}$	15.4$^{cd, xy}$	17.9$^{d, x}$	12.2$^{bc, x}$	13.4$^{bc, y}$	11.0$^{b, x}$	5.07$^{a, x}$	
	Evening	16.4$^{d, y}$	16.1$^{cd, y}$	21.0$^{e, y}$	12.5$^{bc, x}$	12.3$^{b, y}$	10.8$^{b, x}$	5.83$^{a, x}$	
Sucrose	Morning	27.6$^{cd, x}$	34.1$^{d, x}$	22.8$^{abc, x}$	31.3$^{d, x}$	26.2$^{bcd, x}$	16.5$^{a, x}$	18.9$^{ab, x}$	1.75
	Noon	32.9$^{bc, y}$	38.0$^{cd, x}$	27.6$^{ab, y}$	41.7$^{d, y}$	33.1$^{bc, y}$	21.2$^{a, y}$	26.1$^{ab, y}$	
	Evening	41.8$^{b, z}$	52.6$^{cd, y}$	41.9$^{b, z}$	55.5$^{d, z}$	45.7$^{bc, z}$	27.2$^{a, z}$	38.6$^{b, z}$	

[1] See footnotes of Table 2.

Table 4. Concentrations of glucose, fructose, sucrose, fructans and total WSC (g kg^{-1} of dry matter) in regrowth above 7 cm at different time points in August and October across species and sampling day.[1,2]

		Time of day			
Analyte	**Period**	**Morning**	**Noon**	**Evening**	**SEM**
Glucose	August	14.5$^{a, x}$	16.6$^{b, x}$	16.6$^{b, x}$	0.45
	October	7.04$^{a, y}$	9.19$^{b, y}$	10.6$^{c, y}$	
Fructose	August	15.2$^{a, x}$	16.3$^{a, x}$	15.8$^{a, x}$	0.40
	October	9.70$^{a, y}$	11.9$^{b, y}$	13.1$^{c, y}$	
Sucrose	August	31.5$^{a, x}$	39.4$^{b, x}$	50.0$^{c, x}$	0.94
	October	19.2$^{a, y}$	23.6$^{b, y}$	36.6$^{c, y}$	
Fructans	August	51.3$^{b, x}$	46.3$^{ab, x}$	45.1$^{a, x}$	1.73
	October	25.8$^{a, y}$	26.7$^{a, y}$	28.5$^{a, y}$	
Total WSC	August	113$^{a, x}$	119$^{a, x}$	128$^{b, x}$	2.47
	October	62.1$^{a, y}$	71.7$^{b, y}$	89.2$^{c, y}$	

[1] WSC = sum of glucose, fructose, sucrose and fructans; SEM = standard error of the mean.

[2] Values within each row with different subscript letters (a-d) differ ($P<0.05$). Values within each column for each analyte with different subscript letters (x,y) differ ($P<0.05$).

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