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Original Research

Seasonal and Diurnal Changes in Starch Content and Sugar Profiles of Bermudagrass in the Piedmont Region of the United States

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ABSTRACT

Seasonal and diurnal patterns of sugar accumulation in bermudagrass (*Cynodon dactylon*) pastures were monitored to evaluate risk factors for pasture-associated laminitis of ponies and horses. Bermudagrass was collected from four plots in the morning and afternoon on a weekly basis, from mid-July until late August. Tissue was air-dried to simulate hay, or frozen to retain the sugar profiles of fresh pasture. Samples were analyzed colorimetrically for total water-soluble and ethanol-soluble carbohydrates, and electrochemically for starch. In addition, sugars were separated and quantified by high-performance liquid chromatography coupled to pulsed amperometric detection. The dominant sugars in extracts were glucose, fructose, and sucrose. Some minor peaks, corresponding to tri- and tetrasaccharides, were also detected in some extracts. Starch increased over time in fresh and dried tissue, and concentrations varied diurnally in fresh, but not in dried tissue ($P = .021$). Sucrose in dried tissue decreased and then increased, with higher concentrations than in fresh tissue on all sampling dates ($P = .024$). Glucose and fructose exhibited diurnal variation on one and two dates, respectively ($P = .034$ and .0028, respectively). These results reveal trends in carbohydrate concentrations and profiles that may help to evaluate the likelihood of equine laminitis outbreaks on bermudagrass.

1. Introduction

Pasture-associated laminitis (PAL) of ponies and horses is reportedly linked to consumption of nonstructural carbohydrates (NSC), which include starch, simple sugars, and fructose polymers, or fructans [1]. Feeding a high dose of pure starch or fructan can induce laminitis, and horses grazing on pastures high in NSC content may ingest as much starch or fructan in a day as the amounts inducing laminitis in one dose [2]. Simple sugars may play a role in laminitis as well. Insulin resistance, which has been closely linked to PAL [3], has been shown to be exacerbated by fructose [4], and abnormal insulin responses have been found after feeding glucose and fructose to previously laminitic ponies [5].

Recommended practices for reducing the risk of PAL to laminitis-prone horses and ponies are to allow the animals to graze on grasses with a low water-soluble carbohydrate (WSC) content (simple sugars plus fructans), and to decrease WSC and starch intake [1]. Grasses with a low WSC content include warm-season (C_4) grasses, which do

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not accumulate fructan [6] and tend to contain lower concentrations of soluble carbohydrates than do C_3 grasses [7]. Bermudagrass (*Cynodon dactylon*; family: Poaceae; subfamily: Chloridoideae [8]) is a warm-season grass frequently used as a forage in warm climates [9]. However, because the starch and WSC content of bermudagrass vary in response to temperature and the growth stage of the grass [7,10], quantifying those values over a growing season may help to determine whether bermudagrass is indeed safe for grazing at all times.

Previous studies of bermudagrass carbohydrates have included colorimetric analysis of ethanol-soluble carbohydrates (ESC) and WSC [7]. ESC consist of monosaccharides and short-chain sugars, whereas WSC include both short- and long-chain sugars, such as fructans [11]. Starch, along with ESC and WSC, has been measured colorimetrically in bermudagrass [10]. Gas chromatographic analysis of bermudagrass sugars, obtained from ambient-temperature ethanol extractions followed by boiling water extractions, has identified glucose, fructose, galactose, and sucrose [7]. However, these gas chromatographic analyses of bermudagrass were not conducted over multiple sampling periods, and sucrose data were not provided. In addition, sugar contents in the morning and evening were not studied, although those can differ greatly in some grass species [12,13].

High-performance liquid chromatography (HPLC) coupled to pulsed amperometric detection (PAD) can separate and detect picomole amounts of sugars without previous derivatization [14]. This technique has been used to profile carbohydrates in various grass species [15], and HPLC with refractive index detection has been used as well [16]. To our knowledge, no HPLC-based analysis of bermudagrass carbohydrate profiles, looking at both seasonal and diurnal changes, has been reported. In addition, the studies of McKell et al. [10] and Wilson and Ford [7] examined the carbohydrate content of bermudagrass that was frozen or freeze-dried promptly, thereby retaining a profile closely resembling fresh herbage. However, carbohydrate profiles of bermudagrass pasture and hay were not compared. The purpose of this study was to profile and quantify the nonstructural carbohydrates of both fresh and air-dried bermudagrass herbage, sampling in the morning and the afternoon over a 7-week period.

2. Materials and Methods

2.1. Plant Material

An established stand of "Tifton 44" hybrid bermudagrass located at Virginia Tech's Southern Piedmont Agricultural Research and Extension Center, Blackstone, VA, was used for this experiment. The experimental design was a randomized complete block design with four replications. Plot size was 4.6×1.8 m^2. At the start of the experiment, plots were clipped to a residual height of 7.5 cm and fertilized with 112 kg nitrogen, 59.8 kg phosphorous, and 124 kg potassium ha^{-1}, and allowed to regrow for 14 days. Tissue was collected every seven days starting on July 16, 2007 and ending on August 27, 2007, during both morning (8-10 AM) and afternoon (4-5 PM), by clipping grass to a residual height of 7.5 cm from four randomly selected areas within each plot. The tissue samples from the four areas within a plot were combined. A subsample of the pooled tissue was frozen immediately in a −70°C freezer, whereas the rest was allowed to air-dry in a greenhouse for three days to simulate, as best as possible in a place sheltered from rain, the gradual water stress and cell death that occur during air-drying of herbage for hay. The air-dried tissue was then oven-dried for three days at 60°C to remove residual moisture, ground to pass through a 1-mm mesh, and stored at room temperature. The greenhouse did not have climate controls, apart from fans operating at or above 29°C. Very little change in tissue weights occurred between the end of the drying period in the greenhouse and the final oven drying, thus indicating that the tissue was almost completely dried after 72 hours of air-drying (data not shown).

2.2. Weather Data

Daily results for minimum and maximum temperatures and photosynthetically active radiation were obtained from the weather station of the Southern Piedmont Agricultural Research and Extension Center, Blackstone, VA (37° 05′ N and 77° 57′ W, at an elevation of ∼425″). This weather station was located about 15 m from the field plots.

2.3. Colorimetric Analysis of WSC and ESC

Half of each sample was extracted and analyzed by Equi-Analytical Laboratories (Ithaca, NY) for WSC (simple sugars plus fructans) and ESC (simple sugars), using potassium ferricyanide assays to quantify WSC and phenol-sulfuric acid assays to quantify ESC (http://www.dairyone.com/Forage/Procedures/default.htm).

2.4. Starch Analysis

Tissue samples sent to Equi-Analytical Laboratories were analyzed for starch by collecting the residue from a water extraction, autoclaving to solubilize the starch, and hydrolyzing starch to dextrose with glucoamylase enzyme. Dextrose was analyzed by amperometric detection in an YSI 2700 Biochemistry Analyzer (YSI Life Sciences, Yellow Springs, OH) (http://www.dairyone.com/Forage/Procedures/default.htm).

2.5. Reagents and Chemicals for HPLC Analysis

Sodium acetate (molecular biology grade), sucrose, glucose, and fructose were purchased from Sigma-Aldrich (St. Louis, MO). Orchardgrass fructan of a wide range of degrees of polymerization (DP), as well as 6-kestose and neokestose (DP = 3), and 1-nystose, bifurcose, and 6-nystose (DP = 4) were generously provided by Dr. Phil Harrison (USDA Agricultural Research Service, Forage and Range Research Lab, Logan, UT). Sodium hydroxide (NaOH) solution was purchased from Fisher (Thermo Fisher Scientific, Waltham, MA). HPLC solvents (0.1 M NaOH and 1 M sodium acetate in 0.1 M NaOH) were filtered through a 0.2-μm nylon filter and kept under helium.