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Chromatographic profiles of nonstructural carbohydrates contributing to the colorimetrically determined fructan, ethanol-soluble, and water-soluble carbohydrate contents of five grasses

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ABSTRACT

Accurate estimates of forage fructan and mono- and disaccharide content may help with feeding management decisions for horses with increased risk for pasture-associated laminitis. In this study, five forages expected to differ in soluble carbohydrate concentrations were extracted with water or 800 mL/L ethanol. Forages were *Festuca arundinacea* (tall fescue) with (E+) and without (E−) the common endophyte, cold-stressed and clipped, vegetative; *Poa pratensis* (Kentucky bluegrass), cold-stressed and clipped, vegetative; Dactylis glomerata (orchardgrass), air-dried, early reproductive stage; and *Cynodon dactylon* (bermudagrass), air-dried, 21 days of regrowth. Extracts were analyzed colorimetrically for water-soluble carbohydrates (WSC, mono- and disaccharides plus fructans) and ethanol-soluble carbohydrates (ESC, mono- and disaccharides). Soluble carbohydrates were separated and quantified by high-performance liquid chromatography (HPLC) with pulsed amperometric detection (PAD) to compare HPLC-based calculations of WSC, ESC, and fructan to the colorimetrically obtained concentrations. HPLC-PAD analysis of WSC extracts confirmed the presence of fructans in tall fescue and orchardgrass. The fructan content of orchardgrass and E− tall fescue did not differ between colorimetric and HPLC-PAD determinations (P=0.21 and 0.10, respectively), but E+ tall fescue fructan was about 36 g/kg DM higher in colorimetric than in HPLC determinations (P=0.0026). Colorimetric analysis of bermudagrass WSC and ESC indicated a low fructan content, whereas HPLC-PAD indicated a lack of quantifiable fructans. Colorimetrically determined WSC and ESC were greater than the corresponding chromatographically determined values. An exception was bluegrass, for which the colorimetric and chromatographic ESC content did not

Abbreviations: cv, cultivar; DM, dry matter; DP, degree of polymerization; E+, endophyte-infected; E−, endophyte-free; ESC, ethanol-soluble carbohydrate; HPLC-PAD, high-performance liquid chromatography with pulsed amperometric detection; SPE, solid-phase extraction; WSC, water-soluble carbohydrate.

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differ, although both were greater than the chromatographically determined mono- and disaccharide content of water extracts. The results suggest that forage soluble carbohydrate concentrations tend to be higher in the colorimetric assays used than in HPLC-PAD determinations. However, HPLC-PAD analysis of unhydrolyzed water extracts may suffice for quantifying both ESC and WSC, besides providing information on individual sugars.

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1. Introduction

Increased risk for pasture-associated laminitis (PAL), in horses and ponies that are insulin-resistant or have a history of laminitis, has been associated with intake of forage high in nonstructural carbohydrates (NSC), which include starch, mono- and disaccharides (glucose, fructose, and sucrose), and fructans (Longland and Byrd, 2006). Fructans are fructose polymers of variable lengths, with a terminal sucrose (Pollock, 1986). Fructans are thought to increase the risk of PAL by causing disturbances to the hindgut microflora and/or exacerbating insulin resistance (Geor, 2010; Kronfeld et al., 2004). Consequently, knowledge of the NSC content of a pasture or hay may be helpful in making feeding management decisions for these animals.

Laboratory analysis of herbage, a service available from commercial testing labs, is sometimes used by horse owners, nutritionists, and veterinarians to determine NSC content. Analysis of NSC components often relies on their different solubilities in water and ethanol. Mono- and disaccharides and fructans with a wide range of chain lengths, or degrees of polymerization (DP), are all water-soluble, while only mono- and disaccharides and fructans of relatively low DP are ethanol-soluble, a trait sometimes utilized to isolate high-DP fructans (Chatterton et al., 1993a).

Concentrations of carbohydrates in water and ethanol extracts can be determined by colorimetric assays (Lechtenburg et al., 1972; Wilson and Ford, 1973), enzymatic assays coupled to colorimetry (Ciavarella et al., 2000; Udén, 2006; Zhao et al., 2010; Longland et al., 2012), titration (Waite and Boyd, 1953), or chromatography (Lechtenburg et al., 1972; Wilson and Ford, 1973; Longland et al., 2012). The difference between concentrations of water-soluble carbohydrate (WSC) and ethanol-soluble carbohydrate (ESC) provides an estimate of the amount of fructan present (Kronfeld et al., 2004). Fructans also can be measured directly by high-performance liquid chromatography (HPLC) with pulsed amperometric detection (PAD). This method does not require sample derivatization and can detect picomole amounts of sugars (Hardy and Townsend, 1988).

Colorimetry and HPLC, two types of methods commonly used to quantify soluble carbohydrates of grasses, each have disadvantages. Various factors may hinder accurate colorimetric determination of WSC and ESC, and hence of fructan. The maximum DP of fructans present in an ethanol extract varies among forage species, and with the concentration of ethanol used in forage extraction (Grotelueschen and Smith, 1968). For example, although 800 mL/L ethanol extracts of cool-season grasses may be assumed to recover primarily mono- and disaccharides (Kronfeld et al., 2004; Waite and Boyd, 1953), a timothy (*Phleum pratense*) extract prepared with 800 mL/L ethanol may contain fructans with a DP as high as 20 (Grotelueschen and Smith, 1968). In such cases, the difference between WSC and ESC may represent only the fructans of the greatest molecular weight. Because fructans of low molecular weight are sometimes fermented more rapidly than those of high molecular weight (Ince et al., 2005; Perrin et al., 2002), a value representing only high-molecular weight fructans may underestimate the concentration of fructans contributing to laminitis. An under- or overestimation could also occur if recovery of mono- and disaccharides differed between water and ethanol extracts.

HPLC-PAD provides detailed information on forage carbohydrates but is more time-consuming and requires more expensive equipment than the colorimetric methods. An additional challenge of HPLC-PAD is the comparison of data on individual sugars to data from prior studies in the literature. Such comparisons help to determine if results are within the range of those obtained by others. However, if results from a prior study are expressed in terms of total WSC or ESC content, HPLC data are probably best compared to the prior study by converting into similar terms. The sum of chromatographically quantified mono- and disaccharides and fructan from a water extract would be expected to approximate colorimetrically determined total WSC content, but colorimetric assays may detect other compounds besides the ones that are intended to be measured (Avigad, 1968). Similarly, the sum of chromatographically quantified mono- and disaccharides would be expected to approximate colorimetrically determined ESC, but discrepancies may arise if the colorimetric assay detects other compounds besides those expected to be measured.

Previous studies in our laboratories (Kagan et al., 2011b,c) examined colorimetric data obtained from a commercial forage testing laboratory, as well as HPLC profiles of the same samples. Colorimetric data appeared to approximate the sums of chromatographically quantified sugars, but because our extraction protocol differed from those of the commercial laboratory, it was not possible to determine if differences in results were due to differences in the analytical methods. The objectives of this study were to analyze a single extract by colorimetry and HPLC profiling, and to determine (1) the relative amounts of monosaccharides, disaccharides, and fructan extracted by water and 800 mL/L ethanol, and (2) extent of similarity between calculations from colorimetric and HPLC data.