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Selection of an empirical detection method for determination of water-soluble carbohydrates in feedstuffs for application in ruminant nutrition☆

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ABSTRACT

Water-soluble carbohydrates (WSC) are commercially measured in feedstuffs for use in diet formulation for ruminants. However, we lack information as to which empirical detection assay most correctly measures WSC. The objective of this study was to determine which of two commonly used empirical assays was most appropriate for detection of WSC based on equivalency to results from high performance ion chromatography with pulsed amperometric detection plus soluble starch analysis (HPIC) of the water extract. Empirical analyses used were a reducing sugar assay (RSA) that uses *p*-hydroxybenzoic acid hydrazide-based reagent with 50:50 glucose:fructose standards, and the phenol-sulfuric acid assay (PSA) with sucrose standards. Twenty samples including cool season grasses (CSG), legume forages, non-forage feedstuffs, silages, or warm season grasses were used. Air dry samples (0.2 g) were extracted in 35 mL of deionized water for 1 h at 40 °C with continuous shaking. Water extracts for HPIC and RSA analyses were hydrolyzed with 0.037 M H_2SO_4 at 80 °C for 70 min. Theoretically, RSA should give essentially the same results as HPIC, excepting that RSA also detects reducing ends of unhydrolyzed molecules. PSA detects all solubilized or suspended carbohydrates. On average, RSA and PSA values were greater than those found for HPIC by 28.2 g WSC/kg dry matter (DM). The two classes of feeds that showed differences between PSA and RSA were CSG and silages. For CSG, RSA and PSA were respectively 54.1 and 20.6 g WSC/kg DM greater than HPIC; for silages differences were smaller at 8.8 and 15.9 g WSC/kg DM. CSG contain fructans, for which RSA gives higher values than does PSA. However, the elevated RSA values for CSG were in excess of differences predicted based on inflated RSA recovery values for fructose measurement (106.5% of actual). Elevated RSA values obtained for CSG suggest that interference is affecting these grasses to a greater degree than other samples. Distillers grains showed an elevated value with PSA (69.1 g WSC/kg DM greater than HPIC); this is partially explained by the inflated recovery values for glucose (128.2% of actual) noted for PSA. Neither PSA nor RSA perfectly reflected HPIC values, however PSA gave more similar values. Gross differences between RSA and HPIC for CSG are an

Abbreviations: CSG, cool season grasses; DM, dry matter; ESC, 80% ethanol-soluble carbohydrates; ESE, 80% ethanol-soluble extract; HPIC, high performance ion chromatography; PSA, phenol-sulfuric acid assay; RSA, reducing sugar assay; SED, standard error of the difference; WSC, water-soluble carbohydrates; WSE, water-soluble extract; WSEhy, acid hydrolyzed water-soluble extract; WSEun, water soluble extract without acid hydrolysis.

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issue, particularly without clear, resolvable basis for the discrepancy. Accordingly, PSA is preferred over RSA for detection of WSC. Selection of standards to more closely reflect WSC composition could further improve accuracy.

1. Introduction

There has been increased interest in commercial analysis of feedstuffs for soluble carbohydrates as a way of parsing readily fermentable carbohydrates for use in diet formulations for ruminants. However, there has been a lack of information as to which extraction method and which detection method should be used for this purpose. Methods commonly used by commercial laboratories for determinations of sugars and non-starch, nonstructural carbohydrates in feeds include the relatively simple water-soluble (WSC) or 80% ethanol-soluble (ESC) carbohydrate assays. Both use empirical detection methods that allow high throughput of samples. Extraction with 80% ethanol solubilizes carbohydrates with a lower molecular weight than those extractable by water, but may contain oligosaccharides up to 20 monosaccharide residues in length (Asp, 1993), which includes short chain length fructans (Wylam, 1954). The WSC include the carbohydrates found in ESC as well as soluble polysaccharides such as longer chain length fructans. Lactose is sparingly soluble in 80% ethanol, but is soluble in water.

For diet formulation purposes, the choice of using ESC or WSC must rely on the nutritional relevance of the assay. Monosaccharides (mostly glucose and fructose), sucrose, galactooligosaccharides (raffinose and stachyose), and fructan may be fermented by rumen microbes, may yield lactate from that fermentation (Thomas, 1960; Cullen et al., 1986) or be converted to glycogen by rumen microbes (Thomas, 1960; Prins and Van Hoven, 1977; confirmed citations for all but stachyose), though they may differ in rates of fermentation (Thomas, 1960). Further, amounts of these same carbohydrates in fresh forage are used to determine adequacy of fermentable carbohydrate to achieve sufficient acid production to preserve silage (Buxton and O'Kiely, 2003). On the basis of similar microbial action, it seems that these carbohydrates could be grouped into a single feed fraction. To describe this fraction, use of WSC is preferable to ESC because water wholly extracts fructans as well as the lower molecular weight carbohydrates, whereas 80% ethanol incompletely extracts fructans, yet extracts more than just mono- and disaccharides. As yet, there appears to be no ready way to quickly, inexpensively, and accurately analyze for fructans in cool season grasses to separate them from other WSC (Longland et al., 2012).

For analysis of WSC, there remains the question of which carbohydrate detection method to use. Commercial laboratories have commonly used reducing sugar assays (RSA) and the phenol-sulfuric acid assay (PSA) (DuBois et al., 1956) for detection of soluble carbohydrates. However, the methods have some limitations. They all vary in their recovery, or amount detected/actual amount present, of different carbohydrates. This has been shown for PSA (DuBois et al., 1956) as well as for reducing sugar methods using ferricyanide, copper reduction, and *p*-hydroxybenzoic acid hydrazide (Shaffer and Somogyi, 1933; Weinbach and Calvin, 1935; Lever, 1973). In the case of the RSA, the methods consistently show different recoveries of different monosaccharides (e.g., if glucose recovery = 1.00, recoveries of fructose = 1.05, and galactose = 0.75; Weinbach and Calvin, 1935) irrespective of what carbohydrate standard is used. Recovery of WSC other than that used as the standard is also affected by the carbohydrate standard used (Hall, 2013). The PSA and RSA also give different measures of different carbohydrates, with results differing substantially between them for measurement of fructose and inulin, even when equivalent carbohydrate standards were used (Hall, 2013). The requirement for RSA that carbohydrates be hydrolyzed to monosaccharides can also affect results of that analysis. Typically, a relatively gentle hydrolysis method is applied to hydrolyze sucrose, often the predominant soluble carbohydrate, and not destroy the released fructose (e.g., Bach Knudsen, 1997). However, such hydrolysis conditions are not sufficient to completely hydrolyze a variety of carbohydrates including soluble starch, and galactooligosaccharides such as raffinose, though the terminal fructose of that molecule is cleaved (Browne, 1912). Incomplete hydrolysis of oligo- or polysaccharides poses a problem for complete detection of such carbohydrates with RSA, though reducing ends of the larger carbohydrates are detected. Additionally, protein and minerals, or any compound that has reducing properties has potential to interfere with RSA to varying degrees that can differ by assay (Van Der Plank, 1936; Lever, 1973). Presently, WSC methods with RSA detection are being performed in commercial laboratories without use of agents such as lead acetate, which were originally recommended to precipitate and remove interfering substances (Van Der Plank, 1936) but are environmentally hazardous chemicals.

As presently practiced, there is uncertainty as to how well the empirical detection methods quantify the amount of WSC present in feeds. Previous work with purified carbohydrates showed RSA to give better recoveries than PSA (Hall, 2013), but the purified samples lacked the complex composition and potential interfering compounds of actual feeds. The objectives of the present study were to compare the measurement of WSC determined using empirical RSA and PSA detection methods as compared to high performance anion-exchange chromatography (HPIC) combined with analyses for non-hydrolyzable carbohydrates, and to verify the composition of feeds for ESC and WSC using HPIC. The empirical detection methods used were selected based on their current use in commercial feed analysis laboratories (PSA; DuBois et al., 1956) or their reported reduced sensitivity to interference and AOAC method status (Lever, 1973; Official Method 999.03, AOAC, 2012). The feed samples were selected to give a wide diversity of plant WSC amounts and types (e.g., monosaccharides, sucrose, galactooligosaccharides, fructans, etc.). We hypothesized that the detection methods would differ in the degree to which they agreed with the HPIC measurements, and that would vary by sample type due to the varying composition of extractable

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