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Methods for reduction of water soluble carbohydrate content in grass forages for horses

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ABSTRACT

A large number of factors influence water soluble carbohydrate (WSC) concentration in forages. Four of these were studied; effect of conservation method (silage, haylage, hay); effect of using bacterial inoculants in silage and haylage; effect of storage time; and effect of soaking. Grass forage was harvested as silage (400 g dry matter (DM)/kg) and haylage (600 g DM/kg) in laboratory silos and as hay (840 g DM/kg). Silage and haylage were preserved with and without a bacterial inoculant. All forages were sampled at 3, 6, 12 and 18 months of storage. Forages stored for 3 and 12 months were soaked in water for 12 and 24 h to evaluate soaking as a method to reduce WSC concentration. Concentrations of glucose, fructose, sucrose and fructans were analysed using an enzymatic-spectrophotometric method and total WSC concentration calculated as the sum of these. Conservation method influenced concentration of WSC and its components, as silage had lower content of glucose, fructose and WSC compared to haylage and hay; and silage and haylage contained less sucrose and fructans compared to hay ($P<0.001$). Use of inoculants in haylage and silage resulted in lower fructose concentration in silage ($P=0.03$) but not in haylage. No consistent effects of storage time on concentration of WSC and its components were present. Soaking for 12 h reduced concentration of fructose and WSC in silage to approximately half of the initial concentration before soaking ($P=0.001$). For haylage, soaking for 12 h resulted in approximately half of the concentration of initial glucose, fructose and WSC ($P<0.001$), but no further reduction in these components was present after 24 h soaking time. For hay, soaking for 12 h resulted in 50% of glucose, 70% of fructose, 15% of sucrose and 40% of fructan concentrations compared to initial contents ($P<0.02$). Soaking hay for 24 h resulted in further reduction of glucose and WSC concentrations ($P<0.001$). Concentration of WSC was lower in silage compared to haylage and hay due to the utilization of WSC components by lactic acid bacteria during ensiling. Concentration of WSC in silage before soaking (24 g/kg DM) was also lower than in hay after soaking for 24 h (38 g/kg DM), meaning thatpreserving forage as silage was more effective in reducing WSC concentration than soaking of hay. If the goal is to produce forage with low WSC concentration, preservation as silage should be preferred over hay-making.

1. Introduction

Horses diagnosed with equine metabolic syndrome (EMS), laminitis, decreased insulin sensitivity (insulin resistance, IR) (Frank et al., 2010), pituitary pars intermedia dysfunction (PPID) (McGowan, 2008), or tying-up due to polysaccharide storage myopathy (PSSM) (Firshman et al., 2003), have been reported to benefit from a diet low in non-structural carbohydrates (NSC). Among feedstuffs commonly included in the horse diet, grains and concentrates are higher in NSC compared to forages (NRC, 2007). Exclusion of these from the diet is generally not a problem for the majority of horses, as forage can cover the requirements of energy and protein for both idle and high-performing horses (Jansson and Lindberg, 2012; Ringmark et al., 2013). However, forages may also be rich in NSC, but cannot be excluded from the horse diet without increasing the risk of digestive disorders such as colic (*e.g.* Archer and Proudman, 2006) and gastric ulcers (*e.g.* Coenen, 1990) or development of stereotypic behaviour (*e.g.* McGreevy et al., 1995). It is therefore important to select forages with low concentration of NSC for horses suffering from EMS, IR, PPID or PSSM. Frank et al. (2010) suggested that horses with IR should be fed hay with a NSC concentration lower than 10% calculated on a dry matter (DM) basis.

The content of NSC in forages is affected by several different factors during growth, harvest, storage and feeding. A common recommendation for reduction of water soluble carbohydrate

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(WSC, including glucose, fructose, sucrose and fructans) concentration in hay is to soak it in water for 60 min before feeding (Frank et al., 2010). However, soaking hay has been reported to result in variable WSC reduction (Longland et al., 2011) or no WSC reduction when hay was soaked for 10–30 min (Blackman and Moore-Colyer, 1998). Increased soaking time (from 15 min to 12 h) has however been reported to result in larger NSC-reduction in orchardgrass (*Dactylis glomerata L.*) hay cut in flowering stage (Martinson et al., 2012b). Soaking of hay may also result in loss of other important nutrients such as Na, P, K, Mg (Moore-Colyer, 1996), Ca (Martinson et al., 2012 a) and crude protein (CP) or other N-compounds (Warr and Petch, 1993; Martinson et al., 2012a), and Cu (Blackman and Moore-Colyer, 1998). The effect of soaking on WSC concentration in other forage types than hay has not been reported in scientific literature.

A recommendation commonly given to manage laminitic, IR and/or EMS horses is to use hay from last years' harvest. This is based on the assumption that increased storage time results in decreased WSC concentrations. Scientific support for this recommendation is scarce, although Archibald et al. (1951) showed that sugar concentration of hay decreased by 19% after 192 days of storage. However, the largest loss in sugar concentration took place during the first week of storage after harvest. After one month of storage, the sugar content was stable for the remaining period (Archibald et al., 1951). Whether further loss of WSC concentration in hay occurs after 192 days of storage has not been reported.

Another factor with the capacity to reduce WSC concentration in forages is conserving the forage as silage or haylage. During ensiling, sugars in the crop are fermented by epiphytic lactic acid bacteria (LAB) to lactic (and sometimes also acetic) acid (Gibbs et al., 1950). Fermentation of WSC components during ensiling may be further enhanced by adding bacterial inoculants to the crop at harvest (*e.g.* Müller, 2005). However, intensity of ensiling, and thereby WSC reduction, is greatly influenced by DM content of the crop. High DM content decrease the extent and intensity of fermentation in both naturally fermented and inoculated silages, and may result in residual WSC concentration being higher in drier haylages compared to wetter silages (Müller, 2005; Han et al., 2006).

The objective of this study was to investigate (a) the effect of conservation method (silage, haylage, hay), (b) use of inoculants in silage and haylage, (c) soaking of forages (silage, haylage, hay) in water, and (d) storage time on the concentration of total WSC and its components glucose, fructose, sucrose and fructans in forages produced from the same grass crop.

2. Material and methods

2.1. General

An experiment was performed using laboratory silos (stainless steel, 25 L volume) for silage and haylage conservation, and a conventional high-density hay baler for hay production. A grass-dominated sward in its fourth year, consisting of 0.5 timothy (*Phleum pratense*), 0.4 meadow fescue (*Festuca pratensis*) and 0.1 red clover (*Trifolium pratense*), was harvested on the 15th of June in 2010 in Uppsala, Sweden (59°86′N, 17°64′E, elevation 20 m above sea level, clay-dominated soil type, humid continental climate with average yearly precipitation of 576 mm and average yearly temperature 6.5 °C). The grass was cut using a mower conditioner with flails (Kverneland Taarup 4028, Kverneland, Nyköping, Sweden) and left in rows of 2-m width in the field during wilting. Dry matter (DM) content of the grass during wilting was determined by taking samples approximately every third hour during daytime (starting at 06.00 and ending at 21.00), and drying them in a microwave oven for 8–10 min (750 W) until no further weight loss. At an approximate DM content of 400 and 600 g/kg, the crop was collected and transported in 150 L plastic bags from the field to the experimental station (three min transport time), for conservation as silage and haylage, respectively. When the crop had reached an approximate DM content of 800 g/kg, hay was baled. Before collection or baling of the crop started, new samples of the wilted herbage were taken from nine random spots in the field.. The samples were stored at −18 °C before analysis of chemical composition.

Half of the crop for silage and haylage was used as control (24 silos for each forage type), and the other half was used for additive treatment (24 silos for each forage type). The additive consisted of four strains of LAB: *Lactobacillus plantarum* Milab 393 (30%), *Pediococcus acidilacti* (30%), *Enterococcus faecium* (30%) and *Lactococcus lactis* (10%) (Feedtech Silage F3000, DeLaval Sales AB, Tumba, Sweden). Dosing was 0.003 g dry additive/kg wilted grass, giving log 5.7 CFU bacteria/g grass. The additive was dissolved in water before application (0.75 g additive per L water). Inoculation was performed in the wilted plant material just before it was filled in laboratory silos. The additive treatment was performed for an individual mass of wilted grass intended for an individual silo, thus making each silo an experimental unit. Each individual herbage mass heap was sprayed with the additive using a hand held spray pump, and mixed with a hand-fork during spraying. The grass in the silos was compacted using a stationary hydraulic press, and the same filling–compacting–weighing procedure was followed for each silo. For silage and haylage 8 and 6 kg of herbage, respectively, were placed into the silos, which corresponded to approximately 3.5 kg DM. The silos were sealed with lids, using silicone paste and plastic stretch film. Each lid was equipped with a water-filled gas lock to allow gas out of the silo. After completing the sealing procedure, silos were kept in room temperature (16–18 °C) for a total period of 18 months. Hay was baled using a high-density hay baler (Welger AP 730, Lely Maschinenfabrik GmbH, Wolfenbüttel, Germany) producing rectangular bales sized approximately $70 \times 38 \times 46$ cm^3. Bales were put on a barn-drier and dried with cold air until the DM content was 840 g/kg and the water activity (a_w) less than 0.70 (measured at constant room temperature 21 °C using a $BaCl_2 \cdot 2H_2O$-calibrated Lufft Duratherm Kontroll hygrometer 5804, Germany). Thereafter, the hay was covered with bales of straw, to protect the hay and avoid moisture uptake from the surrounding air during storage. The total storage period was up to 18 months, and temperature in the hay storage room followed the ambient temperature.

Silos and hay-bales were opened and sampled after 3, 6, 12 and 18 months of storage. At every sampling occasion, six hay bales, six silos of silage and six silos of haylage were randomly selected, opened and destructively sampled. Samples taken at opening of silos and bales after 3 and 12 months of storage were used for chemical analysis. After sampling the remaining forage was soaked in 17 L of water/kg DM in the 25 L steel silos. The forages were completely submerged in water, and water temperature was 5–7 °C (cold tap water). The forages were soaked for up to 24 h, and the water temperature at the end of the soaking period was 14–16 °C (approximately 2 degrees below room temperature). After 12 and 24 h of soaking, samples were taken for analysis of WSC concentration and its components and for DM content. Sampling was performed using a stainless steel core sampler (1 m × 40 mm in diameter, Medeltida smide, Almunge, Sweden) connected to an electrical drilling machine (DeWalt DW006, Tampa, USA). The core sampler was sterilized between each silo using ethanol (0.96) and an open flame. Samples taken after 6 and 18 months of storage were analysed for WSC concentration and components as well as DM content, but no soaking procedure was performed.