Hydrolyzable and fermentable carbohydrates in North Italian pastures for horses

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Abstract

Digestive and metabolic disorders in the horse were previously associated with hydrolyzable and nonhydrolyzable but rapidly fermentable components of nonstructural carbohydrates, particularly fructans. Aim of the study was to assess the contribution of the carbohydrate fractions to the composition of pastures in specific areas of Italy ordinarily designed for horses feeding. Seventy-seven samples from 11 pastures differing for floristic composition were collected across two growing seasons from April 18th 2008 to May 10th 2009 in the foothills (380 m a.s.l.) of the province of Parma (Italy). All samples were collected during the vegetative growth phase. To identify the bioclimatologic conditions of the growth, the average fortnightly thermohygrometric index (THI) was used. Slow fermentable carbohydrates mean concentration (NDF: 510.6, ADF: 274.7, ADL: 31.5 g/kg DM) reflected the typical chemical composition of high-quality grass and grass-legume mixtures. Among the nonstructural carbohydrates (NSC: 199.9 g/kg DM), hydrolyzable carbohydrates content accounted for about the 18%. Nonhydrolyzable but rapidly fermentable carbohydrates were constituted for about the 23% by fructans (37.7 g/kg DM, range 15.5 to 138.2). The environmental conditions significantly affected the carbohydrate concentration in forages. Among NSC, fructans concentration in pastures was affected by bioclimatologic conditions (P<0.05). The floristic composition influenced the fructans content at a lesser extent. These results indicate a high variability of fructans concentration and reflect what previously observed in other areas. In any case the high levels observed for fructans in some geographic areas are not been achieved. Further interdisciplinary studies in this area between veterinarians and forage researchers are clearly warranted.

Introduction

Carbohydrates constitute the major dietary energy source for horse (Varloud et al. 2004). They are present in plants both as a structural component and as a nonstructural (NSC) but reserve component (Van Soest, 1963). Sugars produced by photosynthesis are used for energy production and as substrates to form other essential components including proteins and structural carbohydrates such as hemicellulose and cellulose. NSC are found in both intra- and intercellular space while structural carbohydrates are part of the plant cell wall (Watts, 2004). From the analytical point of view the NSC can be classified into hydrolyzable carbohydrates (CHO-H), including disaccharides, some oligosaccharides (e.g. maltooltriose) and starch, and nonhydrolyzable but rapidly fermentable carbohydrates (CHO-Fr) comprising oligosaccharides (e.g. fructans, galactans), gums, pectins, etc., and the amount of starch resistant to enzymatic hydrolysis (Hoffman et al., 2001).

In horse, CHO-H is mainly hydrolyzed in the small intestine and, if starch intake exceeds the digestive capacity, it is fermented in the hindgut, similarly to the CHO-Fr fraction (Potter et al., 1992). Fermentative process produces volatile fatty acids (VFA), mainly acetate, propionate, butyrate and to a lesser extent, lactate and valerate. However, the energy efficiency of dietary carbohydrate is increased when it originates from a hydrolytic process rather than from a fermentation, and it is higher when produced from the metabolism of glucose rather than from that of acetate (Kronfeld, 1996).

Previous studies suggest that digestive and metabolic disorders associated with the onset of colic (Hudson et al., 2001) and laminitis (Garner et al., 1975; Longland et al., 1999; Chatterton et al., 2006) may be related with abrupt changes in the diet of the content of hydrolyzable carbohydrates and/or nonhydrolyzable but rapidly fermentable carbohydrates.

In pasture, nonhydrolyzable but rapidly fermentable carbohydrates contents can widely vary due to interaction of plants and their environment (Buxton and Fales, 1994). Among CHO-Fr, fructans are involved in the onset of laminitis (Longland and Cairns, 2000; van Eps and Pollitt, 2006). Fructans are the main storage carbohydrates of temperate (cool season, C3 plants) pasture grasses; they are deposited in the growing tissues from sucrose by the action of specific enzymes (fructosyltransferases). In the vacuole fructans accumulation is not a self-limiting mechanism even to the point of covering more than 70% of the total dry matter content (Vijn and Smeekens, 1999).

In contrast, warm season grass (C4 plants) and legumes do not produce fructans but starch. Starch production occurs in the chloroplasts of leaf and storage is governed by their saturation.

Most plants store starch as reserve carbohydrate, but about 15% of all flowering plant species store fructans. Among the plants that store fructans there are many of significant economic importance, such as forage grasses (Lolium perenne, Festuca sp., Dactylis glomerata, Phleum sp., Bromus sp., etc.). Fructans concentration in pastures is influenced by several factors that act directly on the metabolism of the plant, such as environmental temperature, sunshine, stress conditions (water, salt concentrations of the soil, fertilizers, etc.). It is generally considered that in temperate climates the concentration of fructans in plants is higher in spring and autumn, when the daily temperature range is likely to affect the respiration of sugars and it is lower in winter and summer (Watts, 2004).

In relation to light, Longland and Byrd (2006) reported that the concentration of fructans in the vegetative tissues of different cultivars of Lolium perenne is equal to 160 g/kg DM early in the morning and that it reaches 240 g/kg of DM during the peak daylight. In low light conditions and warm temperatures the changes over the 24
hours are rather negligible.

Despite this, it has not yet been definitively demonstrated a close relationship between their intake and the occurrence of digestive and metabolic disorders in the horse (Kronfeld et al., 2005). Their occurrence may vary from season to season, location to location, and within a location during the day, field topography, and grazing method of individual animals (Harris et al., 2006). Safe concentration of fructans in pasture cannot be well established. While it is possible to experimentally induce metabolic diseases in a horse by administering a single dose of fructan, for grazing animals it is difficult to quantify the dangerous dose of intake even if pastures contain high fructans concentrations. Threshold levels determined under controlled conditions may be very different from what occurs naturally in an individual predisposed to the condition.

Horse typically graze for 50-70% of the day, and thus the fructans do not reach the gut as a bolus, but rather, they are trickle-fed throughout the grazing period (Longland and Byrd, 2006).

It is not known if continued ingestion of high concentrations of storage carbohydrates in grazing horse results in a chronic proliferation of lactate-producing bacteria and consequent lowering in hindgut pH, or if it must be considered a transient effect. In any case, the intestinal microbiota reacts by adapting to the changing concentrations of slowly fermentable carbohydrates in pastures without reaching a steady state conditions. In such a situation, the animals apparently healthy but with an individual predisposition will more easily develop dismetabolic frameworks.

The aim of the study was to assess the contributions of the carbohydrates fractions to the composition of pastures in specific areas of Italy ordinarily intended for horses feeding.

### Materials and methods

The survey involved 11 pastures of the foothills (380 m a.s.l.) of the province of Parma (Italy). Seventy-seven pasture samples were collected across two growing seasons from April 18th 2008 to May 10th 2009. To identify the bioclimatologic conditions of the growth the thermohygrometric index (THI) was used, calculated from the values of temperature (°C) and relative humidity (%) (Unger, 1999) taken calculated from the values of temperature (°C) and relative humidity (%) (Unger, 1999) taken into 4 classes: low: <10.0; medium-low: from 10.1 to 15.0; medium-high: from 15.1 to 20.0; high: >20.0. In relation to floristic composition the sowed or natural pastures have been grouped in: i) site 1: pastures 1, 3, 8, 9 sowed with Festuca arundinacea, Dactylis glomerata and Bromus inermis; ii) site 2: pasture 2 sowed with Lolium perenne; iii) site 3: pasture 4 sowed with Medicago sativa; iv) site 4: pasture 5, natural meadow grass; v) site 5: pastures 6, 7, 10, 11 sowed with grass-legume mixtures (Poa trivialis, Bromus inermis, Festuca ovina, Lolium perenne, Vicia sativa, Trifolium pratense).

In each pasture three replications (0.6 m² areas) were identified. Samplings were taken during the vegetative phase (pre-blooming), at about 4-5 weeks intervals, when the environmental conditions allowed the growth. The cut was made in early afternoon at approximately 3 cm from the ground, using a battery-powered lawn with a cutting width of 10 cm.

The samples were immediately brought to the laboratory in a refrigerated bag, weighed and then placed in ventilated oven at 65°C for 24 hours; after this pre-drying they were ground with a hammer mill fitted with a grid of 1 mm. Proximate analysis included residual moisture, crude protein, crude fat, Ash and crude fiber (AOAC, 1990).

Slowly fermentable structural carbohydrates (neutral detergent fiber, NDF, acid-detergent fiber, ADF; acid detergent lignin, ADL) were determined (Van Soest et al., 1991) while non-structural carbohydrates (NSC) were calculated as: 100 - moisture - crude protein - crude fat - Ash - NDF (AOAC, 1990).

Fructans were quantified out of hydrolyzable carbohydrates (CHO-H) were calculated as: 0.154 * NSC + 0.00136 * NSC² (R² = 0.978) and nonhydrolyzable but rapidly fermentable carbohydrates (CHO-Fr) as: NSC - CHO-H. Fructans were quantified out of CHO-Fr according to the AOAC procedures (Method 999.03) (AOAC, 2005) with minor modifications to facilitate analysis of multiple samples. Briefly, fructans are extracted in hot water. Initial clean-up enzyme digestion (β-amylase, sucrase, pullanase, maltase) degrades sucrose and starch, which are reduced to sugar alcohols. Then remaining fructans are hydrolyzed with fructanase and sugars (glucose and mainly fructose) measured spectrophotometrically (Helios® ThermoSpectronic®, Cambridge, UK) at 410 nm after treatment with p-hydroxybenzoic acid hydrazide. For the color reactions standard of fructose (54.5 μg) were used.

The data collected were then subjected to correlation analysis and to analysis of variance (SAS, 2003), using the following linear model: $Y_{ijk} = \mu + S_i + F_j + \varepsilon_{ijk}$ where $Y$ = dependent variable; $\mu$ = overall mean; $S_i$ = fixed effect of sampling day; $F_j$ = fixed effect of the sowing day; $\varepsilon_{ijk}$ = residual error. The interactions initially provided by the model were not significant and were subsequently removed.

### Results and discussion

Mean concentrations and ranges of carbohydrate fractions of pastures are shown in Table 1. Mean values of NDF, ADF and ADL reflect the typical chemical composition of high-quality pastures of the same species, whereas fructans show high values in the first growth period, as expected by higher concentration of non-structural carbohydrates in these pastures. The comparison of the two growing seasons shows that pastures of the first season were characterized by lower fructans and non-hydrolyzable carbohydrates, while the second season had a higher concentration of these fractions in the sowed pastures, as well as a lower concentration of the other carbohydrates.
The mean CHO-H content accounts for about the 18% of the NSC (199.9 g/kg DM). Hoffman et al. (2001), observed, on 83 samples of pasture in Virginia, a higher concentration of CHO-H (38% NSC) and a consequently lower concentration of CHO-Fr (62% of NSC vs. 82% from our study).

Traditionally, certain digestive and metabolic disorders in horse are linked to overload of cereals, in which the CHO-H represent up to 90% of NSC (Pagan, 1999) but also abrupt changes in the levels of CHO-Fr in pasture are involved (Longland et al., 1999). During the growing season the most significant changes in the chemical composition of pasture are charged to this fraction.

Both the excess of starch (Potter et al., 1992) and of CHO-Fr, when subjected to an anaerobic fermentation in the hindgut, lead to changes in the ratio of volatile fatty acids and lactic acid, with a raise of the latter resulting in a rapid decrease of intestinal pH. This occurs by the action of lactate producing bacterial species that in horses are mainly represented by Streptococcus bovis and Streptococcus equinus (Milinovic et al., 2008), associated with different species of the genus Lactobacillus spp and Mitsubihwa (Jassin et al., 2005).

The mean fructans concentration (37.7 g/kg DM) constitutes only 23% of the CHO-Fr (163.2 g/kg DM), which include other fractions of carbohydrates (such as gums, mucilages, pectins, etc.) that are resistant to digestion by mammalian enzyme but are readily fermentable (Van Soest et al., 1991). These results are similar to those resulting from analysis of 10 pastures different for floristical characterization in Germany (fructan: 37.5 g/kg DM, range 18 to 57) but lower than those derived from pastures obtained in two growing seasons in the United Kingdom (fructan: 177 g/kg DM, range 75 to 279) (Longland and Byrd, 2007).

Based on the minimum and maximum values of fructans in the studied pastures (15.5 and 138.2 g/kg DM, respectively) for a horse of 500 kg of bodyweight that intakes about 8 kg/d of DM (Marlow et al., 1983, Kronfeld et al., 2005), the daily intake of fructans (0.12-1.11 kg/d) results strongly below the minimum quantity (3.5 kg/d) used by Pollitt et al. (2003) to experimentally induce laminitis, but not colic.

While high fructan intake has a negative effects on horse health (Crawford et al., 2007), small quantities might be beneficial. Berg et al. (2005) observed, in Quarter Horse foals, a reduction in the number of Echerichia coli in faeces after intake of 8-24 g fructans/d.

The floristic composition of the different pastures influenced their chemical composition (Table 3). The limited ability to store structural carbohydrates in the cell wall by legumes (Van Soest, 1994) significantly affected (P<0.05) the NDF and consequently the NSC content of the site 3, which was, respectively, lower and higher than the pastures which were in a large part or totally grasses (sites 1, 2, 4). Site 5, with both legumes and grasses showed a mid-range behavior. Fructans content among the pastures ranged between 22.2 and 49.9 g/kg DM, but differences were not significant (P>0.05).

The results show that in pastures growing in the province of Parma and designed for horse feeding, environmental conditions can have a significant effect on carbohydrate concentration in forages. Among NSC the fructans concentration changes in relation to the bioclimatologic conditions more than in relation to the floristic composition. In any case the high observed levels for fructans in other geographic areas are not been achieved. So the impor-

### Table 2. Carbohydrate fractions (g/kg DM) of pastures as affected by sampling day.

| Site | Sampling day | MSE |
|------|--------------|-----|
|      | THI | April 18 | May 20 | June 18 | July 17 | September 22 | March 23 | May 10 |
|      | M-L | M-L | M-M | M-H | M-H | M-M | L | M-L |
| NDF | 467.2ab | 452.2d | 547.3b | 574.3b | 540.7b | 433.2a | 483.1a | 42.37 |
| ADI | 245.8b | 253.2bc | 305.8cd | 308.6d | 291.4cd | 208.8a | 222.2ab | 29.15 |
| ADL | 21.2ab | 26.3bc | 35.9bc | 41.8d | 49.8c | 18.0a | 27.1bc | 10.71 |
| NSC | 215.8b | 256.8b | 126.7b | 135.9a | 221.9a | 228.9a | 221.3a | 49.65 |
| Fructans | 38.1b | 44.6b | 19.4a | 9.2a | 8.3a | 55.8bc | 74.3c | 20.54 |
| CHO-H | 39.9b | 47.5b | 21.8b | 23.8a | 41.0a | 42.7b | 49.9a | 10.48 |
| CHO-Fr | 175.8b | 203.2c | 105.1a | 112.1a | 180.8b | 186.1b | 180.3c | 39.18 |

### Table 3. Carbohydrate fractions (g/kg DM) of pastures as affected by the floristic composition.

| Site | MSE |
|------|-----|
| 1 | 2 | 3 | 4 | 5 |
| NDF | 532.2cd | 521.0cd | 456.4a | 550.1d | 486.9db | 42.37 |
| ADI | 283.9 | 274.6 | 258.2 | 299.5 | 270.5 | 29.15 |
| ADL | 33.1 | 29.8 | 32.1 | 32.3 | 36.7 | 10.71 |
| NSC | 172.8b | 179.2ab | 236.4c | 163.1a | 224.3bc | 49.65 |
| Fructans | 22.2 | 49.9 | 34.1 | 31.9 | 37.2 | 20.54 |
| CHO-H | 31.0b | 32.7b | 44.5 | 29.0b | 41.8bc | 10.48 |
| CHO-Fr | 142.0b | 146.5bc | 191.9b | 134.1a | 182.5bc | 39.18 |

Clostridium tetanomorphus; Dactylis glomerata, Bromus inermis; L. equinum, Vicia sativa; Tribolium pratense; 3, Natural meadow grass; 5, Poa trivialis, Bromus inermis, Festuca alba, Lolium perenne, Vicia sativa, Tribolium pratense.
quence of fructans in pasture for horses must not be considered a real crucial factor.

Further interdisciplinary studies in this area between veterinarians and forage researchers are clearly warranted.

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