

NONSTRUCTURAL CARBOHYDRATES IN OAT FORAGE

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Abbreviations; CP-crude protein; GFS-glucose, fructan, sucrose; ha-hectare; N-nitrogen; NDF-neutral detergent fiber; NSC-nonstructural carbohydrates; P-phosphate

INTRODUCTION

Nonstructural carbohydrates (NSC) fractions found in forage may play a role in equine diseases that involve carbohydrate intolerance, such as laminitis. Starch in seed grains, such as oats (*Avena sativa* L.), barley (*Hordeum vulgare* L.), and wheat (*Triticum aestivum* L.) and corn (*Zea mays* L.), has been used to induce equine laminitis in clinical studies (1).

Sugars in forage may adversely affect equines with dysfunctions of glucose metabolism. Insulin resistance has been associated with laminitis in equines (2), although the mechanisms by which excess sugars can trigger an episode are not yet understood. French and Pollitt (3) were the first to experimentally induce laminitis using fructans. Much of the existing data on NSC concentrations in forage utilize analytical methods that extract and quantify sugars and fructans collectively as water soluble carbohydrates. In this study, purified hydrolytic enzymes were used in the analytical procedures to separate starch, fructan, and sugars, thereby facilitating quantification of individual carbohydrate fractions.

High concentrations of NSC, including sugars (glucose, fructose, and sucrose-GFS), fructan and starch, must be considered when developing feed rations for horses and ponies prone to laminitis. Numerous factors are known to influence NSC concentrations in various plant parts. Nonstructural carbohydrate content and type depends on the plant species, plant part, stage of development, and environmental conditions such as root and shoot temperatures during growth, as well as, light intensity and duration, plant nutrient availability, and water status (4, 5). Nonstructural carbohydrate concentrations vary through time with lesser amounts being present during the morning than afternoon and early evening hours (6, 7). It is often assumed that mature plants are higher in fiber and lower in NSC content than immature plants. Generally, any environmental condition that restricts growth (NSC utilization) to a greater extent than photosynthesis (NSC synthesis) results in increased amounts of NSC in plant herbage (8, 9).

Cool-season grasses, those of temperate origin, grown under cool temperatures, accumulate soluble sugars, starch, and fructan, while warm-season grasses accumulate soluble sugars and starch but no fructan (10). Thus, cool-season and warm-season grasses have different metabolic pathways by which they fix and store carbon. Nonstructural carbohydrates are the sum total of GFS, fructan, and starch. As a cool-season grass that utilizes fructan as a storage carbohydrate, oat forage is an appropriate model for investigating the relationships that influence fructan concentration.

Fructans are water-soluble carbohydrate chains formed from the attachment of multiple fructose molecules (a few to hundreds or even thousands) to a sucrose molecule (11). The fructans that occur in most cool-season grasses, including the small grains wheat, barley and oats, are called phleins. Those found in dicotyledonous plants are known as inulins (12). Although the roles of fructans in plant metabolism are not fully understood, they serve as a carbohydrate reserve (4). Accumulation of fructan occurs within cell vacuoles (13), and is often associated with conditions under which the rates of metabolism and plant growth are lower than the rate of photosynthesis (9). In contrast, starch generally accumulates within leaf chloroplasts in vegetative tissues.

A horse that ingests 10 kg of dry hay per day may be eating nearly 3 kg of sugars and starch (30% dry weight of diet). Thus, information on NSC content of forages is important in determining feed rations for carbohydrate-intolerant equines.

Generalizations about NSC concentrations in forages are often difficult because of the many environmental and plant growth-related factors that influence carbohydrate metabolism and accumulation in forage (14). Generally, NSC is lost by respiration or leaching during drying of forage. Slow drying, as a result of cool or wet weather, generally increases NSC losses.

Because both environmental conditions and stage of plant maturity are thought to influence carbohydrate content of forages, an experiment was designed to quantify changes in carbohydrates in oats grown under field conditions and harvested for dry forage. Oats grown for forage are often planted and grown as an early first crop in the spring, but they are also grown as a late season crop. The objectives of this study were to quantify NSC concentrations at various times during development in oats planted in both spring and summer, and to describe nutritional composition for both immature and mature plants grown in warm and cool environments.

MATERIALS AND METHODS

The oat cultivar 'Monida' was seeded (four replicated plots) with a cone seeder at 100 kg seed/ha into a firm seedbed at Center, CO on two planting dates in each of two years (22 April and 21 June, 2002; 21 April and 18 June, 2003). The soil was a cobbly sandy loam, pH 8.2, organic matter 1.5%, and cation exchange capacity 13 with medium fertility. In 2002, 36 kg N/ha was applied as ammonium nitrate on 27 April and 21 June for a total of 72 kg N/ha. During 2003, 47 kg N and 45 kg P/ha were applied as ammonium nitrate and ammonium phosphate on 21 April and 29 June for a total of 94 kg N/ha.



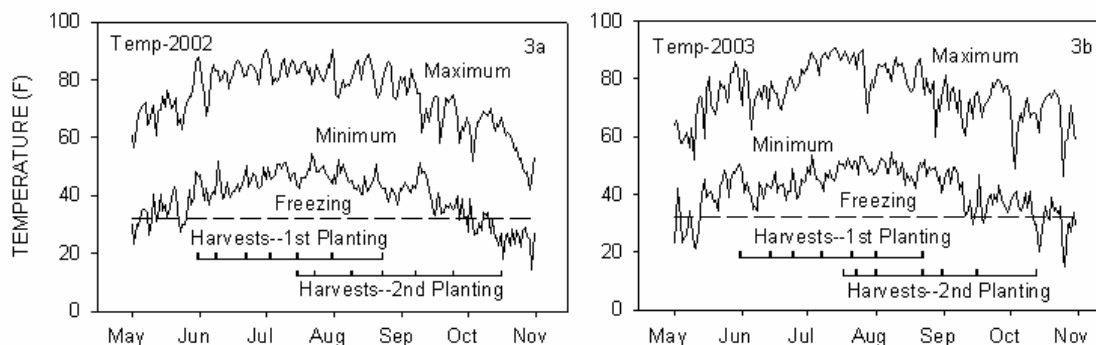
Plots were sprinkler irrigated as needed, generally every three days. Plant samples were harvested in the afternoon (3-5 PM) from each of the replicated plots. Stage of plant growth was standardized across the two plantings in the two years by harvesting samples at specific developmental stages using the Feekes scale (15).

Developmental stages are: tiller, all leaf tissue-no stem elongation; joint, early stages of stem elongation; boot, stem has elongated and reproductive tissue developing but not emerged; flowering, stem fully elongated, reproductive organs present but no seed development; milk, seeds are filled with milky fluid; soft dough, seed tissue is

becoming solid; mature, green color absent from seeds. Planting dates, harvesting dates, and corresponding stages of growth at harvesting are listed in Table 1.

Planting Dates				Feekes Growth Stage	
2 April 2002	21 June 2002	21 April 2003	18 June 2003		
Harvest dates					
31 May	15 July	31 May	17 July	3	Tiller
08 June	23 July	14 June	21 July	7	Joint
22 June	09 August	24 June	01 August	10	Boot
03 July	23 August	07 July	22 August	10.5	Flower
15 July	07 September	21 July	31 August	11.1	Milk
31 July	24 September	01 August	16 September	11.2	Soft Dough
23 August	19 October	22 August	13 October	11.4	Mature

Maximum and minimum air temperatures over the plant growth period.



Maximum and minimum air temperatures for the growing seasons of 2002 and 2003 during which the oat hay samples were grown and collected. Data collected at Center, CO Station (CTR01) Latitude: 37.7067 Longitude: -106.145; Elevation: 7702 ft located at CO State Univ. San Luis Valley Research Ctr.

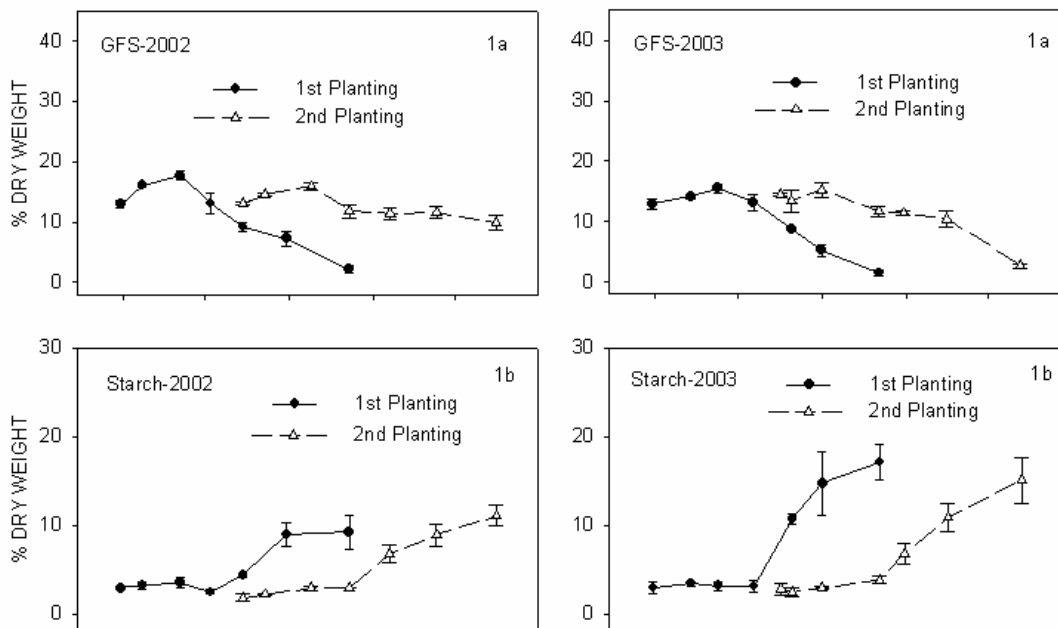
Samples were air dried in mesh cages in the sun (to simulate drying conditions of farmer-harvested hay) for 2-3 days, placed in a freezer at -20 C to keep dry until analyzed. Foliage was double ground, first with a Wiley mill then with a Tecator-Cyclotec tissue grinder to pass through 0.5 mm screen. Carbohydrates were quantified (four replicated measurements per sample) using AOAC procedures (Methods 996.1, 999.03, 76.13, and 32.32) with minor modifications to facilitate analysis of multiple samples using a plate reader. Also, MegaZyme ∇ -glucosidase was used for digestion of sucrose to specifically measure GFS instead of the MegaZyme sucrase/ ∇ -amylase/pullulanase/maltase enzyme specified in AOAC 999.03. Standard curves for

the color reactions were prepared using glucose (for starch) or equal concentrations of glucose plus fructose for other measurements.

Samples were analyzed for N using a LECO CHN-2000 Series Elemental Analyzer (LECO Corp., St. Joseph, MI). Multiplying N x 6.25 established levels of crude protein (CP). Neutral detergent fiber (NDF) was determined using procedures described by Goering and Van Soest (16). An ANKOM-200 Fiber Analyzer (ANKOM Technology Corp., Fairport, NY) was used to determine NDF. Mean values and standard deviations were determined using Microsoft's Excel software.

RESULTS

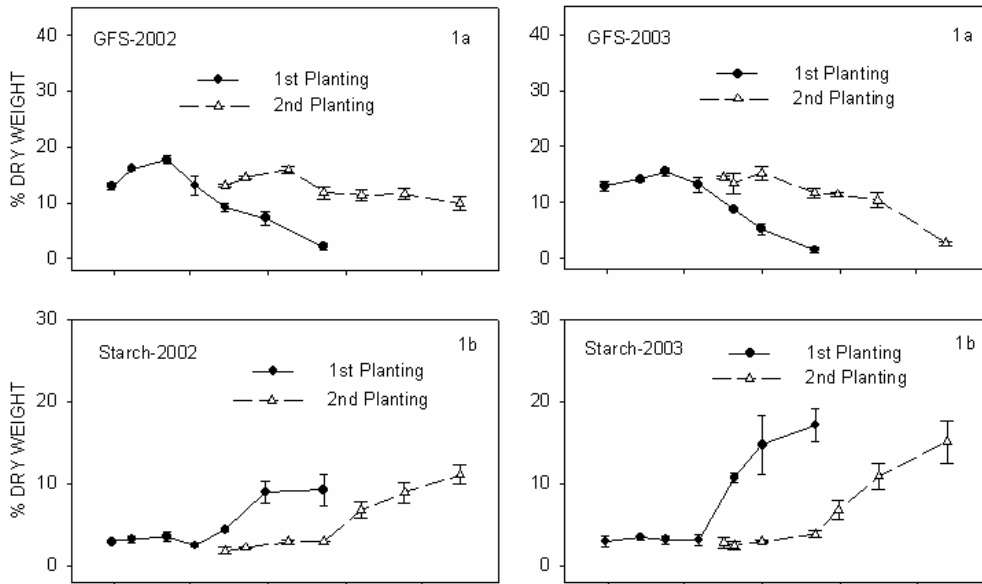
Sugar concentrations in vegetative tissues were generally highest when plants were young (tiller and joint growth stages), and fiber (structural portion of the plant) was relatively low. GFS averaged about 15% dry weight in hay from oat plants in the boot stage at both planting dates and declined to 1 or 2% dry weight when mature.



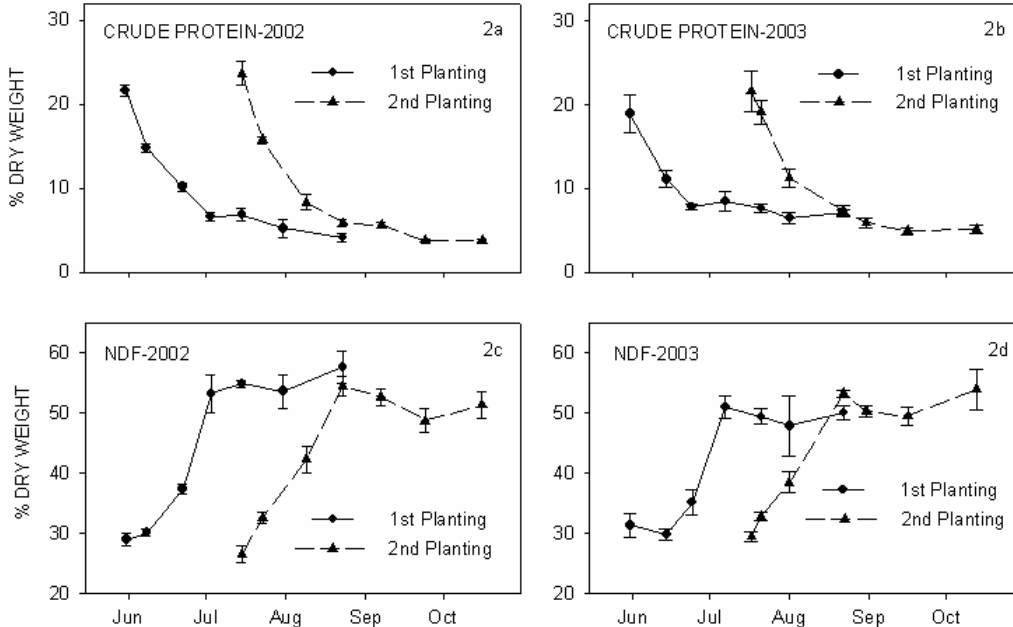
Conversely, starch, a storage carbohydrate, was present in low concentrations in young vegetative tissues (tiller to flowering stages) and then increased with plant maturity. In oat hay, starch increased from 3-4% dry weight early in plant development to 10-15% dry weight in mature plants.

Fructan accumulations in oat hay, when considered across all planting dates, generally were not a function of plant maturity.

In contrast, GFS and crude protein (decreased with plant maturity while starch and neutral detergent fiber increased).



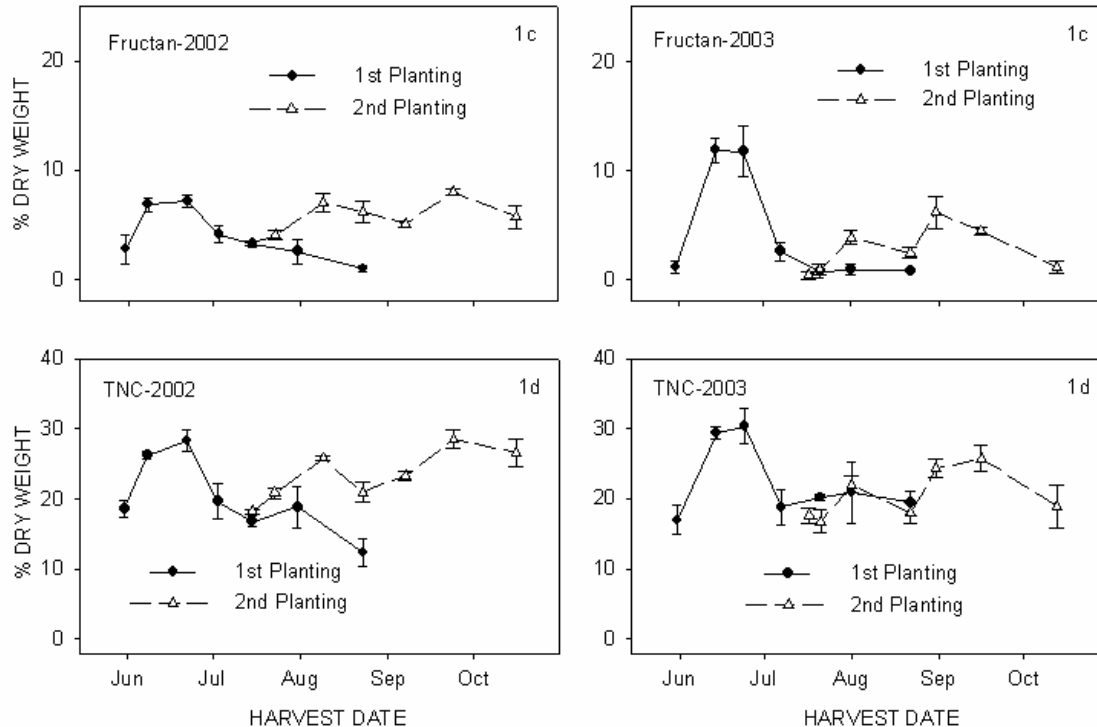
Carbohydrates (GFS, glucose+fructose+sucrose; starch; fructan; NSC, nonstructural carbohydrates) in oat hay harvested on seven dates from early and late plantings in 2002 and 2003, Center, CO. Samples were collected at eight stages of maturity. Bars indicate one standard deviation from mean. n=4



Crude protein and NDF (neutral detergent fiber) in oat hay harvested on seven dates from early and late plantings from samples collected at eight stages of maturity during 2002 and 2003. Center, CO. Bars indicate one standard deviation from mean. n=4

Earlier studies have shown that the amount of fructan present within vegetative tissues of grasses is greatly influenced by day and night temperatures, especially those during the few days preceding sampling (10).

Fructan concentrations were highest at the joint and boot stages of growth in April planted oats. In contrast, fructan concentrations were highest in plants at the milk or soft dough stages in the June planting. High fructan concentrations in plants following cool ambient temperatures agree with prior observations (10).



Nonstructural carbohydrate concentrations (the sum of GFS, fructan, and starch) clearly demonstrated a disjunct relationship with plant maturity in oat hay. Oats planted early in the season had greater concentrations of NSC in immature than in mature oat hay. However, mature late-season planted oats had NSC concentrations similar to immature oat plants from the first planting. Following the joint stage, NSC concentrations decreased with advancing maturity in April planted oats. In contrast, NSC increased (2002) or changed only slightly (2003), depending on the year, in those planted in June.

CONCLUSIONS

The amount of NSC present in harvested and grazed forages varied with several factors including date of planting, season of harvest, ambient temperatures, and plant maturity. Nonstructural carbohydrate contents should be considered when formulating feed for laminitic equines. The ideal approach is to chemically analyze all feeds. When feed analysis is not an option, practitioners should consider the following: 1) the concentrations of the various carbohydrate contents are not always inversely related to plant maturity. In fact, GFS (concentrations up to 15% dry weight), is the only

carbohydrate fraction that always decline with plant maturity; 2) because ambient temperatures at or just prior to harvest have been shown to influence fructan content in approximately 100 cool season grasses when grown in controlled environments (10) the fructan contents of oat forage may also be related to seasonal changes in air temperatures; 3) starch is present in vegetative tissues (up to 10% dry weight) and generally increases with maturity; 4) fructan and starch are the major NSC components in harvested oat hay, however, concentrations of GFS may be high during the joint and boot stages of growth; 5) environmental conditions may be as important as plant maturity in determining NSC content of oat hay.

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