Forage and Pasture Management for Laminitic Horses

Kathryn A. Watts, BS Rocky Mountain Research & Consulting

Supported in part by: The Animal Health Foundation

Abstract

In the past, many cases of laminitis have been of mysterious origin. Recent investigations in veterinary medicine, coupled with the availability of new techniques for analyzing hydrolyzable carbohydrates in forage may provide evidence to solve some of these mysteries. Fructan has been shown to be capable of inducing laminitis in clinical studies. Insulin resistance, a primary feature of Equine Metabolic Syndrome, has consequences to vascular tissue that help explain why many horses with hyperinsulinemia suffer with chronic laminitis. High levels of fructan, as well as simple sugars, may be found in grass that is subjected to stress from rapidly changing environmental conditions. Stressed grass, and hay made from such grass, may contain up to 35% (dm) sugar, starch and fructan, which comprise a fraction defined as Non Structural Carbohydrates (NSC). The factors that may affect the NSC content of forage include: genus and specie of the forage, light intensity and duration, temperature, nutrient and water status, stage of growth, and grazing management. This paper is an introduction to the principles of plant physiology that affect the NSC content of forage can offer veterinarians some practical solutions for the feeding and management of laminitic horses under their care.

Introduction

In the last few decades, forage scientists have implemented breeding programs and management practices that have increased the concentration of hydrolyzable carbohydrates in forages. Under certain conditions, grass and hay may have concentrations of sugar, starch and fructan of up to 35% of dry matter. Because the cattle industry directs and funds most of the research in forage science, the need for increased meat and milk production are the focus of these improvements. Grass breeders intentionally select for increased nutrient density, vigor, extended grazing season, disease resistance, and cold and drought tolerance. Because all these traits are associated with increased levels of hydrolyzable carbohydrates in forage, any selection for these improvements inadvertently increases hydrolyzable carbohydrates as well.

Much of the research on equine nutrition is funded by the performance horse industry, however, their requirements do not represent the needs of the majority of the equine population, which includes categories such as recreational trail horses, typical back yard pets and companion animals, children's mounts and retirees. As current equine health practices extend the average lifespan of the horse, there has been a concomitant rise in the population of older, more sedentary animals. Additionally, the typical high grain and hay diet remains a staple for the average horse and pony as many owners, perhaps out of tradition, follow this widely practiced protocol. These factors, among others, have led to an increasing segment of the equine population being made up of laminitic horses. It is also

generally acknowledged that certain breeds of horses are known for being 'easy keepers', able to maintain body weight with a miniscule volume of food when compared to the average animal used in the majority of equine nutritional studies. Recent focus has been placed on the common link between conditions such as insulin resistance, Equine Metabolic Syndrome, and Equine Cushings Disease, all of which frequently include some manifestation of impaired glucose metabolism. Fructan, which can comprise a major portion of the carbohydrate in certain types of grass under specific conditions, has been implicated in the etiology of laminitis. Consideration therefore needs to be applied to minimizing dietary carbohydrate in the diet, especially forage, the basis of the diet for most of the horses exhibiting the above syndromes. Since glucose intolerance and the presence of fructans are linked to the development of laminitis, this suggests that the form of carbohydrate, and its glycemic index are more important to this segment of the horse population than Digestible Energy, which is calculated from all sources of calories. While several studies have been conducted on glycemic indices of horse feeds, various types of forage were not compared. Given the wide range of sugar content in grasses and hays, this work is clearly needed. While laminitic horses do need optimum levels of protein and minerals to maximize hoof growth, the high concentration of hydrolyzable carbohydrates often associated with premium quality forages should be avoided.

An Overview of Carbohydrate Metabolism in Grasses

Photosynthesis converts CO₂ and water to sugar. The driving force is solar energy, and while photosynthetic rate is correlated to light intensity and CO₂ concentration, it is not dependant on temperature. As long as light is available, and the plant has sufficient water, the process will continue even at near freezing temperatures. Plant respiration, which happens mostly at night, is the process by which the sugars produced by photosynthesis during the day are utilized. These sugars are used for energy production and as substrates to form other essential components including proteins and structural carbohydrates such as hemicellulose and cellulose. The term 'structural carbohydrate' refers to those polysaccharides that are part of the plant cell walls. Non Structural Carbohydrates (NSC) includes simple sugars, disaccharides, fructans, and starch. They are found in both intra- and intercellular spaces. The concentration of NSC in forage is the result of a complex interaction of factors (1).

Factors Affecting NSC Accumulation

<u>C3 vs. C4</u>

Common forage grasses are considered C3- cool season, or C4-warm season. This is based on the photosynthetic pathway by which they fix carbon. The first photosynthetic product in C3 metabolism is a 3-carbon compound, while the first product in a C-4 grass is a 4-carbon compound. While the details of the different metabolic pathways are not essential to this discussion, the consequences to the potential NSC content of the grass are significant. C3- cool season grasses preferentially utilize fructans as the storage carbohydrate. This facilitates better winter hardiness, and allows growth under conditions too cool for C4 grasses. During cool weather, C3 grasses can accumulate large amounts of storage carbohydrate, sequestering it in various parts of the plant, and rapidly converting it to transportable simple sugars as needed. While well adapted to cooler weather, the C3 form of photosynthesis is not as efficient under high temperatures or moisture stress. During hot, dry weather, they grow more slowly or go dormant when temperatures reach 30°C. Examples of C3, cool season grasses include: brome (*Bromus sp.*) fescue, (*Festuca sp*). orchardgrass (*Dactylis glomerata*), perennial rye (*Lolium perenne*), timothy(*Phleum sp*). and small grain hays (oat, wheat, rye, triticale).

C4 plants produce little to no fructan, and utilize starch as their storage carbohydrate. Because starch is not transportable within the plant, starch accumulation under cold stress is self-limiting,

causing the plant to shut down after starch saturation is reached in the organelles producing it. This type of grass will generally go dormant after frost, although gradual acclimation to cold can allow some species of C4 grass to adapt to some degree (2). Under hot, dry, sunny conditions, C4 grasses are more metabolically efficient and achieve high growth rates under conditions that retard the growth of C3 grasses. Because of morphological differences, C4 grasses generally have a greater percentage of dry matter as hemicellulose than C3 grasses.

The implications important to this discussion are that C3 grasses are generally higher in NSC than C4, but even more so under cold conditions. The most intensive study of the quantification and characterization of carbohydrate fractions in 185 types of grass under warm and cool temperatures was done by Chatterton (3). Cool season, C3 type grasses averaged a threefold increase in NSC content when grown under day/night temperatures of 10° day/5 night° C as compared to concentrations while grown at 25° day /15° night C. Certain brome and fescue species reached levels of NSC equivalent to <40% of dry matter content under cold stress. C4, warm season grasses were lower in NSC in general, and had less increase in NSC content under the same conditions. Examples of C4 grasses are Bermuda (Cynodon dactylon.), bahiagrass (Paspalum sp.), bluestem (Andropogon sp.), crabgrass (Digitaria sp.), switchgrass (Panicum virgatum), gramma (Bouteloua sp) and various native prairie grasses that predominate in mid summer.

Cold stress in C3 grasses

During hardening of wheat in cold conditions, NSC increased continuously, and the rate of accumulation correlated significantly with the frost tolerance of the cultivars (4). In a study using timothy, orchardgrass, and perennial ryegrass, potential for regrowth was significantly correlated to the NSC content in all species (5). Those grasses that accumulate fructans do so in the spring and fall when diurnal temperature fluctuations minimize growth and respiration of sugars. The ratio of simple sugars to fructans in cold- stressed C3 plants can vary widely depending on whether the plant was subjected to a gradual change in colder temperature vs. a sudden drop below freezing. Gradual acclimation or First Stage Cold Hardening in C3 grasses occurs at near, but above, freezing temperatures and is characterized by fructan accumulation. Sub-freezing temperatures instigate Second Stage Cold Hardening that stimulates fructan conversion to sugars, which then function as a cryoprotectant (6). Concentrations of fructans are generally lowest in mid winter and mid summer in climates where extended periods of subfreezing temperatures are likely to have a very high sugar concentration.

On Jan. 1 after a month of -20°C night temperatures, intense sunshine, and an absence of precipitation, a whole plant sample of tall fescue (*Festuca arundinacea* var. Fawn) grown by the author at Center, Colorado tested 31.7% NSC (analysis by Dairy One, Ithaca, NY). It is notable that the plants retained viable green tissue at the stem bases. (Fig 1)



Light Intensity

Photosynthetic capacity, and hence production of sugar, is directly correlated to light intensity and duration. NSC concentration will be lowest in early morning if the night was warm enough to allow the sugars produced the previous day to be utilized by respiration. Respiratory rate will be diminished below 5°C. In a field study using *Phalaris* sp. NSC concentration was significantly reduced by shading, and returned to concentrations similar to unshaded grass two to four hours after removal of the cover (7). The same specie of grass will have more potential for high concentrations of NSC in sunnier seasons or climates than under cloudy conditions, other variables being equal. Caution should be exercised when attempting to extrapolate data for NSC content of grass when the data has been collected under different growing conditions.

Concentrations in Various Plant Parts



Fig 2. Meadow foxtail in flower: developing reproductive tissue has priority for allocation of carbohydrates. Concentration of NSC, and particularly fructans, may peak at this stage of growth.

In C3 grasses, NSC are generally higher in grass stems than in leaves, and increase in concentration towards the bottom. A high stem to leaf ratio will generally mean higher NSC, all other factors being equal. The stem base is considered a storage organ. In C4 grasses, excess NSC is in the form of starch in the leaf tissue. While grass leaves are the source of sugars, the developing seed head becomes the sink. Newly emerged seed heads in flower stage may be higher in NSC than the leaves, although not as high as the stems (1). Figure 2 shows a grass head in flower stage. This is a time of peak concentration of NSC throughout the plant, hence the stage when farmers are advised to cut hay for maximum digestible nutrient content. A two year study is being conducted by Rocky Mountain Research, Center, Colorado, and USDA-ARS Forage and Rangeland Research Lab in which oat hay is grown in side-by-side plots under optimum field conditions for accumulation of carbohydrates. The first year data shows that environmental conditions are far more important than stage of growth in determining the concentration of NSC in oat hay. Oat hay cut in an overmature stage, but stressed by cool temperatures in late fall was as high in NSC as pre-heading stage cut in early summer (8).

Drought Stress

Drought stress is a stimulus for NSC accumulation in grass. Respiration is limited by low water content before photosynthesis. This is an adaptive advantage as the NSC in reserves facilitates quick growth after return of optimum moisture (9). The duration of the drought affects which NSC fractions predominate. In simulated water stress of five days duration, fescue leaf bases had a 258% increase in sucrose content, 187% increase in hexose content and a 69% drop in fructan (10). In a study on the

effects of drought in 21 varieties of orchardgrass, NSC and fructan increased as the drought progressed, reaching 35-40% of DM in stem bases after three months of drought (11). Because fructan content is associated with drought tolerance there is interest in utilizing genetic engineering techniques to introduce the capability to produce fructan into other crops like sugar beets (12).

Low Fertility

Forage researchers have shown that nitrogen and phosphorus deficiency causes an increase in concentration of NSC in both grass and legumes (1). Nitrogen deficiency increased NSC content in fescue (13), wheat (14), several varieties of ryegrass and fescue (15). Phosphorus deficiency is also correlated with higher concentrations of NSC in grass (I). There has been little research done on trace mineral fertilizer and its relationship to NSC of grass.

Salty Soil, Anaerobic Conditions

Increasing salt stress caused higher amounts of soluble carbohydrate and fructan to accumulate in wheat plants. Salt tolerance in wheat is associated with the ability to accumulate high NSC (16). High salts in either soil or irrigation water will interfere with the osmotic gradient by which plants absorb water, and will induce conditions similar to drought stress, slowing growth beyond the ability of the plant to fully utilize available NSC.

Oxygen deficiency caused by standing water or ice is another form of plant stress. In timothy subjected to anaerobic stress by enclosure in gas-tight bags, NSC concentration increased. By maintaining higher carbohydrate reserves under oxygen deficiency, timothy has a greater chance of survival under conditions of ice encasement than orchard grass, which had decreased NSC under the same conditions (17).

NSC Content of Hay and Straw

The NSC content of hay will reflect the amount present when it is cut. Therefore hay that is cut under any of the stressful conditions described above may be high in NSC. Hay cut in the morning will be lower in NSC than hay cut in the afternoon. Cut forage will continue to respire and burn off sugars until the moisture content is below approximately 40%. The faster the hay dries; the less sugars will be respired. Therefore hay that cures slowly, in humid, cloudy conditions will be lower in NSC content than if it dried under sunny, dry conditions (18). These principles are reflected in the NSC concentrations of various types of forages listed on the Feed Composition Library, from Dairy One Forage Lab, Ithaca, NY (Table 1).

Table 1 NSC* content of some forages and feeds

	n	Ave.	Normal range		St Dev
Fresh grass forage	374	18.70	10.59	26.82	8.11
Fresh Bermuda forage	6	10.20	6.74	13.66	3.46
Grass Hay	1712	13.26	11.12	15.4	2.15
Bermuda grass hay	374	13.26	9.15	17.37	4.11
Alfalfa hay	3996	11.39	9.96	12.83	1.43
Oat hay	377	23.09	16.29	29.88	6.79
Small grain hay	13	19.09	12.67	25.53	6.4
Straw	52	11.97	6.28	17.66	5.69
Beet pulp-dry	34	12.24	9.44	15.05	2.81
Wheat bran	20	30.19	20.92	39.46	9.27

* sugar, starch and fructan, All units as % of dry matter basis

From Feed Composition Library at Dairy One Forage Lab, Ithaca, NY Complete listing at <u>http://www.dairyone.com/Forage/FeedComp/default.asp</u>

Fresh grass forage averages 18.7% NSC, while grass hay averages 13.26% NSC. Table 1 only shows the normal range of NSC content. Outliers range from <1% to 35% NSC content for hay. It is the author's opinion that forages containing NSC on the lower end of the normal range, (<10% NSC), are most suitable for horses prone to laminitis associated with metabolic abnormalities. Note that hays made from oats and other small grain crops are higher in NSC than grass hays. This stands to reason, as small grains have been selected for their capacity to create large amounts of starch. When cut in early heading stage before the starch has formed, the plant will be high in sugars and other precursor carbohydrates.

The hay pictured in Fig 3 is a wheat, oat and rye mix grown in California that tested 30.1% NSC with 25.3%(DM) as sugar/fructan. The crude protein content of only 3.2% (DM) confirms that it suffered from nitrogen deficiency. The red streaks on the stems are symptom of phosphorus deficiency. As previously mentioned both these nutrient deficiencies are correlated with high NSC concentration in forage. A hyperinsulinemic pony foundered in all four feet after consuming this hay.



Straw may have significant amounts of sugar in it, as shown by table 1, averaging nearly 12% NSC (dm). Standard forage testing will show it to be low in protein and minerals, but sugars can still be high even when other nutrients are low. Given the wide range of NSC content of straw, it may be prudent to use other forms of bedding, or to have straw tested for NSC content before using as bedding for laminitic horses.

Testing for Non Structural Carbohydrates in Forage

It is impossible to estimate NSC content of hay by visual characteristics or conventional forage testing that does not include direct analysis for NSC. Testing for NSC in forage is an emerging technology, and problems in nomenclature are common. Forage scientists and nutritionists often do not speak the same language, which creates confusion. The terms non fiber carbohydrates (NFC), non structural carbohydrates (NSC), acid extractable carbohydrate (AEC), water soluble carbohydrates (WSC) and hydrolyzable carbohydrates, may be used interchangeably, but differ in constituents depending on the analytical procedures used for quantification. Even amongst laboratories, there is a lack of standardization of analytical methods; hence one cannot compare test results from one lab to another without knowing the precise procedures used to obtain the numbers

Clinical Techniques in Equine Practice, March 2004, Volume 3(1) 88-95

(19). As more people discover the value of testing for NSC levels, the National Forage Testing Association should adopt a standard procedure and include testing for NSC as part of their certification process for approved laboratories.

One method currently available to measure NSC on a commercial basis is that developed by Hoover and Webster (20). It includes mono and disaccharides plus fructans as the sugar fraction. A separate procedure using enzymatic digestion quantifies the starch fraction. As equine nutritionists seem to agree that the sugar, starch and fructan fractions as a group are of most concern, this test best meets our needs. For the purposes of this discussion, NSC shall be defined as the total of sugar/fructan plus starch as measured by this test. Non fiber carbohydrates (NFC) is the term adopted by forage scientists to describe a fraction that is calculated by an equation subtracting protein, fat, fiber and ash fractions from the total dry weight. This method is fraught with inaccuracies (21) and should not be considered precise enough when evaluating the safety of feeds for horses with laminitis or other glucose intolerance issues.

Procedures to ensure that samples are representative are extremely important. When pulling pasture samples, time of day and environmental conditions contributing to the NSC content should be noted. Grazing horses should be observed and the samples should reflect those grass species, or portions of the plants that are being selectively grazed. Many samples from various parts of the paddock should be gathered, well mixed and a sub sample sent for testing. Fresh forage samples must be frozen within 15 minutes to stop respiratory loss of sugars. They must remain frozen or very cold until analysis in order that testing truly reflects the amount of NSC being ingested in the field. To retain the most NSC freeze drying of samples is preferred over oven drying.

The NSC content of hay is extremely variable from bale to bale and from one end of the stack to the other. Grab samples from a few bales may provide misleading information. Samples sent for analysis must represent an average of the whole lot being fed. At least 15-20 bales per lot should be sampled with a hay probe and mixed well before sub-sampling.

Soaking Hay to Remove Excess Carbohydrates

Excess carbohydrates in C3, cool season grasses are water-soluble. Sugars and short chain fructans are soluble in cold water, and long chain fructans are soluble in hot water. When 15 samples of various hays were soaked 60 minutes in cold water and drained, an average of 31% of the NSC was leached out, with a range of 0-56% (22). Soaking hay is a first aid measure for horses with laminitis associated with metabolic issues until such time as hay with lower NSC content can be procured.

Managing Pastures for Lower NSC

Species Selection

Horse owners hoping to establish sustainable grazing systems utilizing lower NSC grasses will have to accept that they will be more management intensive. Far too many new horse farm owners naïvely believe that all one needs to do to provide a more natural diet and lifestyle for their horses is land and native grass seed. Native grasses, and possibly any low NSC grass species, will not withstand intensive grazing without intensive management. In the American West, the loss of native prairie grasses to overgrazing has shown this to be inevitable. Since competitiveness is usually linked to the ability to produce and store high levels of NSC, lower NSC grasses will need to be nurtured carefully. With this in mind, forage specialists feel obliged to recommend species that are most competitive when asked to select species for intensive grazing situations. In light of the fact that

many horse owners are not skilled in basic farming and land management techniques, it becomes even more necessary to recommend those grass species that can tolerate abuse and neglect. Hence, those grasses recommended by grazing experts to new horse farm owners may often be those with the highest potential to generate and store high levels of NSC. While there is a large selection of grass varieties with lower potential to accumulate NSC, the research to develop management systems that will be sustainable under intensive grazing has not been done. There will be different species and different strategies for each bioregion. In subtropical climates where C4 grasses like Bermuda thrive, these are the best choice. Better choices for laminitic horses may be found in native species, and those that have been developed for conservation uses such as soil stabilization and re-establishment of wildlife habitat. While native grasses will not withstand heavy grazing, they may be a viable option for those with large acreage and few horses. Grazing experts may be hesitant to recommend these species for grazing because of low palatability, but given the sedentary and obese nature of many chronically laminitic horse and ponies, lower palatability may be a beneficial quality when choosing grass species. Avoid those varieties most popular with local cattle producers focusing on weight gain.

Types of grasses selected will depend on local growing conditions. One should not attempt to grow species in regions where they are not well adapted, as this will put them under stress, which will generally increase NSC. In areas with harsh winters, selection of species that will sustain intensive grazing will be more difficult as lower NSC grasses are inherently less winter hardy. Under these conditions, maintaining a separate paddock of standing hay comprised of warm season summer annuals such as those found in native prairies may provide a safer source of forage during the spring and fall when cool season grasses are high risk. Research on quantification and characterization of NSC fractions of various species of pasture grass under a variety of regional growing conditions is sorely needed.

<u>Shade</u>

Consider planting trees around or within paddocks designated for laminitic horses to shade the grass and limit photosynthetic rate. Paddocks shaded by buildings part of the day will be lower in NSC than those receiving sunlight all day.

Rotational Grazing

While frequently grazed grass may have lower concentrations of NSC, it is less competitive and more apt to succumb to disease (23). After grazing a paddock while at its lowest concentration of NSC, it is imperative to allow the grass to rest, grow, and replenish its reserves. Without this rest period lower NSC grass species will diminish, encouraging more aggressive species to invade. The length of the rest period will vary by many factors inherent to individual bioregions, but should be a minimum of three weeks.

Limiting Intake:

Full time grazing may not be an option for many horses prone to laminitis. If the owner has the time and proper resources, quality of life need not suffer. The availability of a dry lot that is large enough to encourage self-exercise will be very useful in the management of laminitis prone animals. In wet climates the use of French drains, pea gravel, or wood chips may reduce problems with mud. Grazing muzzles and strip grazing with portable fencing, which limits the amount of grass throughout the day may be a viable option for some animals. Overgrazing to limit intake is not recommended. Because the stem base of grass is a storage organ for NSC, short stubble may contain higher concentrations. Over grazing also creates selective pressure for more competitive, higher NSC species. Limit grazing when grass production cannot meet demands to minimize stress to the grass and help sustain a healthy sward.

Restrict Grazing During Time of Highest stress

Most susceptible horse and ponies must be kept completely off any green grass during periods with freezing nights. In many temperate climates this may last six weeks in spring and again in fall. In fall, grass should be completely brown to the ground, and with enough precipitation occurring to leach out accumulated sugars. An insulating layer of snow may keep winter hardy species of grass alive and green, and if light can penetrate the snow, photosynthesis may continue to produce sugars. Several species of grass with high potential for accumulation of NSC contained 30-40% NSC (DM) in mid winter, when grown under conditions optimum for accumulation of NSC, and receiving no precipitation since senescence. (Watts KA, 2004 unpublished data). Extremely sensitive animals have become laminitic while grazing grass in midwinter that is 95% dead in a cold, arid but sunny climate (Ovnicek G, personal communication 2004). Subsequent analysis showed the pasture in question to contain 29.6% NSC (DM). Most susceptible horse and ponies must be kept completely off the first new shoots of growth after rainfall breaks a drought (9). Waiting until the new grass has 2-3 leaves per tiller, allows the sugars accumulated in underground storage organs to be utilized for the production of biomass, thereby lowering the overall NSC concentration.

Mowing

Mowing before head emergence will eliminate the opportunity for selective grazing of heads by horses. Developing reproductive organs secrete plant hormones and exert dominance over carbohydrate allocation. When the source of these hormones is removed by mowing, vegetative growth from stolons or new tillers is instigated. While this may increase the amount of vegetation available, the concentration of NSC is now spread more evenly throughout the plant.

Optimum Fertility

Soil should be fertilized on the basis of soil analysis for optimum levels of N, P and K and limed to pH of 6-7. Over fertilization as well as under fertilization both create stress that can increase NSC levels.

Irrigation

Water as needed to maintain optimum growth, without leaching nutrients or allowing water to stand. Drought stress can increase NSC concentration.

Weed control

Thistles, chicory, dandelion, and quack grass are high in fructan (24) and are very palatable to horses. Certain herbicides in the 2, 4 D family share a mode of action that may increase NSC levels after treatment. Susceptible animals should be kept off treated pastures until affected plants are completely dead.

Summary

Environmental conditions can have a profound affect on carbohydrate concentration in forages, as well as other anti-quality factors, that may contribute to the onset of laminitis in horses. Veterinarians investigating the etiology of laminitis could provide valuable data for epidemiological studies on laminitis by gathering pertinent information on the NSC content of available forage. Equally valuable would be data on the environmental factors that

Clinical Techniques in Equine Practice, March 2004, Volume 3(1) 88-95

contributed to the formation of high NSC, when discovered. Interdisciplinary studies between veterinarians and forage researchers could prove to be invaluable to our understanding of the etiology of laminitis.

References

(1) Smith D: Nonstructural Carbohydrates, in Butler GW, Bailey RW (eds): Chemistry and Biochemistry of Herbage, vol 1 London, Academic Press, 1973, pp 105-155

(2) Pitterman J, Sage RF: The response of the high altitude C4 grass *Muhlenbergia Montana* (Nutt) A.S. Hitchc. To long- and short-term chilling, J. of Exp. Bot, 52 (357):829-838, 2001

(3) Chatterton NJ, Harrison PA, Bennett JH, et al: Carbohydrate partitioning from 185 accessions of gramineae grown under warm and cool temperatures. J. Plant Physiol 34:169-179, 1989

(4) Vagujfalvi A, Kerepesi I, Galiba G, et al: Frost hardiness depending on the carbohydrate changes during cold acclimation in wheat, Plant Sci, 144(2):85-92, 1999

(5) Moriyama M, Abe J: Etoliated growth in relation to energy reserves and winter survival in three temperate grasses, Euphytica, 129(3):351-360, 2003

(6) Dionne J, Castonguay Y, Nadeau P, et al: Freezing tolerance and carbohydrate changes during cold acclimation of green-type annual bluegrass (*Poa Annua* L.) ecotypes. Crop Sci 41:443-451, 2001

(7) Ciavarella TA, Simpson RJ, Dove H. et al: Dirurnal changes in the concentrations of water-soluble carbohydrates in *Phalaris aquatica* L. pasture in spring, and the effect of short-term shading. Aust J Agric Res 51:749-56, 2000

(8) Watts, KA, Chatterton NJ: A review of factors affecting carbohydrate levels in forage, JEVS, (2):84-86, 2004

(9) Busso CA, Chatterton NJ: Nonstructural carbohydrates and spring regrowth of two cool-season grasses: interaction of drought and clipping. J Range Manage 43(4), 1990

(10) Spollen WG, Nelson CJ: Response of fructan to water deficit in growing leaves of tall fescue. Plant Physiol 106:329-336, 1994

(11) Volaire F, Lelievre F: Production, persistence, and water-soluble carbohydrate accumulation in contrasting populations of *Dactylis glomerata* L. (orchard grass) subjected to severe drought in the south of France. Aust J Agric Res 48:933-44,1997

(12) Pilon-Smits EA, Terry N, Sears T, et al: Enhanced drought resistance in fructan-producing sugar beet. Plant Physiol Biochem 37(4):313-317,1999

(13) Belesky DP, Wilkinson SR, Stuedemann JA: The influence of nitrogen fertilizer and Acremonium coenophialum on soluble carbohydrate content of grazed and non-grased Festuca arundinace., Grass Forage Sci 46:159-166,1991

Clinical Techniques in Equine Practice, March 2004, Volume 3(1) 88-95

(14) Batten GD, Blakeney AB, McGrath VB, et al: Non structural carbohydrate: analysis by near infrared reflectance spectroscopy and its importance as an indicator of plant growth. Plant Soil 155/156: 243-246, 1993

(15) Wilman D, Gao Y, Altimimi MA: Differences between related grasses, times of year and plant parts in digestibility and chemical composition. J Ag Sci 127:311-318, 1996

(16) Kafi M, Stewart WS, Borland AM: Carbohydrate and proline contents in leaves, roots, and apices of salt-tolerant and salt-sensitive wheat cultivars. Russ J Plant Physiol 50(2):155-160, 2003

(17) Bertrand A, Castonguay Y, Nadeau P, et al: Oxygen deficiency affects carbohydrate reserves in overwintering forage crops. J Exp Bot 54(388):1721-30

(18) Trevino J, Centeno C, Ortiz LT, et al: Changes in the non-structural carbohydrates associated with the field drying of oat forage. Sci Food Agric, 67: 393-397,1995

(19) Hoover W H, Miller-Webster, TK: Are you confused about carbohydrates? Proceedings, 44th Ann. New Eng. Dairy Feed Conf., West Lebanon, NH, 2003

(20) Hoover, W H, Miller-Webster, TK: Nutrient analysis of feedstuffs including carbohydrates. Anim. Sci. Report No. 1 Div. Of Animal & Vet. Sci, West Virginia Univ. Morgantown, WV,1998

(21) Hall, MB: Interpreting feed analyses: uses, abuses, and artifacts. 8th Ann. Florida Ruminant Nutr.. Symp. Gainesville, FL pp. 71-19, 1997

(22) Watts, KA: Soaking hay to remove excess soluble carbohydrate, (abstr) in: Proceedings: 2nd Ann. Intnl Equine Conf On Laminitis and Diseases of the Foot, Palm Beach, FL 2003, Full text at <u>www.safergrass.org/articles</u>

(23) Donaghy DJ, Fulkerson WJ: The impact of defoliation frequency and nitrogen fertilizer application in spring on summer survival of perennial ryegrass under grazing in subtropical Australia, Grass Forage Sci 57(4):351, 2002

(24) Suzuki M, Chatterton NJ: Science and Technology of Fructans, Boca Raton, FL, CRC Press, 1993