

## Pasture Nonstructural Carbohydrates and Equine Laminitis<sup>1-3</sup>

Annette C. Longland\*<sup>4</sup> and Bridgett M. Byrd<sup>†</sup>

\*IGER, Aberystwyth, Wales, UK and <sup>†</sup>Department of Animal and Poultry Sciences, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061-0303

**ABSTRACT** Fresh forages constitute a majority of the diet for many horses and ponies that graze on pastures during the growing season in many parts of the world. Grasses generally predominate in such pastures, with varying proportions of legumes. Nonstructural carbohydrates (NSC) (simple sugars, starch, and fructan) can induce laminitis experimentally, and NSC can accumulate to >400 g/kg of dry matter (DM) in pasture grasses. In this article we discuss the environmental factors affecting NSC accumulation in pastures and estimate the potential daily intakes of pasture NSC by grazing horses. We also discuss strategies for both reducing the NSC content of pastures and management practices that can help reduce intakes of pasture NSC by equines at risk of developing laminitis. This study reveals the importance of accurate forage analysis in the development of feeding regimens for equines at risk of laminitis. *J. Nutr.* 136: 2099S–2102S, 2006.

**KEY WORDS:** • laminitis • pasture forage • sugar • grass fructan • starch • nonstructural carbohydrate • water soluble carbohydrate

Laminitis is a painful, disabling, common, and costly disease of the horse and pony that has many causes; the digestive and metabolic forms are linked to equine nutrition. Overall, annual incidence of laminitis in the U.S. is reported to be 2%, but this rises to ~5% in the spring and summer (1), and nearly half of all reported cases of laminitis in the U.S. occur in animals at pasture (2). Current research is focused on 3 main areas: pathological changes and related treatments of the disease once it has already occurred, the identification of equine genotypes that predispose animals to laminitis, and the avoidance of laminitis through the management of risk factors in pastures. The goal of this last area of research is to provide information to the equine community on both pasture and equine management, which is likely to help reduce the risk of laminitis to equines predisposed to this disease.

### Nonstructural carbohydrate accumulation in pastures

During photosynthesis, green plants “fix” atmospheric carbon dioxide in the presence of light, resulting in the production of simple sugars. When sugars are produced in excess of the energy requirement of the plant for growth and development,

they are converted into storage, or “reserve” carbohydrates. The vegetative (nonreproductive) tissues of temperate (cool season, C3 plants) pasture grasses accumulate fructan (oligo- and polyfructosyl sucrose) as their reserve carbohydrate, which is translocated from the leaf vacuoles to those of the stem for storage until required by the plant as an energy source. The sum of the simple sugars and fructans comprise the water-soluble carbohydrate (WSC) fraction of the plant. By contrast, starch is the storage carbohydrate of temperate grass seed and the seed and vegetative tissues of legumes. The sum of the simple sugars, fructan, and starch comprises the nonstructural carbohydrate (NSC)<sup>5</sup> fraction of the plant. Typically, warm season grasses (C4) have lower NSC contents than cool season species (C3) (3). Because there is no self-limiting mechanism for the production of fructan in C3 species, high concentrations of it can accumulate. In contrast, C4 plants and legumes accumulate starch as their storage carbohydrate and do not produce fructan. Starch production and storage occurs in the chloroplasts of the leaf, and this is a self-limiting process in which starch production ceases once the chloroplasts become saturated with starch. Therefore, in C3 grasses, the stems contain the highest concentrations of fructan, whereas in C4 plants and legumes, the leaves are the primary sites of NSC accumulation. As both C3 and C4 plants mature, the developing seed head becomes a sink for NSC.

Storage carbohydrate concentrations in plants are constantly changing, a function of photosynthesis on the one hand, and utilization for growth and development on the other. This results in diurnal variations in storage carbohydrates, with concentrations tending to rise during the morning, reach maxima in the afternoon, and decline overnight (4–6). Similar diurnal patterns of storage

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<sup>4</sup> To whom correspondence should be addressed. E-mail: annette.longland@bbsrc.ac.uk.

<sup>5</sup> Abbreviations used: DM, dry matter; NSC, nonstructural carbohydrate; WSC, water soluble content.

carbohydrate accumulation have been reported for legumes (7). There are also seasonal variations in the storage carbohydrate content of grasses and legumes associated with varying energy demands at different stages of growth, with concentrations being highest in late spring, lowest in mid-season, and intermediate in autumn (8,9). Although these patterns of diurnal and seasonal variations in storage carbohydrates are observed under ideal conditions, they may be subject to change in the field as a result of the cumulative effects of various environmental factors, including varying light intensity, temperature, fertilizer, and water status, being superimposed upon "normal" profiles.

#### Possible mechanisms for induction of laminitis by NSC

There is meager evidence of association and stronger evidence of causation between laminitis and rapid intakes of NSC, whereby excessive intakes of NSC have been implicated in acute digestive disturbances associated with their rapid fermentation, and chronic metabolic disorders associated with high glycemic and insulinemic responses (10,11). Thus, laminitis in equines can be caused experimentally by the administration of high concentrations of starch that exceed the digestive capacity of the small intestine, the undigested material flowing into the hindgut (11). Furthermore, fructans are thought not to be digested by mammalian enzymes (12), but while some may be partially susceptible to acid hydrolysis (J. Ince, IGER, Aberystwyth, personal communication) or fermentation (13) in the foregut, it is probable that much passes into the hindgut relatively unchanged. Administering high concentrations of fructan to horses as a bolus results in laminitis (14). The appearance of large amounts of starch or fructan in the hindgut is believed to be the result of a proliferation of lactic acid-producing amylolytic and saccharolytic bacteria. This may result in reduced hindgut pH, which, in addition to hindgut acidosis, may lead to a cascade of events culminating in compromised blood flow (and thereby reduced nutrient supply) to the foot, resulting in laminitis. Laminitis is also associated with insulin resistance in equines, whereby the uptake of circulating glucose by tissue cells normally potentiated through insulin is reduced, leading to impoverished glucose supply to cells (or its metabolism within them), including those of the foot. Insulin resistance is often seen in very fat horses and ponies, and may be exacerbated by high intakes of sugars and/or starch (15). Clearly, pastures contain each of the carbohydrate types that have been implicated in the elicitation of laminitis.

#### Environmental factors that affect NSC accumulation

Studies indicate that not only does the plant genotype affect NSC status, but that environmental conditions can also cause large and significant changes in the amounts of NSC that accumulate. Thus, the WSC content of a given plant species can range from 95 to 560 g/kg DM with a corresponding range of fructan 32–439 g/kg DM depending on the temperature at which it is grown and with higher and lower values being associated with cooler (5–10°C) and warmer (15–25°C) temperatures, respectively (3). This probably occurs because photosynthesis and subsequent storage of photosynthate continues at temperatures below the threshold for vegetative growth (16). Light intensity also affects the NSC status of forages, thus shading *Phalaris aquatica* L. pastures for an average of 43 h resulted in NSC contents of 62 and 126 g NSC/kg DM for shaded and unshaded pastures, respectively (17). Furthermore, in a study on the effects of drought on varieties of orchard grass in the Mediterranean, fructan content increased as drought conditions progressed, reaching 350–400 g/kg DM in stem bases at the end of

a 3 mo drought (18). Thus, factors that reduce growth but do not affect production of photosynthate result in the accumulation of elevated concentrations of NSC. Conversely, factors that encourage growth generally result in a reduction of NSC content. For example, increased growth in response to an application of N fertilizer reduced the WSC content of forage (19).

#### Diurnal and seasonal variation in pasture NSC

Both diurnal and seasonal variations in pasture WSC content have been found in a number of studies. For example, diurnal variation in WSC content of vegetative tissues of various *Lolium perenne* cultivars was such that, under suitable conditions, the WSC concentrations were observed to double during daylight hours with increases in WSC content from 160 to 240 g WSC/kg DM occurring within 3 h; the lowest and highest concentrations were recorded in the early morning and late afternoon or early evening, respectively (A. Longland, P. Thomas, and S. Jones, unpublished data). However, under conditions of reduced light and warm ambient temperatures, there was comparatively little diurnal fluctuation of sward WSC content.

Seasonal variation in pasture WSC constituents is well documented (8,9). Thus, in a study of mixed-species pastures in 10 horse farms in Germany from April to November, concentrations of fructan ranged from 18 to 57 g/kg DM, with highest concentrations being found in May, lowest in August, and intermediate concentrations in October (20). However, in another study, the WSC contents of various ryegrass monocultures at vegetative growth stages were determined throughout the growing season for 3 consecutive years at 9 sites in 5 Northern European countries (Germany, Ireland, Norway, Sweden, and the U.K.) with sites as far north as the Arctic circle and as far south as latitude 52. Over the 3-y study, concentrations of WSC ranged from <100 g/kg DM to >385 g/kg DM, the highest concentrations being associated with cooler temperatures (M. Halling, A. Longland, S. Martens, L. Neirsheim, and P. O'Keily, unpublished data). The various WSC fractions were measured in the swards at the U.K. site across 2 growing seasons. The fructan content ranged from 75 to 279 g/kg DM, accounting for 55 to 75% of the WSC fraction. After fructan, sucrose was the next most abundant component of WSC, accounting for 16 to 22% of the total WSC fraction, with proportions of fructose and glucose being 6–12%, and 3–10% of the WSC fraction, respectively (A. Longland, S. Jones, and P. Thomas, unpublished data).

#### Estimated intakes of pasture NSC

The intake of fresh pasture by horses is reported to range from 1.5 to 5.2% of BW/d, (21,22). The highest concentration of reported intake was exceptional, but such amounts have also been observed for pelleted feed intake by ponies (23). The potential intakes of total WSC, fructan, and simple sugars by a 500 kg horse grazing pastures containing the highest and the lowest concentrations of WSC and fructan observed in the N. European study at a range of intake levels are given in **Table 1**. At the lowest level of intake from the low WSC pastures, horses would have ingested at least 0.75 kg of WSC/d, and at the highest concentrations of intake of the high WSC pastures 5–10 kg of WSC. At the higher levels of intake (2.5 and 5.2% BW/d) from swards with elevated WSC and fructan content, horses could have ingested between 3.5 and 7.3 kg fructan/d, respectively. These amounts are similar to and almost double the amount of fructan known to activate laminitis when delivered as a single dose (14). It may be relevant that, to avoid hindgut dysfunction, Meyer et al. (24) and Potter et al. (25)

**TABLE 1**

*Estimated mean intakes of WSC, fructan, and simple sugars by a 500 kg horse grazing pastures with either high or low concentrations of WSC and constituent fractions<sup>1</sup>*

% BW (DM basis)	Intakes, kg/d						
	DM	WSC		Fructan		Simple sugars <sup>2</sup>	
		High	Low	High	Low	High	Low
1.5	7.5	2.9	0.75	2.1	0.56	0.8	0.19
2.0	10.0	3.9	1.00	2.8	0.75	1.1	0.25
2.5	12.5	4.8	1.25	3.5	0.94	1.3	0.31
5.2	26.0	10.0	2.6	7.3	1.95	2.7	0.65

<sup>1</sup> High and low concentrations are 384 g or 100 g WSC/kg DM, respectively.

<sup>2</sup> Simple sugars are the sum of glucose, fructose, and sucrose.

respectively recommended that the maximum amounts of starch that should be fed to horses in a single meal were 2 and 4 g of starch/kg BW. This equates to 2 or 4 kg starch/d for a 500 kg horse; the higher concentration is similar to the 3.75 kg of fructan used by Pollit et al. (14) to experimentally induce laminitis. Concentrations of simple sugars that could have been ingested by a 500 kg horse grazing the pastures range from 0.19 to 1.3 kg simple sugars/d, and 2.7 kg if the highest intake were achieved (Table 1). Some of the swards in the N. European study contained as much as 100 g/kg DM simple sugars, which might contribute to the onset of insulin resistance in susceptible animals. Indeed, horses are selective feeders, and are known to find feedstuffs with elevated sugar content highly palatable, a fact exploited by horse-feed manufacturers who add sweeteners to many of their products. It is not known whether horses that suffer from laminitis are particularly assiduous in their selection of forages with high concentrations of WSC or starch.

Although starch is a minor component of vegetative tissues of temperate pasture grasses (usually <35 g/kg DM) it is a major component of grass seed, ~330–440 g starch/kg seed DM (26). Yields of perennial ryegrass seed may be as high as 900 kg/ha, thus a yield of 360 kg starch/ha, could theoretically be achieved from the seed over a relatively short period of the growing season. The grazing behavior of domesticated horses has been shown to be highly selective, with some areas of the pasture being used for grazing, and others as latrines; the latter often containing long, mature, grasses that have gone to seed during the summer (27). Depending on the stocking density, latrine areas may account for as much as 50% of the available grazing, and seed heads may be abundant in fields loosely stocked by horses. Many grazing horses have been observed to preferentially “strip” seed heads from grasses (28), and although there are no records of intakes of grass seed heads by horses, it is conceivable they may ingest considerable amounts of starch in this way. Furthermore, temperate grass seeds are small, and, at a few millimeters long, many of the seeds may not be significantly disrupted during mastication of ingested forage but may pass into the GI tract relatively intact. The starch of such seeds may be Type 1 Resistant starch (29), whereby, due to encapsulation within plant cell walls that are largely undigested in the foregut, the resistant starch would flow directly into the large intestine. Thereafter, degradation of the cell walls to various degrees by the fibrolytic microflora of the large intestine would expose varying amounts of starch to the amylolytic, lactate-producing microflora. Intakes of grass seed at only 5% of the total intake for a 500 kg horse consuming 2.5 or 5.2% of BW/d

could result, respectively, in ~250 or 500 g of starch being delivered relatively rapidly into the hindgut.

### Strategies for avoidance of large intakes of pasture NSC

Although a direct relation between the onset of laminitis and the ingestion of one or more of the storage carbohydrates of pasture plants through grazing has not been unequivocally demonstrated, it is probable that storage carbohydrates can play a significant role in eliciting this debilitating condition. Thus, until pasture NSC have been shown not to activate the onset of laminitis or exacerbate an existing condition, it would be prudent to reduce the perceived risk for animals predisposed to this disease. This can be achieved through a combination of both pasture and horse management practices to reduce the concentrations of WSC that accumulate on the one hand, and to prevent the ingestion of high concentrations by the grazing equine on the other. Therefore, where possible, horse pastures should contain either C4 species, or those C3 species or varieties that tend to accumulate low concentrations of WSC. Maintaining short, leafy grass swards, either by grazing or mowing (30), together with maintaining appropriate soil moisture and fertility, will encourage growth and utilization of NSC as opposed to storage. Restricting grazing to the early morning will avoid intakes of the highest concentrations of NSC encountered during the day, and the grazing of shaded pastures will help reduce NSC intakes. Susceptible horses should be prevented from grazing pastures at times of high light intensity and low temperatures, or other conditions that favor slow growth but high levels of carbon assimilation. Such conditions are often found in the spring and autumn, and particular care should be exercised at these times of the year. Furthermore, pastures that have “gone to seed” should be avoided, as well as recently harvested stubble fields where high concentrations of fructan may be present in the stem bases. Physical prevention of excessive herbage and NSC intakes by horses at pasture can be achieved through the use of grazing muzzles. The use of such devices reduces herbage bite mass and restricts intakes to the tops of leaves where the concentrations of NSC tend to be lowest; both of these characteristics reduce the overall intake of NSC. Animals on restricted grazing regimens require an alternative source of forage, and this is most commonly given in the form of hay. However, forage hays can contain high concentrations of NSC, and therefore all forages fed to susceptible animals should be accurately analyzed for their total NSC content, which includes sugars, fructan, and starch. In this context it is appropriate to discuss the different terms used to describe, and the various analytical procedures used to measure, the total nonstructural carbohydrate fraction and constituent components.

### NSC analysis and nomenclature

Forage laboratories in the U.S. were established initially in association with universities to support the Dairy Herd Improvement Association (DHIA). Their analysis of carbohydrates was designed to be appropriate to the digestive physiology and metabolism of bovids rather than equids (31). Furthermore, some commercial forage laboratories measure WSC and report it as sugar, which is misleading, because this so-called sugar consists of free sugars and fructan (31) (see Eq. 1 below). Other DHIA laboratories use the term sugar to describe the free sugar fraction only. Measurement of nonstructural carbohydrate has been undertaken in a number of ways by many analytical laboratories. The term NSC is used to describe 2 quite different fractions. NSC may be estimated by difference

(see Eq. 2), with the NSC value representing the sum of all starch, sugars, fructans, pectins, gums, and mucilages; or the NSC may be used to describe the sum of measured sugar (which, in this context, constitutes free sugars plus fructan, i.e., WSC) plus starch (see Eq. 3). Nonfiber carbohydrate (NFC), replaced NSC (Eq. 2), where crude protein (CP), neutral detergent fiber (NDF), and neutral detergent insoluble crude protein (NDICP) are taken into account (see Eq. 4). Although more definitive techniques for measuring fructan by enzymatic methods or chromatographic techniques have been developed (32), until they are routinely adopted by commercial laboratories, such detailed information is unlikely to be available to the majority of the equine community. Therefore, in the analysis of forage for equines, it is imperative that the user fully understands which fractions are included in the value to enable the development of suitable feeding regimens for equines predisposed to laminitis:

$$\text{Sugar} = \text{Free sugar \%} + \text{Fructan \%} \quad (1)$$

$$\text{NSC} = 100 - (\text{water} + \text{CP} + \text{Fat} + \text{Ash} + \text{NDF}) \quad (2)$$

$$\text{NSC} = \text{Starch \%} + \text{Sugar \%} \quad (3)$$

$$\text{NFC} = 100 - [\text{CP \%} + (\text{NDF \%} - \text{NDICP \%}) + \text{Fat \%} + \text{Ash \%}] \quad (4)$$

### Other considerations

Although a few animals within a herd may develop laminitis on a given pasture, the majority of the herd does not. This may suggest that some animals are either more predisposed to the condition, whereby the threshold for eliciting laminitis is reduced, or laminitic animals preferentially graze areas high in storage carbohydrates, or a combination of both. It is possible therefore; that the dosage of fructan (or total WSC or starch) required to elicit the onset of laminitis in susceptible animals may be somewhat lower than that used to induce the disease experimentally in healthy animals. However, horses typically graze for 12 to 17 h/d (33,34), and thus the intakes of storage carbohydrates are not delivered to the gut as a bolus, but rather, are trickle-fed throughout the grazing period. However, it is not known if continued ingestion of high concentrations of storage carbohydrates results in a chronic proliferation of lactate-producing bacteria and lowering in hindgut pH, or if it is a transient effect. Due to the continually changing concentrations of storage carbohydrate in some pastures, however, it might be difficult for the hindgut microflora to ever reach near steady state conditions. It is conceivable that, in susceptible animals that may already have a chronically perturbed hind-gut environment, the effects of acute ingestion of significantly elevated concentrations of storage carbohydrates may tip the balance toward a laminitic episode.

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