



## Finding and Testing Low-Sugar Forage

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Did the brown, stemmy, over-mature hay you thought was perfect for your easy keepers make them even fatter? Are increased sugar concentrations in your pasture causing your pony's recent bouts of laminitis, or increased muscle soreness in your equine polysaccharide storage myopathy (EPSM)-afflicted horse? Could high fructan levels caused by recent frosty nights be the reason why several of your pastured horses experienced gas colic or diarrhea recently?

If you find yourself asking these questions, you'll need to know how to sample grass and hay for testing, and be able to wade through the quagmire of myth, misunderstanding, and new science that is emerging in the area of carbohydrates in forage. Several studies conducted by Rocky Mountain Research & Consulting Inc. will help you get test results you can believe.

### Sample Selection

**Pasture** If a horse got too much of something sweet in the pasture, it's important to make sure you sample what is being eaten, which is frequently different from a random, representative sample of the whole pasture. If your horse seeks out clover first, it might be really interesting to see if the clover was higher in sugar and starch than the grass surrounding it. You might be tempted to cut the grass that's easy to collect while randomly walking around, but it's not worthwhile to sample tall grass that is headed out if the horses are selectively grazing short, new growth as the sugar content is probably very different. You might need to spend some time on your knees with clippers to get the pound of fresh grass that testing labs require.

**Hay** Grabbing handfuls of hay from the windrow in the field, or from individual bales, will give you extremely variable samples, not reliable test results.

### Editor's Note

Rocky Mountain Research and Consulting has conducted a good deal of research on carbohydrates in forage, primarily focusing on levels of nonstructural carbohydrates (NSC). As defined by plant scientists, this fraction includes sugar, starch and fructan--those carbohydrates found inside plant cells. These are high-energy, easily digestible carbohydrates that have been implicated to exacerbate some equine diseases such as laminitis and equine polysaccharide storage myopathy. However, nutritionists and forage analysis laboratories are moving away from using the term "NSC" to evaluate carbohydrates because they might define the term differently than plant scientists do. To avoid confusion, there is a new trend in favor of breaking this dietary component into more specific measures of water-soluble carbohydrates (WSC) and ethanol-soluble carbohydrates (ESC; for more information, see [www.TheHorse.com/ViewArticle.aspx?ID=9380](http://www.TheHorse.com/ViewArticle.aspx?ID=9380)).

When this article uses the term 'NSC,' it uses the definition that plant scientists have always agreed on. Its information on sampling forage and factors affecting carbohydrate levels in forages is extremely valuable for horse owners, particularly those with laminitic or metabolically-challenged horses.

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Hay on top of the windrow that is exposed to the sun might accumulate more sugar before it dries down, while hay underneath in the shade will respire (convert sugar to energy to maintain its cells), decreasing its sugar content. Variations in soil and plant density can change sugar content dramatically from one spot to another. Much more believable sample results can be obtained after the hay is baled and stacked, using a hay probe and taking at 15 - 20 samples from around the stack, or preferably from the end of the stack that you will be purchasing. A hay probe slices through a whole bale, averaging out the variability from individual clumps of grass.

### Fresh Sample Handling

Cut grass is not dead. It will continue respiration if kept in the dark, or might make more sugar if left in bright light until it dries down enough to stop the enzymes that drive these processes. It also can start to ferment if sealed inside a warm sample bag. That's why fresh grass samples need to be frozen immediately to correctly reflect the sugar content they had when fresh--i.e., when your horse eats them. But freezers are not always close by, and shipping samples overnight is costly.

**Q:** How much does it matter how we handle fresh grass samples when we analyze for nonstructural carbohydrates (NSC)?

**A:** It matters a lot!

To answer this question, a pure stand of Italian ryegrass from a small area was cut with a motorized swather. The grass was mixed well, and four samples each for four different treatments were placed in gallon Ziploc bags (16 samples total). Two sets were placed in a freezer at -20°C (-4°F) within 15

Treatment	WSC (% of DM)	Starch (% of DM)	Total NSC (% of DM)	Change (%)
Freeze, ship overnight on dry ice (best method)	14.0 a	1.55ab	15.55 a	
Three hours to freeze, ship overnight on dry ice	11.1 b	1.7 a	12.9 b	-17%
Freeze, priority mail, no ice	9.3 bc	2.1 a	11.3 bc	-27%
Never frozen, priority mail	8.8 c	0.8 b	9.6 c	-38%

\* Mean of four reps, analysis by Dairy One/Equi-analytical, Ithaca, N.Y.

\* Numbers followed by the same letter do not differ significantly at  $p=.05$ . Numbers followed by a different letter are significantly different.



minutes of cutting. Of these, one was shipped on dry ice overnight, and the other was sent priority mail without ice. One set was placed in the back of a car with a min-max thermometer (which showed a temperature range of 78-94°F) for three hours while running errands, then placed in the freezer and shipped overnight while frozen. We'll get to the fourth sample shortly.

Overnight shipments were double-boxed in Styrofoam coolers and cardboard boxes with approximately two pounds of dry ice and were received by the lab about 19 hours after being taken out of the freezer. The lab reported that they arrived cold, but not frozen. The fourth set of samples was taken directly to the post office and mailed priority mail with no cooling. Priority mail shipments took 2.5 days to reach the testing lab. The results show that sample handling is very important to get believable test analysis for NSC on fresh forage; mishandling can lead to underestimation of as-fed NSC and thus underestimation of the forage's danger to a carb-sensitive horse.

If you are going to ship pasture samples, ask your vet for a vaccine box, or buy an inexpensive Styrofoam cooler. Many grocery stores carry dry ice (check your shipper's regulations regarding dry ice), or add a frozen gel pack. Take a cooler with wet or gel ice to the field to collect fresh forage samples if you cannot get them to a freezer quickly. Careful handling can produce test results that might enable you to properly quantify the carbohydrates your horse is eating, so you can act to prevent flare-ups of any carbohydrate-related diseases when the conditions that produce high-sugar grass reoccur (more on these conditions in a moment).

### High fiber does not ensure low NSC

You might have heard that NSC can be roughly estimated if you know the fiber content of the forage--that if fiber is high, NSC is low and vice versa. However, this is not at all true. Let's investigate why.

Old-school nutrition is based on proximate analysis. This a method of quantifying fractions in forage by subtracting out things like fiber, protein, fat, and ash and calling the leftover non-fiber carbohydrates (NFC). NFC is not the same as NSC. If based on calculation or estimation instead of direct analysis, the resulting fraction will be NFC. While NFC does include sugar and fructan, it also includes pectin and organic acids, so it cannot be used interchangeably with NSC, although unfortunately this continues to occur. No wonder we get confused! This is why forage laboratories are moving away from using either term.

Nutritionists like to express forage components as a percentage of dry matter, and based on proximate analysis, assume that if one fraction is higher it will result in the other fractions being lower. The two biggest variables in proximate analysis are fiber and NFC. This leads a lot of lay people to assume that high fiber will insure lower sugar content, and unfortunately some nutritionists still believe this as well.

### Carbohydrate Definitions

**Structural carbohydrates** Cellulose and hemicellulose, a.k.a. fiber, found in plant cell walls.

**Nonstructural carbohydrates (NSC)** Sugar, fructan, and starch found inside plant cells.

**Nonfiber carbohydrates (NFC)** A fraction of plant nutrients calculated by proximate analysis that includes NSC plus pectin and organic acids. This term is used mostly by nutritionists.

**Neutral detergent fiber (NDF)** A fraction determined by proximate analysis that contains cellulose, hemicellulose, and lignin.

**Dry matter (DM)** The only fair way to compare different forages because the amount of water varies so much.

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Forage scientists quantify NSC in relationship to the amount of structural carbs, fully acknowledging that the association is highly variable. Units of NSC will be expressed as milligrams of non-structural carb per gram of structural carb. This might seem to be just a technical difference in nomenclature, but it goes right to the root of the misunderstanding.

The fiber in plant cells is found in the cell walls; hence the term Structural Carbohydrates. NSC is stored in organelles called chloroplasts and vacuoles, found inside the cells. We can make an analogy to a warehouse with storage bins. The warehouse can be empty or can have all the storage bins full, without changing the mass of the walls. NSC can still be high when fiber is high. They are not mutually exclusive because they don't fill up the same spaces. If you pick out hay for your carb-intolerant horse because it's overly mature and high in fiber, expecting it to be low in NSC, you could be disappointed. The following studies confirm this concept.

### **NSC vs. Fiber**

Fresh grass samples from 13 grass species, four replicates each, were collected from 3:30-6:30 pm on July 24, fully a month after local first cutting hay is usually made. The timothy and redtop, being later maturing, still had some heads in flower stage, hence they were not as mature as the other varieties. Samples were frozen within 15 minutes at  $-20^{\circ}\text{C}$  ( $-4^{\circ}\text{F}$ ), and shipped to Dairy One/Equi-analytical in Ithaca, N.Y., overnight with dry ice. The results show the correlation between NSC and neutral detergent fiber (NDF) is only 0.66.

In plain language, this means that while fiber content is a large factor in the NSC content of forage, it is far from the whole story. Grass that is very over mature and very high in fiber (60%+ NDF) can still be very high in NSC; in this set of high-fiber samples it comprised up to 20% of dry matter. These samples were pulled during warm weather, properly fertilized, and irrigated to prevent drought stress (which can cause accumulation of NSC).

In another study, two plots of oats were planted side by side, one in early spring and the other in early summer, and the study was repeated over two growing seasons. The spring planting was subjected to freezing nights in early stages of growth, while the other got frosted in late maturity. Four replicated samples were pulled at eight different stages of growth from each plot, all the way to full maturity, when all the green was gone from the straw, and when the grain was fully mature. These were analyzed by USDA Forage and Rangeland Research Lab in Logan, Utah, using purified enzymes to quantify sugar, starch and fructan separately. The oat hay made when nights were below  $40^{\circ}\text{F}$  was highest in NSC, ranging from 25-30% of dry matter, regardless of stage of growth. Fully mature, high-fiber straw and oats had as much NSC as oats in boot stage in early spring, due to high levels of fructan (a type of storage carbohydrate associated with laminitis episodes) that form under cold stress. Environmental conditions just before cutting affect NSC content more than stage of growth when cut.

### **You Can't Tell by Looking**

Sugar content of grass hay is not related to color, texture, amount of stem, or age of the haystack. Contrary to popular myth, the NSC content of well-cured, properly stored hay will not change over time. The only reliable way to choose appropriate hay for your carbohydrate-intolerant horse is to get it



tested using a direct analysis method.

In your quest to find low-NSC hay, an interview with the grower (to get information about pre-harvest weather conditions) will be far more useful than any visual characteristic of the hay. Some conditions help reduce NSC in forages, and hearing that such conditions were present can help you narrow down which stack to test, if the hay grower has not already done that.

Seek hay that is:

- **Properly fertilized**--Slow growth due to nutrient deficiency can cause high NSC; fertilized stands should have grown quickly and avoided this

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Grass variety (grouped by significant difference in NSC content)	NSC (% of DM)	NDF (% of DM)
Roadcrest crested wheat-grass	20.2 a	61.7 cd
Garrison meadow foxtail	19.9 a	57.3 e
Climax timothy	17.2 ab	59.6 de
Potomac orchardgrass	15.4 bc	61.9 cd
Cache meadow brome	13.8 bcd	66.5 abc
Sherman big bluegrass	13.4 bcd	67.5 a
Regar meadow brome	13.0 cd	64.7 abc
Ginger Kentucky bluegrass	12.8 cd	63.8 abc
Fawn tall fescue	12.8 cd	62.3 cd
Wideleaf orchardgrass	12.4 cd	61.9 cd
Manchar smooth brome	12.0 cd	63.3 bcd
Redtop	10.7 d	64.5 abc
<i>Least significant difference (p=.05)</i>	3.55	3.37

\* Mean of 4 reps

\* Values followed by the same letter do not differ significantly.

problem. Also, thick stands shade lower leaves from the sun, thereby reducing their ability to make sugar.

- **Cut after a period of cloudy weather**--Plants synthesize sugars during sunny weather, and periods of cloudy weather will result in reduced NSC.
- **Rain after cutting may be a plus** if humidity is low and hay dries without mold. Some carbohydrates might dissolve in water and leach out of the hay.
- **Mixed with legume**--If your horse has not previously shown sensitivity to alfalfa, grass mixed with a little alfalfa may be low in NSC as the alfalfa provides needed nitrogen (fertilization) and shades the grass. Be careful; some laminitic horses seem to react to some unknown triggers in alfalfa and clover.
- **Already tested for NSC**--Let your hay grower know that this information



is valuable to you so you can choose hay that is appropriate for individual classes of animals. With proper testing, he may be able to sell his high-sugar hay at a higher price for dairy cattle or performance horses. He may be able to sell his rained-on, but not moldy hay for a better price if tests low in NSC and therefore is more suitable for easy keepers and metabolically challenged horses. Let him know that horse owners are requiring appropriate testing, just like his dairy clients do.

Hay is likely to be higher in NSC if:

- It was cut after drought or cold nights, especially when cured under cloudless skies.
- It comes from thin, sparse stands of grass that have more sunshine on every leaf.
- It was grown under growth-limiting levels of nitrogen, phosphorus or potassium (inadequate fertilization).
- It is made from cereal grains such as oat, wheat, rye or barley that tend to be higher in NSC, even if the grain is not developed yet. The stems collect sugar in preparation for seed development.

### **Take-Home Message**

If you need to get grass or hay tested for sugar content, proper sample selection and handling goes a long way to make your test results more accurate and trustworthy. If it's worth doing, it's worth doing right. Freeze fresh grass samples immediately and ship them fast and frozen. Sample 15-20 bales of hay with a hay probe, and let your hay grower know that horse folks are now educated enough to insist on a hay test.

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