Carbohydrate Testing of Plants and Feeds

Introduction

There is an increased interest in evaluating the nutritive quality of forage samples for the purpose of improving ruminant animal production particularly in the high value dairy sector. Carbohydrates are an important component of all forages as they provide a major source of energy to grazing ruminants such as cows, sheep and deer and they also contribute to the efficient utilisation of feed and to the maintenance of healthy animals. This technical note introduces the major categories of carbohydrates that are present in plant tissue as well as providing brief notes on the carbohydrate methods used at Hill Laboratories.

The Function and Classification of Plant Carbohydrates

Photosynthesis and Energy

Carbohydrates are present in all material of vegetable origin and fulfil a number of vital structural and metabolic functions. Energy for growth is derived from photosynthesis which uses UV energy from sunlight combined with water and carbon dioxide to synthesis **glucose** which provides an immediate form of energy to plants. Glucose is a six carbon sugar with the atomic formula of $C_6H_{12}O_6$ or $C_6(H_2O)_6$ - hence the classical name of "carbo-hydrate". It is one of several simple sugars which are present in all plants. Plants are also able to store energy in the form of **starch** which is a polymer composed of glucose units joined together in long chains which interlink with each other. Glucose is classified as a **monosaccharide** and using this nomenclature, starch is known as a **polysaccharide**. These carbohydrates are known collectively as **non structural carbohydrates.**

- **Plant Cell Wall Carbohydrates**

The physical and structural integrity of all plants is provided by the polysaccharides which constitute the plant cell walls and these are termed the **structural carbohydrates.** These are high molecular weight polysaccharides composed of monosaccharides such as glucose, xylose, arabinose, mannose, galactose; and sugar acids such as galacturonic and glucuronic acids. **Cellulose** is the most important and abundant of these polysaccharides and it forms the backbone of all plant tissue. It is chemically very inert, in contrast to starch, but is partly digestible by ruminant animals.

Cell wall polysaccharides give plant stems, stalks and trunks their physical rigidity and the ability to stand up, so it is not surprising that the cell wall content of all plants increase with increasing maturity and as the plants grow taller.

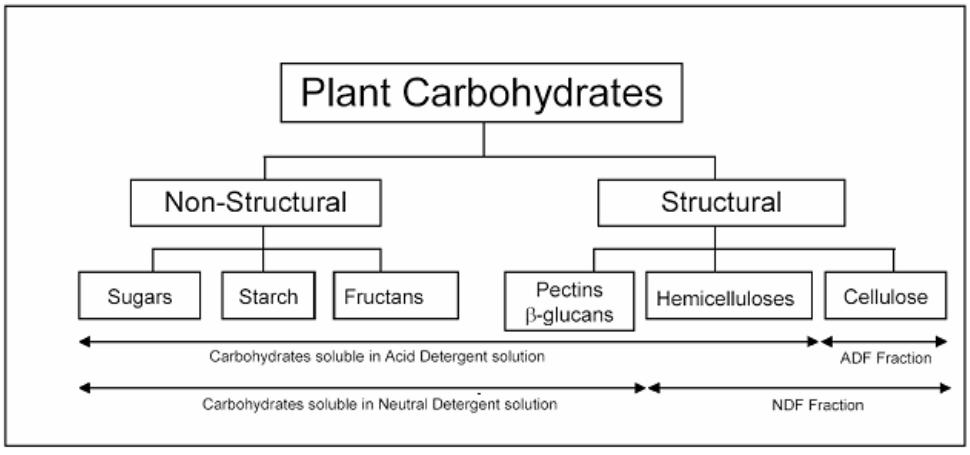

Figure 1. Empirical Fractionation of Plant Carbohydrates (Modified diagram ex Mary Beth Hall)

Carbohydrate Fractions in Pasture and Forage Materials

The classification of carbohydrates in vegetable matter is complex and quite dependent on species.

A simplified view of the major plant carbohydrates of relevance to pastures and forages is shown in Figure 1 and these fractions will be discussed in more detail below.

Non Structural Carbohydrates

Non structural carbohydrates include the **simple sugars** (e.g., glucose, fructose, sucrose, raffinose etc), **starch** and **fructosans**. Fructosans are storage polysaccharides composed entirely of fructose sugars and are generally found in temperate grasses only. They are also much less abundant than starch and often ignored in terms of forage quality. The sugars are soluble in water or alcohol/water mixtures and for this reason, they are usually described as "water-soluble carbohydrates". The higher molecular weight polysaccharides are generally insoluble in cold water although some fructosans are partially soluble in alcohol/water mixtures.

Non structural carbohydrates are easily digested by ruminants and form an important part of their diet. Sugars are important for stimulating and maintaining microbial activity for efficient digestion in ruminant animals and starch provides a readily available source of energy. The levels of sugars and/or starch present in forages and feeds vary widely and some typical examples are given in Tables 1 and 2.

Table 1 Approximate Soluble Sugar levels in some Forages, Feeds and Concentrates

Sample matrix and examples	Present	Range
Temperate pastures (ryegrass, fescue)	moderate levels	5 – 15 %[1]
Fruit wastes (apple pomace etc)	moderate levels	> 10 %[1]
Legumes (clover, lucerne)	low levels	< 5 %[1]
Molasses	high levels	> 60 %[2]
Fodder and sugar beets	high levels	> 60 %[1]
Some tubers (kumara etc)	moderate levels	> 10 %[1]
Forage maize	low levels	< 5 %[1]
Fruit juice concentrates	high levels	up to 70 %[2]
Malt extract syrups	high levels	variable
Confectionary wastes	low levels	variable

Table 2 Approximate Starch levels in some Forages, Feeds and Concentrates

Sample matrix and examples	Present	Range
Cereal grains (maize, barley, wheat)	high levels	65 - 75 %[1]
Potatoes	high levels	< 70 %[1]
Other root crops (taro)	high levels	> 75 %[1]
Temperate pastures (ryegrass, fescue)	none	> 0.5 %[1]
Legumes (clover, lucerne)	low levels	> 5%[1]
Bread and bakery wastes	moderate levels	variable
Forage maize	high levels	10 – 35 %[1]

1. Dry matter basis
2. Fresh weight basis or ‘as received’

- **Analysis of Soluble Sugars**

Soluble or ‘free’ sugars in plant tissue are analysed by extraction with aqueous ethanol, followed by determination by the phenol-sulphuric acid colorimetric procedure which gives a response for almost all carbohydrates in solution. Further information on this method can be found in the references of Dubois *et al* and Hall.

Note: Soluble sugars in animal feeds containing lactose (including whey permeate) may be under-estimated by this aqueous ethanol method, due to the low solubility of lactose in ethanol.

- **Analysis of Starch**

The method is based on the principle that starch is completely broken down to its constituent glucose sugars while still physically located in the sample. This degradation step is carried out using starch degrading enzymes (α-amylase, β-amylase and several dextrinases etc), collectively known as ‘amyloglucosidase’, and which are specific for starch only. The starch

content is then calculated from the amount of glucose produced. The first step in the method requires that any soluble glucose present in the sample must be removed prior to the starch 'hydrolysis' so that it does not contribute to the final starch result.

Structural Carbohydrates

These carbohydrates form an essential dietary "fibre" component of all grazing ruminant animals, but are generally less digestible than the non structural carbohydrates. In order of importance they include **cellulose, the hemicelluloses, pectins and β-glucans**. Cellulose, like starch, is composed of glucose units although the linkages between the glucose units are different from those in starch molecules. For this reason, cellulose is extremely resistant to chemical breakdown although it can be hydrolysed to its component glucose units by cellulase enzymes produced in the rumen (and elsewhere) by micro-organisms. Hemicelluloses are broadly similar to cellulose although they are generally composed of more than one of a number of different sugars. Common examples include the arabinoxylans (composed of arabinose and xylose) and the galactoglucomannans (composed of galactose, glucose and mannose). The β-glucans (composed of glucose) and pectins (composed of galcturonic acid units) are present in plant cell walls at much lower levels and do not impact on forage quality to the same extent as cellulose and the hemicelluloses. The β-glucans are found in some cereal grains such as barley, and pectins are an important component of citrus fruit. Although not a carbohydrate, **lignin** is an equally important constituent of plant cell walls. It is a complex three-dimensional polymer based on repeating aromatic units (as in phenyl groups) and it acts as the cement which binds all of the polysaccharides into a semi-rigid structure. It is chemically very inert and not digestible.

- **Analysis**

Most of the plant structural polysaccharides are very difficult to analyse using direct wet chemistry methods because they are difficult to isolate and extract from the sample matrix in relatively pure form and without being subjected to molecular degradation. Furthermore, the procedures that are available for polysaccharide analyses are very time consuming and complex and not suited to the routine testing of forage samples. These polymers are generally not soluble in organic solvents, and selective fractionation schemes using aqueous based solvents such as weak acids and/or strong alkalis tend to be non-specific and often partially degrade the target polysaccharide fractions during the extraction sequence. Because of this, empirical methods based on the use of hot detergent solutions have been developed for estimating particular carbohydrate fractions of interest to feed and forage quality, and these methods form the basis of the neutral detergent fibre (**NDF**) and acid detergent fibre (**ADF**) tests respectively. Figure 1 has been taken from a publication from Mary Beth Hall, University of Florida, and shows a diagrammatic presentation of the important carbohydrates found in plants and how they are fractionated by hot detergent solutions.

- **Neutral Detergent Fibre**

The NDF extraction involves treatment with hot detergent adjusted to a pH of 7.0 and is assumed to "dissolve" all of the sugars, starch, pectins, soluble β-glucans, protein, oils, minerals, and secondary metabolites leaving only the hemicelluloses, cellulose and lignin to remain as the "neutral detergent fibre". In practice, the residual NDF fraction may contain small residues of protein and ash. As with all empirical procedures, obtaining repeatable and reproducible results is dependent on absolute adherence to the method and any deviation, however minor, may affect the results.

- **Acid Detergent Fibre**

The acid detergent reagent employs dilute sulphuric acid to break down or hydrolyse the cell wall hemicelluloses so part of this degraded carbohydrate fraction is also dissolved and washed away during the filtration step as with the other "detergent soluble" constituents leaving only the cellulose and lignin remaining as "acid detergent fibre". As with the NDF fraction, the ADF may contain a small amount of residual protein and ash. The ADF value for a sample should always be less than the NDF value and the two results can be used to produce a reasonable approximation of the hemicellulose fraction of the sample;

Hemicellulose content (%) = NDF – ADF

The ADF procedure may be carried out directly on the sample and is referred to as **ADF (direct).** Alternatively, the ADF procedure may be performed on a sample that has already been tested for NDF; reported as **ADF (sequential**).

The purpose of sequential ADF is to remove pectin and biogenic silica that cause a positive bias in the direct method. A variety of forages commonly used in New Zealand contain relatively high levels of pectin and/or biogenic silica e.g. brassica, plantain, chicory, lucerne. The ADFsq method has been adopted as the default test for "ADF" at Hill Laboratories (from autumn 2017).

Glossary of Terms

The following listing defines some important carbohydrate terms:

Acid detergent fibre (ADF)	cellulose and lignin fraction in forages estimated by empirical method
arabinose	a pentose sugar which occurs naturally in all plant tissue
Crude fibre	empirical method for estimating plant based fibre in meat products including petfoods
degree of polymerisation	number of sugar units in oligosaccharides or polysaccharides
dextrins	oligosaccharides and higher molecular weight polysaccharides usually derived from the malting process or from maize glucose syrups
dextrose	an alternative name for glucose
disaccharide	a carbohydrate composed of two sugar units, eg, sucrose, maltose, lactose
fibre	a term implying “carbohydrate fraction related to structural polysaccharides”
fructose	a hexose sugar which occurs commonly in plant tissue as a “soluble sugar”
β-glucan	a water-soluble polysaccharide composed of glucose sugars – occurs commonly in the cell walls of barley and oats grain
glucose	a hexose sugar which occurs commonly in all plant tissue – can be present as a soluble or “free sugar” and is the sole sugar constituent of starch, cellulose and β-glucans – it also occurs in other hemicelluloses such as glucomannans
hemicelluloses	cell wall polysaccharides which are composed of one or more sugar units and/or uronic acids
hexose	a six-carbon sugar, eg, glucose, fructose, galactose, mannose, rhamnose, fucose etc
hydrolysis	The chemical cleaving or breaking of the bonds (usually known as glycosidic bonds) that link individual sugar units together in oligo- and polysaccharides
lignin	The complex three-dimensional phenolic matrix or “cement” that binds cell wall polysaccharides together
mannose	a hexose sugar which is a common constituent of some hemicelluloses
monosaccharide	another name for a simple sugar – the building brick of all polysaccharides
Neutral detergent fibre (NDF)	fraction containing hemicelluloses, cellulose and lignin in forages and estimated empirically
oligosaccharide	a carbohydrate composed of a small to moderate number of sugar units, usually four or more sugars up to approx 20 units
pentose	a five-carbon sugar which occurs commonly in plant hemicelluloses, e.g. xylase
pectin	a polysaccharide composed of a backbone of galacturonic acid units
photosynthesis	the combining of carbon dioxide, water and UV energy from sunlight to form the simple sugar glucose in green plant tissue
polysaccharide	naturally occurring complex polymeric carbohydrates composed of large numbers of sugar units
raffinose	a trisaccharide (three sugar units) which occurs in trace amounts as a “soluble sugar” in many plant species
reducing sugar	a simple sugar which contains a free aldehyde functional group readily oxidisable to a carboxylic acid group, eg, glucose, fructose, maltose, lactose etc
sucrose	the most commonly occurring “soluble sugar” in most plant species – a disaccharide composed of single units of glucose and fructose
sugar	generic name for a simple carbohydrate, see “monosaccharide”
sugar alcohol	a simple sugar derivative where all carbon substituents are protons or hydroxyl groups, eg, sorbitol, mannitol etc
xylose	a commonly occurring pentose sugar which forms the backbone of hemicelluloses termed “xylans”, “arabino xylans etc

References

1. Dubois M, Gilles K A, Hamilton J K, Rebers P A and Smith F, **Colorimetric determination of sugars and related substances,** Analytical Chemistry, 1956, **28,** 350 – 356.
2. Hall M B, Hoover W H, Jennings J P and Miller-Webster T K, **A method for partitioning neutral detergent-soluble carbohydrates,** Journal of the Science of Food and Agriculture, 1999, **779**, 2079 – 2086.