

# Non-Fiber Carbohydrate Analysis: Where it is Headed

## Updating Terms, Methods, & Labelling

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For the last two years or so there's been an ongoing effort to allow labeling of animal feeds for carbohydrate content for various carbohydrates perceived as having impact on animal health and performance. Driven primarily by the horse and petfood industries and run through the Association of American of Feed Control Officials (AAFCO), this effort opened the way for some serious discussions on what carbohydrates matter in rations and how we measure them. AAFCO asked me and other scientists and people from industry to participate. For a carbohydrate to "matter", I mean that it is nutritionally relevant, differing from other carbohydrates in how it digests in the rumen or small intestine, and so could differ in what nutrients it supplied to the animal. The carbohydrates also had to have relevance across species (think cows, dogs, cats, horses). To figure out which carbohydrates mattered, I contacted nutritionists/analysts here in the US and internationally who were working with carbohydrates with a variety of animal species. We actually achieved a consensus on which carbohydrates mattered (which also agreed with recommendations for human nutrition on carbohydrates), and then sent our recommendations back to AAFCO where industry and regulators weighed in.

Non-fiber carbohydrate analyses currently used such as water-soluble or ethanol-soluble carbohydrates were too much of a mixed bag to be used. They contained too many different carbohydrates (like WSC = sugars + fructans together) that differed in digestion characteristics, and by feed source to be as specific as a good nutritional fraction. Now, that doesn't mean that WSC and ESC aren't useful, but they didn't meet the set criteria for regulatory use.

### Definitions

The definitions that have been adopted or are in the process of being adopted by AAFCO cover sugars, fructans, and starch. Trying to define starch was a hoot. The problem was that "starch" is already taken as a word describing a carbohydrate in plants. So, we came up with "dietary starch" to define what we really measure.

**Sugars** – The sum of all free disaccharides & monosaccharides such as: sucrose, lactose, maltose, glucose, fructose and galactose or others digestible by enzymes found in an animal's digestive tract. (*Note: Looking at only disaccharides potentially digestible by enzymes in the small intestine. MBH*)

**Fructans** – Polysaccharides and oligosaccharides in which fructose is the major constituent and glucose is the minor constituent. Glucose content is 33% or less. (*These carbohydrates are found in cool season grasses like timothy, ryegrass, etc. and in chicory. Included in pet foods as "pre-biotics", have been associated with potential for laminitis in large quantities. MBH*)

**Dietary Starch** (nutrient) An alpha-linked-glucose carbohydrate of or derived from plants, animals, or microbes from which glucose is released through the hydrolytic actions of purified alpha -amylases and amyloglucosidases that are specifically active only on alpha-(1-4) and alpha-(1-6) linkages in samples that have been gelatinized in heated, mildly acidic buffer. Its concentration in feed is determined by enzymatically converting the alpha-linked-glucose carbohydrate to glucose and then measuring the liberated glucose. This definition encompasses plant starch, glycogen, maltooligosaccharides and maltose/isomaltose.

**AAFCO Task force comments:** In layman's terms these are carbohydrates that digest like starch. They do not include sugars like sucrose in molasses or more resistant carbohydrates (e.g., resistant starch) that are included in dietary fiber. Current definitions for starch only talk about plant sources but the lab methods used to measure them also pull the compounds specified in this new feed term.

Sugar analysis is being approached through chromatography (HPLC and HPIC) to allow analysis of these carbohydrates as analytes. We are evaluating methods of extraction (see below). Fructan analysis will probably be by extraction, measurement of sugars, and then by acid or enzyme hydrolysis and measurement of the simple sugars released from the fructans. Dietary starch analysis will be very similar to methods currently in many labs.

#### **Current Research: Sugar & Fructan Analysis**

A concern that I have had is, if regulatory agencies use analyses that are not used in commercial feed analysis laboratories, what manner of havoc will follow when tag values don't match up with purchased analyses? So, a continuing focus is getting a handle on how the values of methods used by commercial labs compare with the "gold standard" methods used by regulatory labs. Through collaboration with USDA-ARS colleagues in Utah who have focused on fructan analyses, we are evaluating the choice of method of extraction of sugars (a relatively short list focussing on dried feedstuffs), and the comparability of colorimetric analyses (phenol-sulfuric acid assay, anthrone, reducing sugar) to the gold standard method of High Performance Anion Exchange Chromatography with Pulsed Amperometric Detection. We will also be evaluating options for analysis of grass fructans -- it seems likely that a mild acid hydrolysis will be used rather than enzymatic hydrolysis, based on the lab's evaluation of efficacy of available enzymes. Better if we understand how the regulatory and typical feed analyses agree or relate.

#### **AOAC: Starch Analysis of Animal Feeds**

The AOAC method 14.075 for starch in animal feeds is no longer valid because of discontinued production of the enzyme "Rhozyme-S" (Rohm and Haas, Philadelphia, PA) specified in the procedure. As of December 2007, our 5 year plan of research was approved that includes putting an assay for analysis of starch in animal feeds through the AOAC collaborative study process. The starch analysis that I think is most robust that I am recommending for this effort is the modified Bach Knudsen assay (only sodium acetate buffer used, two stage  $\alpha$ -amylase and amyloglucosidase hydrolysis, measurement of released glucose; free glucose can be measured and subtracted, or pre-extracted). I will be seeking labs to be a part of this collaborative study.

#### **Comparability of Ethanol-Soluble vs Water-Soluble Carbohydrate Measures**

I have received a number of questions regarding the comparison of water-soluble carbohydrates (WSC) and ethanol-soluble carbohydrates (ESC; typically 80% ethanol). The main concerns

were when WSC was less than ESC, or when the two values were closer than expected. Theoretically, WSC should be bigger than or equal to ESC, because water should extract more and larger carbohydrates than ethanol, but that also depends on extraction conditions and the substrate. Typically, different methods are used to measure the extracted carbohydrate with ESC (often the phenol-sulfuric acid assay) and WSC (usually reducing sugar assays). To measure WSC, the carbohydrates are typically hydrolyzed with acid to release the monosaccharides for measurement (sucrose, fructans, etc. will not be completely measured without hydrolysis). Fructans can be found in ESC or WSC, depending on the length of the fructan.

Some possible explanations of why ESC could be greater than WSC:

- ◆ When the values are small (1 - 2% of dry matter) the normal variation in the analytical results that we get from sampling and even in the method itself may not allow accurate comparison between the methods. Making some numbers up for example if you are measuring a feed that has 4% ESC and 5% WSC and either method is good for + or - 2%, the analyzed values may not look like they are very different, or ESC could even be bigger than WSC (range of values for ESC of 2 to 6% and for WSC 3 to 7%).
- ◆ Because ESC and WSC use different detection methods, it is possible that the reducing sugar analysis used on the WSC could give different values than the phenol-sulfuric acid assay used on the ESC for the same quantity of sugars. The standard sugar used for the phenol-sulfuric acid assay should be the predominant sugar in the feed (why we typically use sucrose). But with a blend of extracted carbohydrates (monosaccharides, disaccharides, oligosaccharides), the use of a single carbohydrate as the standard won't be precisely accurate. The selection of standard could become more of a problem if a greater diversity of carbohydrates are extracted (as would likely be the case with water over ethanol extractions, but that would also depend on the substrate). The reducing sugar analysis can have interference from extracted proteins and reducing substances.

So, the values for ESC and WSC may or may not be exactly comparable.

