



FOSS

eBook

Fibre analysis of animal feed

Crude fibre, neutral detergent fibre
and acid detergent fibre – the standards
and the automation options

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ANALYTICS BEYOND MEASURE

PREFACE

It's a simple fact that fibre is critical for well-balanced feed, but from there on, it gets a little involved.

There's the trend from crude fibre to detergent fibre with corresponding standards to consider, especially for labelling purposes or in the case of trade disputes. Then there's the question of methods to choose, both in terms of fulfilling those standards while maintaining lab efficiency and throughput. Last, but not least, there's the essential role of near infrared analysis in routine testing of fibre with reliable effective calibration against reference methods.

While the ultimate fibre solution is always going to be elusive, this e-book bring together relevant angles on the subject based on articles, whitepapers and interviews conducted over recent years of product development at FOSS. With progress on standards, automation of chemical methods and effective calibration of NIR, it becomes clear that a global harmonized approach to fibre analysis in feed is now a reality for the modern feed laboratory.

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Fibre analysis overview: From crude to detergent fibre and the creation of a global fi- bre reference

The demands for fibre content in feed are growing. For monogastric animals a proper proportioning of fibre fractions increases the utilisation of the compounded feed, while for ruminants, fibre is an important part of the metabolism in the rumen. Fibre is a determining factor for the hydrolysis of all nutritional ingredients in the feed.

Plant fibre comes from the material that constitutes the cell walls. Inside are the fibre constituents such as cellulose, hemi cellulose and lignin. The rest is undegraded protein, pectin, water and ash.

Defined by the way the analysis is done

As you probably have guessed fibre is by no means clearly defined by a single group of components – basically fibre is defined by the way we historically have done the analyses.

This statement from AAFCO – Association of American Feed Control Officials sums-up the situation: “Because there is no guarantee of direct correspondence between chemical solubility and nutritional availability, in reality, **fiber is defined by the method used to isolate it.**

The actual definition of fiber becomes method-dependent, which explains why there are so many different fiber analyses.” Quote is from ***Critical Factors in Determining Fiber in Feeds and Forages*** AAFCO’s Laboratory Methods and Services Committee, Fiber Best Practices Working Group, February 2017 (Revision 1).

Detergent fibre overview

Though developed in the beginning of the 19th Century, many estimates of the nutritive value of vegetables and forages are still calculated on basis of crude fibre values using the so-called Weende method. Even though there are several challenges with the crude fibre method as an

estimate of the amount of fibre or plant cell wall. However, in recent years, livestock nutritionists have begun using Neutral Detergent Fibre (NDF), Acid Detergent Fibre (ADF) and Acid Detergent Lignin (ADL) as indicators of dietary energy and intake, especially for ruminant rations. As a result, these fibre fractions have replaced crude fibre (CF) in ration formulations in many parts of the world. Today ADF and NDF values are frequently used to estimate the amount of forage that can be digested by animals; the total digestible nutrients and other energy values, as well as the relative feed value (an index used to allocate the correct forage to specific animal performance) to price hay and to assess forage management, harvest and storage skills.



For ruminants, fibre is an important part of the metabolism in the rumen.

The detergent system of feed analysis was developed by Peter Van Soest at the United States Department of Agriculture in the 1960s and is today one of the most important sets of feed assays in ruminant nutrition, but also, increasingly, in non-ruminant research.

The concept behind detergent fibre analysis is that plant cells can be divided into less digestible cell walls (comprising hemicellulose, cellulose and lignin) and mostly digestible cell contents (comprising starch and sugars). These two components can be separated by using two detergents: a neutral detergent and an acid detergent. Neutral Detergent Fibre is a good indicator of bulk and thus feed intake. Acid Detergent Fibre is a good indicator of digestibility and thus energy intake.

In summary, with the van Soest method, improvements were made to reduce errors from poor recoveries of

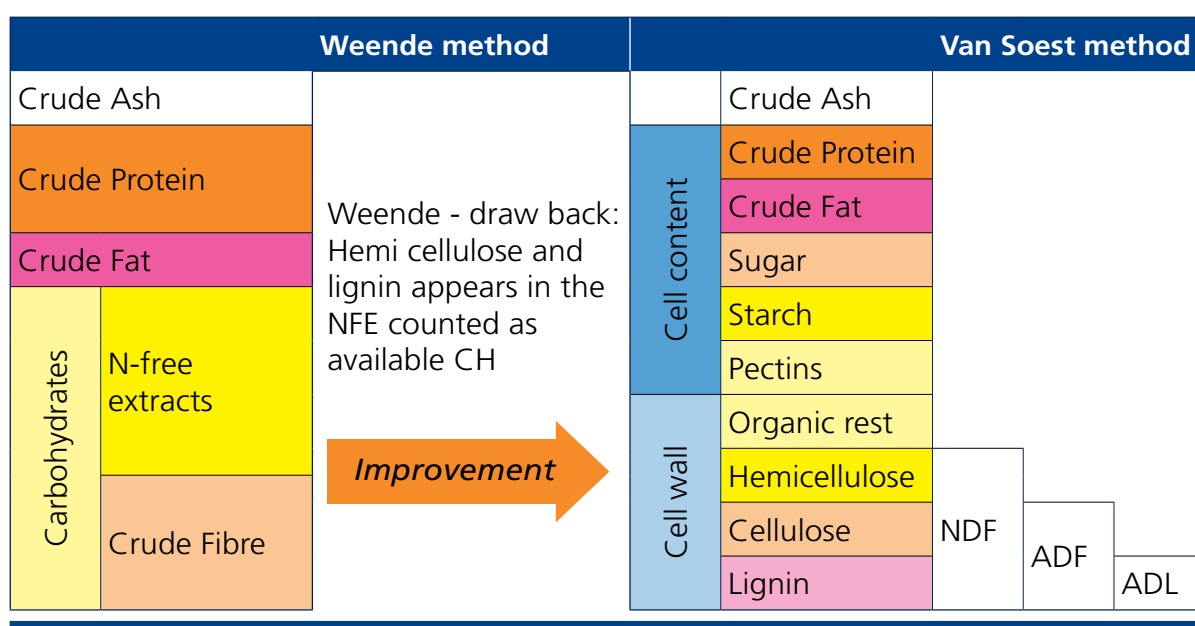


Figure 1 The trend from crude to detergent fibre.

hemicellulose and lignin. The method allows for a sequential fractionation of the fibre fractions into NDF, ADF and ADL, Acid Detergent Lignin (see figure 1) and thus is a better method to calculate for instance animals energy intake from feed.

Some definitions

Crude Fibre (CF) – a chemical method used to describe the indigestible portion of plant material. However, some of these substances can be partially digested by microorganisms in the rumen of cattle. The higher the fibre, the lower the energy content of the feed. It is not a very useful value. The practice of analyzing for it in feeds for ruminants is declining, but it is still commonly used for monogastrics (for example, pigs).

Neutral Detergent Fiber (NDF) – The NDF value is the total cell wall which is comprised of the ADF fraction plus hemicellulose. NDF values are important because they reflect the amount of forage the animal can consume. As NDF percent increases, the dry matter intake generally decreases.

Acid Detergent Fiber (ADF) – The ADF value refers to the cell wall portions of the forage that are made up of cellulose and lignin. These values are important because they relate to the ability of an animal to digest the forage. As ADF increases the ability to digest or the digestibility of the forage decreases.

Acid Detergent Lignin (ADL) – The Lignin fraction of ADF.

CHAPTER 2

Crude and detergent fibre technique

Crude fibre technique

In the so-called Weende method, (fig. 1) Crude Protein, Crude Fat and Crude Ash are determined and the moisture and carbohydrate content can then be calculated by difference: Carbohydrates = amount of total sample – moisture – Crude Protein – Crude Fat.

- History:
Developed in Möglin, Germany (1806) by the chemist Heinrich Einhof (1777-1808)
- Use:
Quality estimate of foods of plant origin in feed trading
- Content:
Indigestible substance – Cellulose, hemicellulose, lignin
- Extraction:
Hot H₂SO₄ (1.25%, w/v) – removes free sugar, starch
Hot NaOH/KOH (1.25%, w/v) – removes protein, saccharides

Deficiency:

Much of hemicellulose (up to 80%) and lignin (50-90%) are removed in the sequential acid and alkaline extractions. As a result, crude fibre will be underestimated

In addition, Crude Fibre determination by acid hydrolysis with 1,25% H₂SO₄ is used for the extraction of sugars and starch, followed by alkaline hydrolysis with 1,25% NaOH, which removes proteins and some hemicellulose and lignin (fig. 1). Crude Fibre is commonly used to estimate

the quality of foods of plant origin on the premise that it constitutes their least digestible fraction. The Nitrogen-free Extracts (NFE) are calculated by forming the difference (total) carbohydrates minus Crude Fibre.

On average, 80% of the hemicellulose or pentosans and from 50 to 90% of the lignin are removed by the acid and alkaline sequential extraction, while cellulose recovery is 50-80%. Thus, much of the hemicellulose and lignin appears in the Nitrogen-free Extract (NFE) to be counted as available carbohydrate. The NFE of straws and grasses may contain as much as 90% of these substances. Because of the failure of the crude fibre method to recover indigestible substances, NFE appears less digestible than crude fibre in a significant number of cases. In the case of vegetables and cereals, the error is less because of the relatively lower content of hemicelluloses and lignin. However, it may be substantial.



Automation options can accelerate fibre analysis while making it safer and more consistent

Detergent fibre technique

There have been various attempts to replace the Crude Fibre method with a system of analysis which gives a better characterization of the less nutritive fraction of food. Most successful has been the concept of Detergent Fibres developed by van Soest and colleagues.

History: Developed in 1963 at Cornell University by Dr. Peter J. Van Soest

Parameter: Neutral Detergent Fibre (NDF)

- Cellulose
- Hemicellulose
- Lignin

NDF extraction:

- Heat-stable α -amylase – hydrolyses starch
- Sodium Lauryl Sulfate (SDS) – form soluble complexes with proteins
- Triethylene glycol – remove non-fibre soluble material
- EDTA – prevent insoluble calcium-pectin matrices to form, and thus dissolves pectin
- Borate and phosphate buffer – maintain pH 7 and prevents hydrolysis of Hemicellulose

In a first step, the sample is treated with a neutral detergent solution (NDS) and rinsed with a heat-stable amylase to make the sugars, starches and pectins

soluble. The remaining residue consists of the non or less-digestible cell wall substances hemicellulose, cellulose and lignin. In a second step, hemicellulose is made soluble using an acid detergent solvent (ADS). The residue, consisting of cellulose and lignin is then treated with concentrated sulfuric acid, dissolving the cellulose and leaving the lignin in the residue. These steps can be performed consecutively or separately to determine the Neutral Detergent Fibre (NDF), Acid Detergent Fibre (ADF) and Acid Detergent Lignin (ADL).



For monogastric animals, a proper proportioning of fibre fractions increases the utilisation of the compounded feed.

Overview of global standards

In the field of standards, developments have been made to keep pace with trends in fibre analysis, but inevitably, somewhat retrospectively.

The most recent development of note is a new international standard for acid detergent fibre, issued in 2008 which compliments an existing standard for neutral detergent fibre. The new standard is entitled 'EN ISO 13906:2008 Animal feeding stuffs – Determination of Acid Detergent Fibre (ADF) and Acid Detergent Lignin (ADL) contents'.

The significance of this is that, while discussion continues about analysis methods, we now have a worldwide standard for ADF and ADL to refer to alongside the well-established Crude Fibre, thereby allowing feed industry players to achieve results that are valid on a worldwide basis. This is particularly relevant for labelling and trade of feed raw material and compound feed.

For ruminant feed labels:

ADF and NDF guarantees

For non-ruminant feed labels:

CF guarantees

For both ruminant and non-ruminant feed labels:

ADF, NDF, and CF guarantees

More background about the ADF standard and the study results behind it can be found in this whitepaper entitled: Animal feeding stuff: “Global Standard for the Determination of Acid Detergent Fibre (ADF) and Lignin” by Dr Jürgen Møller in 2008.

[Read more here](#)

Global Standards overview:

The development of the 2008 standard now gives us a set of global standards for CF, NDF, ADF, ADL

EN ISO 6865 (AOAC 978.10) refers to Analysis of Crude Fibre (CF) in Feed, describes an analytical procedure based on the crucible or Fibertec™ method.

EN ISO 16472 (AOAC 2002:04) refers to Analysis of Neutral Detergent Fibre (NDF) in Feed, describes an analytical procedure based on the crucible or Fibertec™ method.

EN ISO 13906 (AOAC 973.18) refers to the Analysis of Acid Detergent Fibre (ADF) and Lignin (ADL) in Feed, describes an analytical procedure based on the crucible or Fibertec™ method.

Related global standard for measurement of fibre with NIR

For near infrared analysis in routine testing of fibre with reliable calibration against reference methods, there are also guidelines to follow in 'ISO 12099: Animal feeding stuffs, cereals and milled cereal products – Guidelines for the application of near infrared spectrometry.'

This provides definitions and guidelines including how calibrations should be checked against reference measurements (section 11). This does not state exactly which method to use, but the guidelines provide a common international reference.



Standards are particularly relevant for labelling of feed raw material and compound feed for trade.

CHAPTER 4

The importance of automation: speed, safety and reduced opportunity for error

In addition to methods and standards, another important aspect of fibre analysis is the possibility to automate the steps involved. To illustrate this, the following example based on a classic method for crude fibre shows the typical time-savings per step.

Previous market leading solution*	HOW LONG DOES IT TAKE?	Fibertec™ 8000
0.5 min.	Insert crucibles	0.5 min.
–	Choose program and start	1 min.
6 min.	Add acid, antifoam and mix sample	–
9 min.	Heating up to boil, keep gentle boiling	–
10 min.	Draining and rinsing	–
6 min.	Add alkali, antifoam and mix sample	–
9 min.	Heating up to boil, keep gentle boiling	–
10 min.	Draining and rinsing	–
0.5 min.	Remove crucible	0.5 min.
51 min.	TOTAL OPERATOR TIME	2 min.

* FOSS Fibertec™ 2010

The fully automated apparatus, compared to the manual (refluxing in beakers) also reduces potential human error and improves safety by containing the sample throughout the procedures, minimising the handling of reagents and ensuring fast, efficient filtration.

Automation options – Crucible versus filter

So far, we have looked at a little history of fiber analysis, the steps involved in determining crude and detergent fibre and the global standards – all hopefully quite clear. However, when we start looking at different methods of automating the procedure, the waters are easily stirred by differing approaches.

Fibre was first determined by boiling a test material in a beaker and filtering through a Gooch crucible. Many labs still use this method. Tecator introduced the Fibertec[®] extraction system in 1976 that allows the simultaneous digestion and sequential filtration of six test portions thereby eliminating the need to transfer the solution to a filtering crucible.

An alternative is the so-called filter bag system. ANKOM introduced its system in 1992 that allows the determination of up to 24 test portions placed in filter bags in a pressurized kettle. Gerhardt followed this with its Fibretherm system that allows simultaneous determination of 12 test portions placed in filter bags in a reflux kettle.

In the filter bag methodology, the feed samples are placed inside polyester filter bags and treated therein with an acid detergent (cetyl trimethyl ammonium bromide) solution. The residue of the treated sample is considered the ADF. The filter bag approach can offer a significant boost to throughput in the laboratory. Specifically, some equipment allows up to 24 samples to be processed at once.

With automated crucible method solutions offering only six sample-processing, they may appear, at least on paper, to be much slower. However, when automation options are taken into consideration, the picture changes. Time-

to-result for an individual sample is comparable and when a fully-automated solution is used, it leads to significant operator time savings in the laboratory compared to the semi-automatic filter bag method.

An operator can literally load a set of up to six samples press a start button and walk away. The actual operator time is as little as two minutes and equipment can even run overnight. For more, see the time savings comparison in chapter 5 and the case story video in chapter 6.



With an automated crucible method, an operator can literally load a set of up to six samples press a start button and walk away.

Differences in results for some sample types

The different methods for the determination of fibre have been evaluated by analyzing results reported with the proficiency testing scheme of the Association of American Feed Control Officials (www.aafco.org). Significant differences were found for reported ADF and NDF values for the calf replacer sample between the Fibertec values and the Ankom teabag method values. Also the reported NDF values for the corn protein concentrate sample show significant differences.

[Read more here](#)

Methods and standards

Perhaps the biggest consideration is the standards aspect.

While filter bags of a standardized pore size have gained worldwide acceptance, it is the official acceptance of the crucible method (See Chapter 3) that sets the scene for valid fibre results on a worldwide basis. This is particularly relevant for labelling and trade of feed raw material and compound feed.

A consideration for evaluating fibre methods is therefore not only the recovery of indigestible plant residues, but also the analytical performance of the methods and

their official status. Although alternative methods allow higher samples volumes to be run, the adherence to official methods is mandatory in cases of disputes and for labeling purposes. Furthermore, automation options for the official methods add further reliability while making them highly resource efficient.

CHAPTER 6

Automated fibre analysis as a reference for routine analysis with NIR



A new fully-automated fibre analysis system shows how maximum automation and minimal human intervention helps to make reference analysis of fibre more consistent. In turn, the stable reference data can help to improve NIR instrument calibrations.

We humans are good at lots of things, but when it comes to doing repeated reference analysis of fibre in a laboratory, an automated system is more consistent. For each and every analysis, things are done in the right order at the right time and with exactly the right temperature settings and the same dosage of solvents and chemicals.

This consistency is especially important if the analysis is the cornerstone for quality control with NIR as is the case at the busy ADM laboratory at Europort in the Netherlands.

With a view to improving the consistency of reference analysis, the laboratory has been using a fully-automated Fibertec 8000 system for the analysis of fibre in soymeal. The idea is to eventually replace an old manual system that is both labour-intensive and inconsistent. “With this new system we plan to reduce the lab error so that the data is more consistent and we can then use this data for reference data for our NIR calibrations,” says Laboratory Manager, Jeffrey Smith. “It is the goal of our production to produce as close to the limits of our specifications and therefore we need to have a very low standard deviation so that production has the chance to get near that specification.”

See the video here:



CHAPTER 7

Fibre analysis options from FOSS



Fibertec 8000 series

Crude Fibre (CF), Neutral Detergent Fibre (NDF), Acid Detergent Fibre (ADF) and Acid Detergent Lignin (ADL).

The Fibertec™ 8000 provides official method results (ISO, AOAC), with the safest fibre analysis solution available for Crude fibre, ADF, ADL and NDF in feed, feed ingredients, forage, pet food, grain, cereal and oilseeds.

Non-attended measurement of up to six samples simultaneously releases staff to do other things – it can even run overnight.

Total analysis time 2 hours, operator time 2 minutes.



NIR™ DS2500 F

Near Infrared (NIR) analyser providing rapid indirect measurement of Crude Fibre in feed and feed ingredient samples in ground or unground forms. Possible to develop advanced models for parameters such as NDF and ADF. Analysis time: 30 seconds.