

## Feed Control:

# New standard for determination of amylase-treated neutral detergent fibre

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As the nutrient content of feed can vary substantially, especially in forages and silages, raw material testing is essential:

- i For determining the right price corresponding to the quality of the feed,
- ii For efficient feeding of livestock, and
- iii For optimizing production of raw materials, e.g. fertilization, harvesting.

For maximum animal performance at lowest feed costs, what is needed is improved feed efficiency on the basis of improved quality parameters. For instance, still gross energy and crude protein values might be declared, though it is e.g. the amount of energy metabolized or the amount of protein digested that is of interest for efficient feeding.

Fibre is an important nutritional parameter as it represents non-digestible fractions of the feed or fractions that are most difficult to digest. Traditionally, crude fibre content has been an important quality parameter in feed trading, though it does not recover all fractions of indigestible cellulose, hemicellulose or lignin.

### Neutral Detergent Fibre

The concept of detergent fibre was



*A semi-automated extraction system for simple determination of crude fibre, detergent fibre and related parameters in plant materials, compound feeds, foodstuffs etc.*

introduced by van Soest in the 1960s. Neutral detergent fibre is the fraction that remains after solubilization of non-fibrous components with a neutral detergent solution. This fraction contains 100 per cent of the indigestible plant material. On the other hand some soluble fibre, i.e. material that is not digested by mammalian enzymes, is obtained. However, neutral detergent soluble fibre is often fermented by bacteria and digested

in the gut of ruminants.

The concept has been used successfully for analysis of fibre in forages, and the calculation of energy values and dry matter intake.

Originally, fibre was related to the cell wall fraction of plants. However, it is apparent that fractions that are difficult or impossible to digest occur in all feeds and food. Numerous modifications have therefore been proposed to extend the application of NDF to



grains, concentrate feeds and human foods. Most of these modifications have been based on conceptual improvement in total fibre analysis without ruggedness testing, to determine their suitability for all feed matrices or practical application as a routine method. Unfortunately, this resulted in methods that differed, yet all results were called NDF.

Figure 1 shows a typical exam-

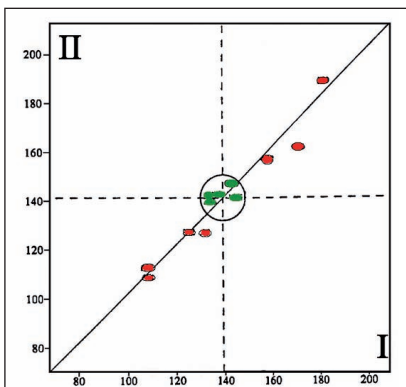


Fig. 1: Typical Youden plot of paired results from a PTS on NDF.

ple of the results of a proficiency testing scheme (PTS) for NDF, with twelve participating laboratories and using seven different modifications of the method. Plotting the results of sample I vs sample II for each individual lab, the Youden plot clearly shows differences between the method modifications.

The difficulty in extracting and washing/filtrating fibrous residues in some materials, and the variety in modifications of the method, have led to the perception that NDF is a “difficult parameter”.

Empirical methods of analysis, such as fibre, require that method protocol is followed exactly. Any method modification can lead to different results as it will mean a different measure for the determination of fibre.

The amylase-treated NDF (aNDF) method was therefore developed as a standard procedure for measuring total insoluble fibre in all types of feed [1,2].

### Collaborative Study

During years of method optimization and ruggedness studies the following factors were evaluated:

- 1 Time of refluxing:  $60 \pm 5$  min of refluxing at boiling temperatures shown to be optimum.
- 2 Neutral detergent concentration: Sodium lauryl sulphate at near saturation concentrations maximized the solubilization of non-fibre components using a minimum volume of liquid to be filtered.
- 3 Neutral detergent pH: As alkaline or acid conditions could solubilize fibre, pH should be maintained within the range of 6,95 to 7,05.
- 4 Triethylene glycol replaced ethylene glycol monoethylether for removal of non-fibre soluble material from concentrate feeds.
- 5 Sodium sulphite was included as a reagent to remove proteinaceous material from fibre residues.

Material	Description	aNDF (%)	Repeatability Sr (%)	Reproducibility SR (%)
Alfalfa silage	Forage, fermented legume	40,8	0,9	0,9
Brewer's grains	Concentrate, heated by-product	49,2	1,7	2,2
Citrus & beet pulp	Concentrate, pectic by-product	29,9	1,0	1,4
Corn grain with cob	Concentrate, containing starch	21,7	0,6	0,8
Corn silage	Forage, grass with starch	37,2	0,8	0,9
Corn stalks	Forage, high-fibre grass	73,1	0,6	1,1
Dairy mixed feed	Concentrate, mixture with fat	12,9	0,8	1,3
Grass hay	Forage, temperate grass	58,2	1,1	1,5
Milk replacer	Concentrate, animal fat	0,0	0,7	0,7
Roasted soybeans	Concentrate, plant fat	13,5	0,8	1,6
Sawdust	Reference, high cellulose	90,0	1,7	2,1

Table 1: Materials used and results of the collaborative study.

6 Heat-stable  $\alpha$ -amylase. Both amylase source and method of using the enzyme were shown to alter NDF values, and pre-extraction of starch via incubation had the potential for solubilizing fibre. In addition, many amylase solutions were crude extracts that contained enzymes that could degrade fibre and must be deactivated by near-boiling temperatures. Heating samples and gelatinizing starch also improved amylase effectiveness in removing starch. The new standard uses two additions of heat-stable  $\alpha$ -amylase, at start of boiling and during washing steps. It also gives a simple visual assessment method for determining the activity of any heat-stable  $\alpha$ -amylase source.

7 Washing method: Multiple soakings of fibrous residues were required to obtain consistent results and uncontaminated fibrous residues.

Sample grinding, drying and modifications for problem samples that are high in starch, pectin, fat or protein and difficult to filter have also been evaluated, and optimum procedures described.

After familiarization with the method the twelve laboratories, representing research, regulatory, industrial and commercial testing labs, analyzed eleven different materials as blind duplicates in a collaborative study.

The most important performance data are the precision limits  $r$  (repeatability) and  $R$  (reproducibility). They are calculated as  $2,8 \times \text{sd}$  and can be used directly as the measurement uncertainty.

They are usually also depicted, in the form of a graph, as a function of the concentration (see Fig. 2). The data for this method shows that the standard is very robust, i.e. differences between  $r$  and  $R$  are

small; different labs can achieve the same results. Absolute values are also reasonable.

As a number of laboratories using FOSS Fibertec instruments participated in the study, the standard describes procedures for both the manual beaker method and the Fibertec crucible method. The latter is published as a FOSS Application Note [4] for the Fibertec, with a validated version for the FiberCap [5].

On the basis of the study results, the aNDF method has been accepted as AOAC International Official Method 2002.04 [2]. In addition the ISO and CEN have decided to approve the method as an EN ISO standard [3].

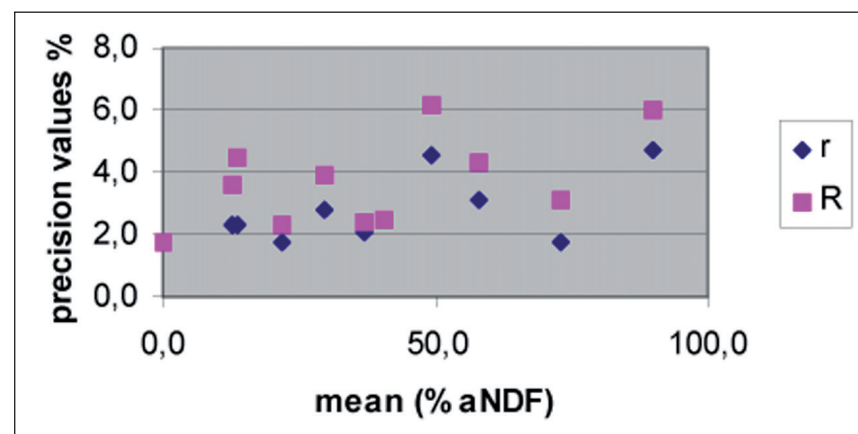


Fig. 2: Precision data for the aNDF method.

## Conclusions

For the first time a global AOAC/ EN/ ISO standard for NDF is now available, a parameter that had not been standardized before, not at least because of all the different modifications. It is anticipated that the amylase- treated neutral detergent fibre (aNDF) method will become the method of choice for measuring insoluble fibre in feeds. As the method has been collaboratively studied on material representing the full range of animal feed matrices including animal products, high protein feeds, high fat feeds, high pectin feeds, oil

seeds, grains, heated by-product feeds, and legume and grass hays and silages, the method has the potential to become the basis for global feed calibrations and more uniform calculations of energy value.

## References:

- [1] David R. Mertens: "Gravimetric Determination of Amylase-Treated Neutral Detergent Fiber in Feeds with Refluxing in Beakers or Crucibles: Collaborative Study", *J. AOAC Int.*, Vol 85, No 6, 2002, pp 1217 - 1246
- [2] *Official Methods of Analysis*,

- AOAC International, Gaithersburg, MD: Method 2002.04*
- [3] *EN ISO/FDIS 16472:2005: Animal feeding stuffs – Determination of amylase-treated neutral detergent fibre content (aNDF)*
- [4] *FOSS Application Note 3434*
- [5] *FOSS Application Note 3805.*

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