APPLICATION NOTE

F&F-F-002-2018/A1

# aNDF Determination in Pig Ration

Reference: **ISO 16472:2006, AOAC 2002.04** Animal feeding stuffs — Determination of amylase-treated Neutral Detergent Fibre content (aNDF)

Tested with **VELP Scientifica FIWE 6 Fiber Analyzer** (Code F30520200).


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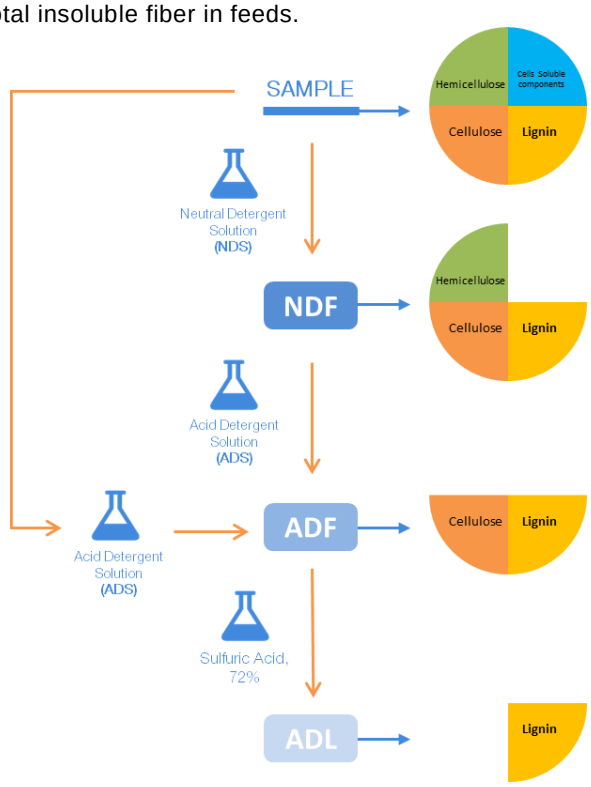
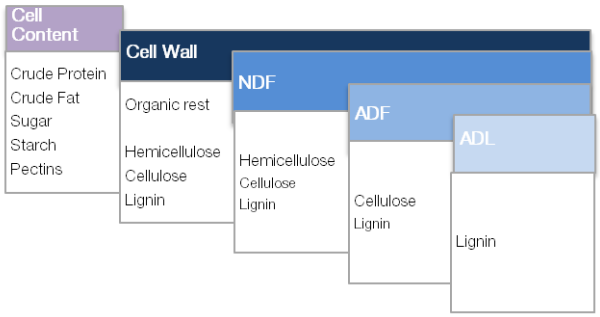
# aNDF DETERMINATION IN PIG RATION

## Introduction

Forage quality is a direct reflection of its essential nutrient content and availability to the consuming animal.
The concept behind the detergent fiber analysis is that plant cell substances can be divided into less digestible cell walls (made of hemicellulose, cellulose and lignin) and the highly digestible cell contents (containing starch and sugars).
Hemicellulose, cellulose and lignin are indigestible in non-ruminants, while hemicellulose and cellulose are partially digestible in ruminants. NDF is a good indicator of the "bulk" fiber and has been used to predict feed intake, in other words, how much an animal will eat before its stomach is full and it stops eating.
The amylase-treated NDF (aNDF) method, therefore, was developed as an accurate and precise method of measuring total insoluble fiber in feeds.




## Determination of aNDF content in Pig ration

The feed sample is boiled in the Neutral Detergent Solution NDS with heat-stable α-amylase-treated enzyme to separate the neutral detergent soluble fraction (sugars, starches and pectine soluble, filtered) from the neutral detergent insoluble fraction (cell walls substances, hemicellulose, cellulose and lignin, residues). The cell contents are highly digestible (about 98 %) and include various sugars, starches, pectins and other soluble carbohydrates, proteins, non-protein nitrogenous compounds, lipids, water-soluble minerals and vitamins. The remaining dry matter is estimated and the proportion gives the neutral detergent fiber (NDF).

## Reagents

1. Neutral detergent solution NDS:
   a) Sodium borate decahydrate ($Na_2B_4O_7 \bullet 10\ H_2O$) 6.81 g
   b) Disodium ethylenediaminetetraacetate (EDTA, $C_{10}H_{14}N_2Na_2O_8$) 18.6 g
   c) Triethylene glycol ($C_6H_{14}O_4$) 10 ml
   d) Sodium lauryl sulfate neutral ($C_{12}H_{25}NaO_4S$) 30 g
   e) Disodium phosphate anhydrous ($Na_2HPO_4$) 4.56 g
   f) Distilled water 1000 ml.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Pour between 400 ml and 500 ml of water into a 1 l flask. Add 18,6 g of disodium EDTA, 4,56 g of disodium phosphate anhydrous ($Na_2HPO_4$), and 6,81 g of sodium borate decahydrate ($Na_2B_4O_7$*10 H2O) and mix until dissolved (heat if necessary). Under a safety hood, add 30 g of sodium lauryl sulfate and, after dissolution, add 10 ml of triethylene glycol (anti-foaming aid). Add water to about 950 ml and mix. Adjust the pH to between 6,95 and 7,05 with concentrated hydrochloric acid (HCl) or sodium hydroxide (NaOH) and dilute to 1000 ml with water. If the pH is outside the range by more than 0,5 pH unit, discard the solution. Store the NDS at room temperature. If precipitation occurs, warm the solution to 25 °C and mix before use.

2. Heat-stable alpha-amylase, as a solution or a water extract of lyophilised enzyme powder (approx. 1 g of powder extracted in 100 ml of water).
3. Sodium sulfite anhydrous ($Na_2SO_3$)
4. Acetone, technical grade

Note: to simplify filtration, 1.00g of celite can be added to the crucible.

## Sample

| | |
|---|---|
| **Standard FAPAS** | **Pig ration** |
| Ref. Number | T10150QC |
| Quantity | 150 g |
| Analyte | aNDF |
| Units | g/100 g |
| **Assigned value** | **17.6** |
| **Satisfactory range** | **13.4 – 21.8** |

## Analysis Procedure

The diagram below shows the steps involved in the procedure:


1. Weigh 0.5 g ± 0.05 g of sample portion into each crucible containing 1 g of celite ($M_{sample}$).
2. Add 0.5 g ± 0.1 g of sodium sulfite and place the crucibles in the FIWE unit.
3. From the top of the glass columns pour 50 ml ± 5 ml of NDS to each crucible and mix using back pressure.
4. Add 2 ml of heat stable alfa-amylase and heat to boiling within 10 min (set heating power at 10). Use back pressure to mix the amylase with the NDS and the sample.
5. Boil for 60 min. Samples may foam vigorously for a couple of minutes. 5 - 10 minutes after adding the amylase, rinse the sides of the flask with a minimum amount of NDS, (twice max).
6. Connect to vacuum to start the filtration through crucibles
7. Add 30 ml of hot water (about 80 °C) and 2 ml of α-amylase solution. Use back pressure to mix the amylase in the initial water soak. Remove amylase-water soak after a minimum of 60 sec. of reaction.
8. Wash with 30 ml of hot water, soak for 3 min to 5 min, and filtrate. If plugged, the crucibles may be backflushed using minimum back pressure

9. Fill crucibles with 25 ml of acetone and use minimum back pressure to disperse the particles. Soak for 3 min to 5 min and evacuate. Repeat the acetone wash.
10. Remove the crucibles from the unit and air-dry for 10 min to remove acetone
11. Dry the crucibles at 130 °C ± 2 °C for 2 h or at 105 ± 2 °C for at least 8 h. Leave to cool in the desiccator and weigh to the nearest 0,000 1 g ($M_{dry}$ and $B_{dry}$).
12. Ignite the crucible with the residue in a furnace at 525 ± 15 °C for at least 3 h or until carbon-free. Leave to cool in a desiccator and weigh to the nearest 0,0001 g ($M_{ash}$ and $B_{ash}$).
13. Remove ash and if necessary clean the crucibles by an oxidizing procedure.

## Calculation

$$\text{aNDF \%} = (M_{dry} - M_{ash} - (B_{dry} - B_{ash})) * 100 / M_{sample}$$

$M_{dry}$ = sample weight after drying

$M_{ash}$ = sample weight after ashing

$M_{sample}$ = sample weight

$B_{dry}$ = blank weight after drying

$B_{ash}$ = blank weight after ashing

## Results

| $M_{sample}$ (g) | $M_{dry}$ (g) | $M_{ash}$ (g) | aNDF % |
|---|---|---|---|
| Blank | 31.1442 | 31.1362 | |
| 0.4985 | 31.6998 | 31.5990 | 18.62 |
| 0.5035 | 30.8374 | 30.7344 | 18.87 |
| 0.5091 | 31.2289 | 31.1265 | 18.54 |
| 0.4972 | 31.8134 | 31.7135 | 18.48 |
| 0.5002 | 31.4048 | 31.3037 | 18.61 |
| | | **Average ± SD%** | **18.62 ± 0.15** |
| | | **RSD% *** | **0.8** |
| Satisfactory range aNDF: 13.4 – 21.8 g/100 g | | | |

***** RSD% = (Standard Deviation * 100) / Average

aNDF Blank ($B_{dry}$-$B_{ash}$) results + 0.0080 g

## Conclusion

The obtained results are reliable and in accordance with the expected range.The use of an extraction apparatus purposely devised for this method as FIWE unit, makes very easy the standardization of analytical conditions. The FIWE Series is suitable for Crude Fiber (CF), Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF) and Acid Detergent Lignin (ADL).

Benefits of FIWE are:

- 3 or 6 positions simultaneously: FIWE units can support up to 3 (FIWE 3) or 6 (FIWE 6) crucibles. Samples can also be processed individually
- Easy to use: convenient filtration, with pump and air pressure
- Precision and accuracy: high reproducibility of the results: **± 1%** relative or better
- Results in accordance with official procedures.

*In order to avoid losses of fiber, it's important to remember that crucibles life is around 20-30 analysis, because the fritted filter could be damaged from basic and acid solutions. Hence it's suggested to change them after 20-30 analysis.*


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