

Application Fibre

D.7.d. FIBRE THERM ADF in Animal Feed



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1 Principle

The part of components which are not eluted by an acid detergent solution as described below is called acid detergent fibre (ADF). In vegetable feed, the content of ADF indicates the content of cellulose and lignin.

The insoluble residue is dried, weighted, and then incinerated. The difference between the ash content and the insoluble residue is the ADF content. The process of dilution and filtration is simplified by use of the FibreBags. The most important part in fibre analysis is the exact observation of the respective times for the boiling stages as well as the weighing procedures.

2 Method

The chemicals as well as the boiling times correspond to:

- AOAC Official Method 973.18, Fiber (Acid Detergent) and Lignin (H_2SO_4) in Animal Feed, First Action 1973, Final Action 1977
- Methodenbuch des Verbandes Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten, Band III, Die chemische Untersuchung von Futtermitteln; 6.5.2 ADF determination

This application document is intended to be a guide to assist users in the initial use of C. Gerhardt analytical equipment. It is not a definitive method. Users may have to adapt this method to the ambient conditions and to suit their own analytical requirements.

3 Chemicals and Accessories

Quality p. a.

1. Water distilled or demineralised
2. Acid detergent solution or ADF-solution: 200 g N-cetyl-N,N,N-trimethylammoniumbromide are diluted in 10 l sulphuric acid c (H_2SO_4) = 0.5 mol/l. This solution should be stored in the dark at 18 - 20 °C.
3. Petroleum Ether, Boiling Range 40 to 60 °C
4. Acetone p.a
5. Antifoam Solution for Fibre determination, Order No.: 10-0143, 5-10 drops for 1.3 l of detergent solution
6. Glas Spacer for FibreBags, to open and fix the bags, Package with 6 pieces, Order No.: 10-0124
7. FibreBags for ADF/NDF determination, bag with 100 pcs, cat. no. 10-0127
8. Incineration Module for FibreBags, 12-place, complete with handle and 12 quartz glass crucibles, Order No.: 13-0092
9. Degreasing module/ ADL- module made of PTFE, for the simultaneous degreasing of up to 6 samples, incl. handle and beaker 1000 ml, OrderNo.: 13-0045

4 Instruments

- FIBRE THERM FT 12, cat.no. 13-0026, with accessory
- FRITSCH Pulverisette P14, sieve ring 1mm
- Desiccator with a drying agent as silica gel
- Drying chamber, electric driven, temperature 100°C +/- 5°C
- Muffle furnace, temp. 500 °C +/- 25 °C
- Timer or alarm clock
- Fume cabinet

5 Sample Type and Preparation

Approx. 5 - 100 g of the feed are grinded down to a particle size of approx. 1 mm. The glass spacer and the crucibles are marked with a heat- proofed marker.

- It is important to ensure the mass constancy of the FibreBags, if necessary, it is recommended to dry the necessary number of FibreBags in the drying chamber at 105 +/- 1 °C for 1 hour. The weight of the FibreBags is value M1 for the balance protocol. When storing the FibreBags in the desiccator they only have to be dried once and then, can be weighed directly.
 - The glass spacers are put into the FibreBags.
 - The weight of the sample to be put into the FibreBag should be around 1 g and should be weighed with 1 mg preciseness; this gives value M2 for the weighing protocol.
 - The glass spacers with the samples are inserted into the FIBRE THERM.
 - The blank value is determined in parallel to the regular analysis.
 - The dry matter of the sample should be determined separately and is important for the calculation of the fibre content (result related to the dry matter).
- De-fatting of the sample is important especially for samples with a fat content higher than 10 %:
The carousel is immersed three times in a row into 40/60 petroleum ether. By turning it as well as moving it up and down the sample is defatted. This facilitates the washing and filtration process, which will follow. Furthermore, no fibre content is lost. The first petroleum ether fraction is thrown away but the following can be re-used. After a short drying process in the fume cupboard (about 2 minutes) the samples are inserted into the FIBRE THERM.

Table 1: Analysis Parameter FIBRE THERM for ADF

Settings Rinsing

1.	Water filling	1.3	Liter	
2.	Circulation Time	9	Seconds	
3.	Circulation Pause	4	Seconds	
4.	Suction	2 to 3 min	min / s	The boiling vessel shall be empty.
5.	Wash out time	1 min	min / s	

Settings Preheating

1.	Circulation Time	10	Seconds	
2.	Circulation Pause	30	Seconds	
3.	Heating delay after start	30	Seconds	
4.	ADF / H ₂ SO ₄ heat up to	80 to 85	°C	The sample solution shall boil.
5.	NDF / KOH heat up to	80 to 85	°C	
6.	H ₂ O-Wash heat up to	80 to 85	°C	The sample solution shall boil.
7.	ADF / H ₂ SO ₄ cool down to	85	°C	
8.	NDF / KOH cool down to	85	°C	
9.	H ₂ O-Wash cool down to	85	°C	

Method Settings

1.	Method Definition	X	No.	
2.	<A> Detergent	ADF-Solution	Type	
3.	<A> Add	1.3	Liter	
4.	<A> Heating Power	30 to 40	%	The sample solution shall gently boil.
5.	<A> Circulation Time	10	s	
6.	<A> Circulation Pause	20	s	
7.	<A> Boiling Time	1 h 0min	h / min	

8.	<A> Suction	2 to 3 min	min / s	
9.	<A> Rinsing	2	Cycles	
10.	 Detergent	----	Type	
11.	 Add		Litre	
12.	 Heating Power		%	
13.	 Circulation Time		s	
14.	 Circulation Pause		s	
15.	 Boiling Time		h / min	
16.	 Suction		min / s	
17.	 Rinsing		Cycles	
18.	<C> Detergent	H ₂ O-Wash	Type	
19.	<C> Add	1.3	Liter	
20.	<C> Heating Power	55	%	
21.	<C> Circulation Time	10	s	
22.	<C> Circulation Pause	15	s	
23.	<C> Boiling Time	0 h 5 min	h / min	
24.	<C> Suction	2 to 3 min	min / s	
25.	<C> Rinsing	0	Cycles	
26.	<D> Detergent	H ₂ O-Wash	Type	
27.	<D> Add	1.3	Liter	
28.	<D> Heating Power	55	%	
29.	<D> Circulation Time	10	s	
30.	<D> Circulation Pause	15	s	
31.	<D> Boiling Time	0 h 5 min	h / min	
32.	<D> Suction	2 to 3 min	min / s	
33.	<D> Rinsing	1	Cycles	
34.	Meth. X defined Time :		h / min	

Drying of the FibreBags

The FibreBags are taken out of the carousel. The glass spacers are removed from the FibreBags rinsing them carefully with water. Alternatively, the incineration module (s. Application_D.7.b.1. FibreBag Procedure with Incineration Module_01-2021_english) can be used to standardize the method.

The drained FibreBags are put into crucibles, which has been pre-ashed at 500 °C +/- 25 °C and are dried in the drying chamber at 105 °C for minimum 4 hours or overnight. Then, the crucibles with FibreBags are put into the desiccator to cool down to ambient temperature and are weighed. The weight of FibreBag and crucible after digestion and drying is value M3.

Incineration of Samples

The FibreBags are incinerated at 500 °C +/- 25 °C for at least 4 hours or overnight. The FibreBags should be placed in the cold muffle furnace if possible, especially if the FibreBags are incinerated together with the glass spacers. This procedure considerably increases the service life of the glass spacers. The vapours produced during the combustion process are harmless.

After incineration, the crucibles are left to cool in the drying chamber at 105 °C for 30 minutes and then in the desiccator to cool down to ambient temperature. Then, they are weighed and value M4 (for crucible and ashes) is obtained for the weighing protocol.

6 Calculation

The ADF is calculated either automatically by use of the Excel calculation macro ADF or traditionally by the formulas shown in this macro:

Table 2: Calculation Macro ADF



Calculations ADF

Formula for the calculation

$$\% \text{ ADF} = ((M_3 - M_1 - M_4) - (B3 - B1 - B4) \times 100) / M2$$

Blank value = B3 - B1 - B4

Denominations:

- M1 = FibreBag [g]
- M2 = Initial sample weight [g] (ev. correct with dry content)
- M3 = Incinerating crucible and dried FibreBag after digestion [g]
- M4 = Incinerating crucible and ash [g]
- B1 = Weight FibreBag blank value
- B3 = Incinerating crucible and dried FibreBag Blank value after digestion [g]
- B4 = Incinerating crucible and ash Blank value [g]

CLR

Operator

B1 [g]	B3 [g]	B4 [g]	Blank value [g]

Date / Time 19.01.2021 / 13:00

FibreBag number	Sample number	M1 [g]	M2 [g]	M3 [g]	M4 [g]	% ADF

7 Reasons for Failures

Result too low:

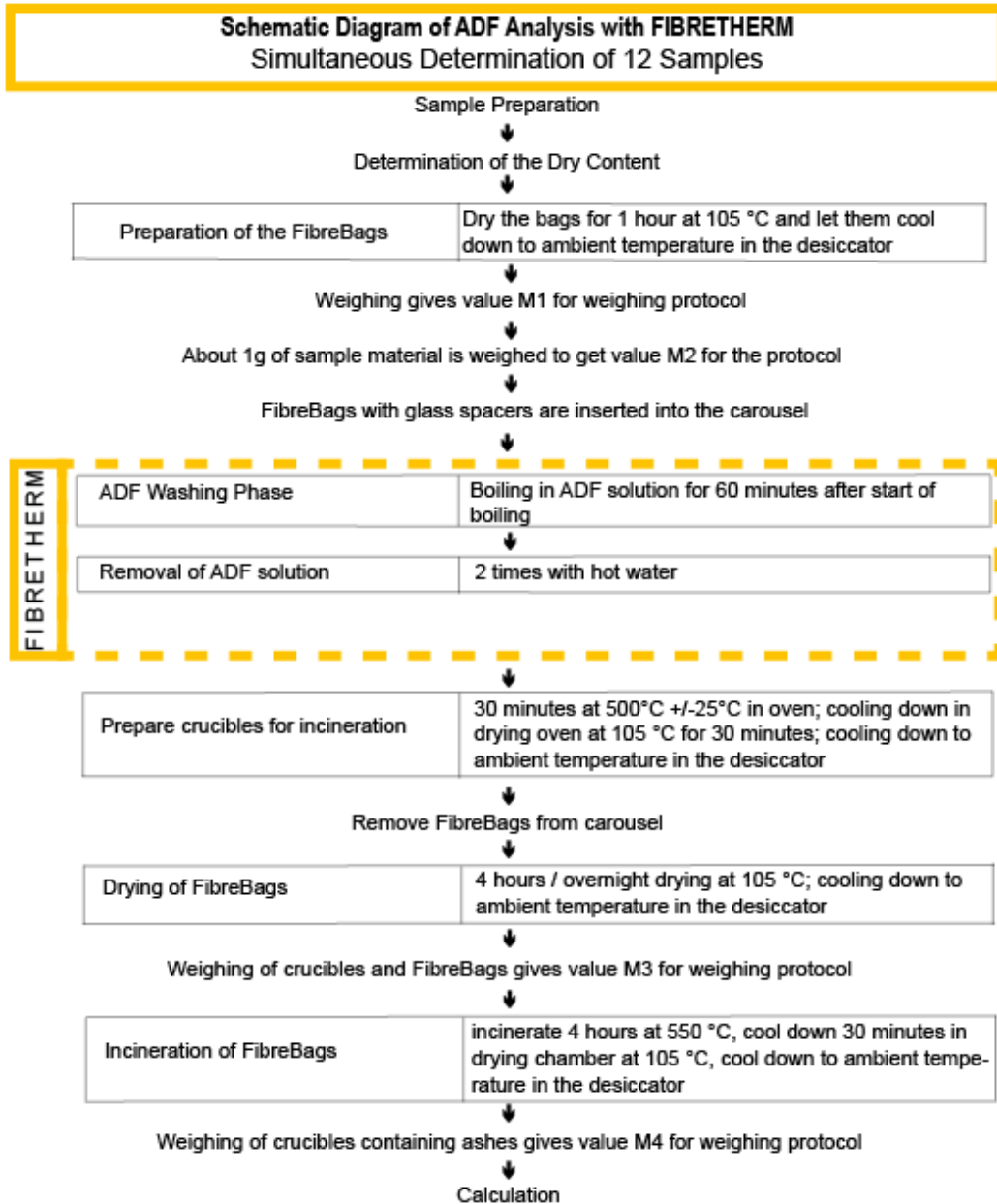
Reason	Reaction
Cooling time	Insufficient cooling time will lead to minute errors in weighing (weight loss). A strict observance of the weighing procedure is recommended.
Broken FibreBags	Sample is lost from the FibreBag due to leaking or broken bags. Check FibreBag before using it for the analysis

Result too high

Reason	Reaction
Desiccation error	The desiccator might not be filled with a sufficient amount of desiccant or it is wet. Humidity influences sample. Exchange desiccant or regenerate it.
Incomplete desiccation	Check the desiccator and the drying time.

Varying results:

Reason	Reaction
Inhomogeneous sample	Check the sample collection and preparation. No inhomogeneity should be seen during initial weighing.
Poor washing	Ensure that on every wash all bags are completely filled and then are completely emptied so that all solubles are removed.
Poor drying	The samples must always be dried for 4 hours or overnight. Different drying times result in different weights.
Varied analysis conditions or instruments	Changing analysis conditions such as varying characteristics of additives, different apparatus, temperature etc must be avoided. All changes have to be checked thoroughly.





COMPREHENSIVE APPLICATION DATA BASE

C. Gerhardt offers a wide range of application notes for many methods and procedures. Please contact our application lab team via application@gerhardt.de for deeper information on:

- Nitrogen in food and feed samples according to Kjeldahl and Dumas
- Crude fibre, ADF and NDF in feed
- Fat in food and feed
- Alcohol determination
- Total cyanide in water
- Trace metal in soil and sludge
- COD determination in water
- Total nitrogen determination in water, soil and plants
- Many more application notes on request.

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