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Animal feeding stuffs — Determination of acid detergent fibre (ADF) and acid detergent lignin (ADL) contents

Aliments des animaux — Détermination des teneurs en fibres au détergent acide (ADF) et en lignine sulfurique (ADL)

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Foreword

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Animal feeding stuffs — Determination of acid detergent fibre (ADF) and acid detergent lignin (ADL) contents

WARNING — The use of this International Standard may involve hazardous materials, operations and equipment. This International Standard does not purport to address any safety risks associated with its use. It is the responsibility of the user of this International Standard to establish appropriate safety and health practices and determine the applicability of local regulatory limitations prior to use.

1 Scope

This International Standard specifies a method for the determination of acid detergent fibre (ADF) insoluble residue and acid detergent lignin (ADL) in all types of animal feeding stuffs. The limit of determination is 1 % mass fraction for ADF and 1,5 % mass fraction for ADL.

A gravimetric routine and reference method is used.

2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 6498, *Animal feeding stuffs — Preparation of test samples*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

3.1
acid detergent fibre content
ADF content
mass fraction of fibrous residue obtained after treatment with cationic detergent in 0,5 mol/l sulfuric acid, primarily consisting of cellulose, lignin and insoluble protein complexes

NOTE The ADF mass fraction is expressed as a percentage.

3.2
acid detergent lignin content
ADL content
mass fraction of residue remaining after cellulose and other organic matter is solubilized by 72 % mass fraction (12,00 mol/l) sulfuric acid

NOTE The ADL mass fraction is expressed as a percentage.

4 Principle

ADF is determined in the first stage of the method.

Cationic detergent solution is used to remove acid-labile carbohydrates, protein that is not complexed into Maillard products (heat damaged), and fats. The remaining fibrous residue is primarily cellulose and lignin (plant products) or insoluble protein complexes (animal products and heat-damaged feeds). The residue is weighed for the determination of ADF.

In the second stage, the remaining residue is solubilized by 72 % mass fraction (12,00 mol/l) sulfuric acid, leaving the lignin (ADL) which is determined gravimetrically.

5 Reagents

Use only reagents of recognized analytical grade, unless otherwise specified, and only distilled or deionized water or water of at least equivalent purity.

5.1 Acid detergent solution. Add 20 g cetyl(trimethyl)ammonium bromide (technical grade) to 1 l of 0,5 mol/l sulfuric acid, previously standardized. Agitate to aid dissolution.

5.2 Sulfuric acid, 72 % mass fraction (12,00 mol/l).

Standardize sulfuric acid (H_2SO_4) to a relative density of 1,634 at 20 °C or 12,00 mol/l as follows.

Calculate the mass, in grams, of acid, $m(H_2SO_4)$, and the mass, in grams, of water, $m(H_2O)$, needed to prepare 1 000 ml of solution using Equations (1) and (2):

$$m(H_2SO_4) = \frac{100 \times 98{,}08 \times 12}{w(H_2SO_4)} \quad (1)$$

where $w(H_2SO_4)$ is the assay mass fraction of sulfuric acid, expressed as a percentage.

$$m(H_2O) = (1\,000 \times 1{,}634) - m(H_2SO_4) \quad (2)$$

where 1,634 is the relative density of 72 % mass fraction sulfuric acid.

Weigh water into a 1 000 ml volumetric flask and add the calculated amount of sulfuric acid slowly with occasional swirling. Cool the flask in a water bath while adding the required mass of acid. Cool to 20 °C and verify the volume. The meniscus should be within 0,5 cm of the calibration mark at 20 °C. If volume is too large, remove 5 ml water and add 4,55 ml sulfuric acid. If volume is too small, remove 1,5 ml and add 2,5 ml water. Repeat if necessary.

5.3 Filtration aid, diatomaceous earth[1)].

5.4 Acetone, technical grade.

5.5 *n*-Octanol, antifoaming agent.

1) Celite, acid washed, and Celite 545 AW are examples of suitable products available commercially. This information is given for the convenience of users of this International Standard, and does not constitute an endorsement of these products by ISO. Alternative products may be used if they can be demonstrated to give comparable results.

6 Apparatus

Usual laboratory apparatus and, in particular, the following.

6.1 Analytical balance.

6.1.1 Analytical balance, capable of weighing to the nearest 1 mg, with readability to 0,1 mg.

6.1.2 Analytical balance, with a measuring range up to 2 500 g, with readability to 1 g, for weighing sulfuric acid (5.2).

6.2 Mill, a cyclone mill or cutter mill or rotary mill or equivalent, giving a mean particle size of 0,22 mm to 0,26 mm.

6.3 Drying oven.

6.3.1 Air-ventilated oven, capable of operating at 103 °C ± 2 °C or 130 °C ± 2 °C.

6.3.2 Air-ventilated oven, capable of being maintained at 60 °C ± 2 °C.

To speed up the drying of moist samples without creating artefact fibres, a **vacuum oven**, maintained at 60 °C ± 2 °C may also be used.

6.4 Refluxing apparatus, with individual heating units and cold water condensers. Any conventional apparatus suitable for crude fibre or amylase-treated neutral detergent fibre (aNDF) determinations is acceptable. Calibrate heating unit settings so that 50 ml of water boils in 4 min to 5 min when using cold water condensers. A Fibertec[2)]-type apparatus can be used and should boil 50 ml of water within 10 min.

NOTE This setting can be expected to result in significant particle movement during refluxing.

6.5 Fritted-disk crucibles. Coarse porosity (pore size 40 µm to 60 µm) crucibles, 40 ml to 50 ml capacity, or Fibertec P2[2)] (pore size 40 µm to 100 µm, 26 ml to 28 ml capacity). Clean new crucibles and ash at 525 °C ± 15 °C for 1 h. Clean crucibles after each use by ashing at 525 °C ± 15 °C for 3 h, removing ash by inverting in a detergent solution and sonicating for 7 min to 10 min. Rinse crucibles in hot water, and soak in water at room temperature for at least 30 min.

Occasionally test filtration rate as follows. Fill each crucible with 50 ml of distilled water (25 ml for Fibertec P2[2)] crucibles) and record the time required to drain completely without vacuum [should be 180 s ± 60 s for Gooch[2)] or 75 s ± 30 s for P2]. If drain time is < 100 s (or < 30 s for P2), discard crucible. If it is < 120 s (or < 45 s for P2), check for cracks in fritted disk. If filtration takes > 240 s (or > 105 s for P2), clean crucible with acid or alkaline cleaning solution (Reference [4]). If cleaning does not improve filtration rate, discard crucible.

6.6 Vacuum filter manifold. Suitable apparatus [e.g. Fibertec[2)]-type cold extraction unit] that allows adequate soaking of fibrous residues.

6.7 Incineration furnace, 525 °C ± 15 °C.

6.8 Reflux beakers. As an alternative to a reflux apparatus (6.4), 600 ml Berzelius beakers with condensers, e.g. made from 500 ml round-bottom flasks, may be used.

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2) Example of a suitable product available commercially. This information is given for the convenience of users of this International Standard, and does not constitute an endorsement of this product by ISO.

7 Sampling

A representative sample should have been sent to the laboratory. It should not have been damaged or changed during transport or storage.

Sampling is not a part of the method specified in this International Standard. A recommended sampling method is given in ISO 6497.

8 Preparation of test sample

Prepare the test sample in accordance with ISO 6498.

Reduce laboratory sample to approximately 100 g (dry weight equivalent) and place half in a moisture-tight, sealed container for total moisture determination. Dry remaining wet (> 15 % mass fraction moisture) materials to < 15 % mass fraction moisture in an air-ventilated oven (6.3.2) at < 60 °C. Drying at < 60 °C is necessary to prevent formation of artefact fibre and lignin. Grind dry (< 15 % mass fraction moisture) test samples using a mill (6.2).

Grinding segregates materials with the most fibrous matter grinding through the screen last. Do not discard material in the grinder, but add it to the ground test sample.

Pre-extraction is required for test samples containing > 10 % mass fraction fat, and is recommended for test samples containing > 5 % mass fraction fat.

Weigh test samples into previously tared, oven-dried crucibles, place crucible on filtering manifold, extract four times with 30 ml to 40 ml of acetone, allowing the material to soak in acetone for 3 min to 5 min each time, vacuum to remove all traces of acetone, air-dry for 10 min to 15 min, and transfer residue to a reflux beaker for fibre analysis. Use the same crucible to collect the fibre residue for each test portion after acid detergent extraction.

For the Fibertec[2)]-type apparatus, place the crucible in a cold extraction unit and fill the crucible with 25 ml acetone (5.4). Leave for 3 min to 5 min and filter by applying vacuum. Repeat three times.

NOTE To simplify filtration, 1,00 g of filtration aid (5.3) can be added to the crucible before the sample.

9 Procedure

9.1 ADF

9.1.1 General

Dry empty crucibles in an oven (6.3.1) at 103 °C ± 2 °C for > 4 h [1 h if moved from the incineration furnace (6.7)] and record tare mass, m_1.

9.1.2 Conventional apparatus

Weigh, into a Berzelius beaker (6.8), a test portion of dried or as-received material of mass 1 000 mg ± 2 mg, and record the mass as m_2.

Materials with > 15 % mass fraction moisture should have the mass adjusted to provide an equivalent mass of dry matter. If conversion of results to the dry matter basis is required, weigh a test portion for moisture determination of the test sample at the same time.

Immediately before refluxing, add 100 ml of acid detergent solution (5.1) at room temperature. Heat to boiling over 5 min to 10 min and, if necessary, reduce heat slightly and add 2 drops to 4 drops of *n*-octanol (5.5) to avoid foaming, but provide moderate particle agitation. After 5 min to 10 min of refluxing, rinse down the sides of the beaker using a fine stream of acid detergent solution (add < 5 ml). Reflux for 60 min ± 5 min from the time when the solution has reached the boiling point.

Remove each beaker from the heating unit, swirl, and filter into the crucible (6.5). Without inverting the beaker, use a fine stream of boiling water to rinse all particles into the crucible. Remove acid detergent and rinse water using minimum vacuum. Close vacuum and fill crucible with about 40 ml of water at 90 °C to 100 °C, stir to break up the residue filter mat, and allow to soak for 3 min to 5 min. Repeat the water soaking twice and vacuum dry. Rinse the sides and bottom of the crucible to be sure that all traces of acid are removed (any residual acid will be concentrated during drying and cause charring of residues and low fibre values).

Add 30 ml to 40 ml of acetone (5.4), stir to break up all clumps and expose all particles to acetone, soak for 3 min to 5 min and repeat until no colour is removed (typically two acetone soaking cycles are sufficient). Remove residual acetone with vacuum, dry for > 5 h, preferably overnight, at 103 °C ± 2 °C in an air-ventilated oven (6.3.1), cool to room temperature in a desiccator and weigh. Record the mass as m_3.

9.1.3 Fibertec[2)]-type apparatus

Weigh, into a pre-dried crucible (6.5) of tared mass, m_1, a test portion of dried or as-received material of mass 1 000 mg ± 2 mg, and record the mass as m_2. To simplify filtration, add 1,00 g of filtration aid (5.3) to the crucible before the sample. Place the crucible in the Fibertec[2)] hot extraction unit and add 100 ml of acid detergent solution (5.1). Add 2 drops to 4 drops of *n*-octanol (5.5) to prevent foaming and heat to boiling point. Adjust heater and allow to boil for 60 min ± 5 min. Measure the boiling time from when the solution reaches boiling point.

Remove acid detergent solution and wash three times with water at 90 °C to 100 °C. Use 30 ml portions of water, and vacuum dry between washings.

Place the crucible in the cold extraction unit and fill the crucible with 25 ml acetone (5.4). Filter. Repeat once.

Evaporate solvent and dry crucibles at 130 °C ± 2 °C for 2 h or at 103 °C ± 2 °C for at least 5 h. Cool to room temperature in a desiccator and weigh to the nearest 0,000 1 g. Record the mass as m_3.

9.2 ADL

9.2.1 Conventional apparatus

Place the crucible containing acid detergent fibre in a 50 ml beaker for support or arrange crucibles in a shallow enamel pan. Cover the contents of the crucible with sulfuric acid (5.2) cooled to 15 °C and stir with a glass rod to a smooth paste, breaking up all lumps. Fill the crucible about half-way with acid and stir. Leave the glass rod in the crucible; refill with sulfuric acid (5.2) cooled to 15 °C and stir hourly as acid drains, keeping the crucible at 20 °C to 23 °C (cool if necessary). After 3 h, filter as completely as possible with vacuum, and wash with hot water until acid-free to litmus paper. Rinse the sides of the crucible and remove stirring rod. Dry the crucible at 130 °C ± 2 °C for 2 h or 103 °C ± 2 °C for at least 5 h, cool to room temperature in a desiccator and weigh. Record the mass as m_4. Ignite the crucible at 525 °C ± 15 °C for at least 3 h or until carbon-free. Cool to room temperature in a desiccator and weigh. Record the mass as m_5.

9.2.2 Fibertec[2)]-type apparatus

Place a glass rod into the crucible for stirring. Add 25 ml sulfuric acid (5.2) cooled to 15 °C. Stir with glass rod and filter off after 3 h. Stir at hourly intervals. Alternatively, use reverse pressure for breaking lumps. Wash with water until free from acid. Dry crucibles at 130 °C ± 2 °C for 2 h or at 103 °C ± 2 °C for at least 5 h. Cool to room temperature in a desiccator and weigh. Record the mass as m_4. Ignite the crucible at 525 °C ± 15 °C for at least 3 h or until carbon-free. Cool to room temperature in a desiccator and weigh. Record the mass as m_5.

9.3 Quality assurance

9.3.1 Include at least one in-house reference or quality control (QC) sample and two blanks for the first 20 to 30 samples in a run and add 1 QC and 1 blank for each additional 20 to 30 samples analysed.

9.3.2 Include at least one set of duplicates in each run if single determinations are being made. Duplicates should not be run consecutively, but one at the beginning and one at the end of the run.

9.3.3 Change in masses of blank crucibles should be < 0,010 0 g after either extraction or ashing. If masses of blank crucibles change by more than 10 mg or crucible masses after ashing are less than those of empty crucibles, cleaning of crucibles is likely to be inadequate and/or there are problems with the weighing technique.

10 Calculation and expression of results

10.1 ADF

Calculate the ADF content on an as-received basis, w_1, expressed as a percentage mass fraction, by using Equation (3) or ADF content on a dry matter basis, w_2, expressed as a percentage mass fraction, by using Equation (4).

For undried materials (test sample has same moisture basis as laboratory sample):

$$w_1 = 100 \times \frac{(m_3 - m_1) - (\overline{m}_{b2} - \overline{m}_{b1})}{m_2} \quad (3)$$

For dried materials (laboratory sample is dried to prepare test sample):

$$w_2 = 100 \times \frac{(m_3 - m_1) - (\overline{m}_{b2} - \overline{m}_{b1})}{m_2 w_d} \quad (4)$$

where

m_1 is the mass, in grams, of the crucible;

m_2 is the mass, in grams, of the test sample;

m_3 is the mass, in grams, of the crucible and residue;

$\overline{m}_{b1}$ is the average mass, in grams, of all blanks after oven drying before acid detergent extraction;

$\overline{m}_{b2}$ is the average mass, in grams, of all blanks after oven drying after acid detergent extraction;

w_d is the percentage mass fraction of dry matter divided by 100.

10.2 ADL

Calculate the ADL (H_2SO_4 lignin) content on an as-received basis, w_3, expressed as a percentage mass fraction, by using Equation (5) or ADL (H_2SO_4 lignin) content on a dry matter basis, w_4, expressed as a percentage mass fraction, by using Equation (6).

For undried materials (test sample has the same moisture basis as laboratory sample):

$$w_3 = 100 \times \frac{(m_4 - m_5) - (\overline{m}_{b3} - \overline{m}_{b4})}{m_2} \quad (5)$$

For dried materials (laboratory sample is dried to prepare test sample):

$$w_4 = 100 \times \frac{(m_4 - m_5) - (\overline{m}_{b3} - \overline{m}_{b4})}{m_2 w_d} \quad (6)$$

where

$\overline{m}_{b3}$ is the average mass, in grams, of all blanks after oven drying before ashing;

$\overline{m}_{b4}$ is the average mass, in grams, of all blanks after oven drying after ashing;

m_4 is the mass, in grams, of the crucible and residue after drying;

m_5 is the mass, in grams, of the crucible after ashing.

10.3 Expression of results

Mass fraction results should be reported to the nearest 0,1 %, and results < 1,0 % ADF or < 1,5 % ADL should be reported as “ADF < 1,0 %” or “ADL < 1,5 %”.

11 Precision

11.1 Interlaboratory test

Details of an interlaboratory test on the precision of the method are summarized in Annex A. The values derived from this interlaboratory test cannot be applied to other concentration ranges and matrices than those given.

11.2 Repeatability

The absolute difference between two independent single test results, obtained using the same method on identical test material in the same laboratory by the same operator using the same equipment within a short interval of time will in not more than 5 % of cases exceed the repeatability limits calculated using Equations (7) and (8).

$$r_{ADF} = 0{,}029\,\overline{w}_{ADF} + 0{,}715 \quad (7)$$

$$r_{ADL} = 0{,}058\,\overline{w}_{ADL} + 0{,}450 \quad (8)$$

where $\overline{w}_{ADF}$ and $\overline{w}_{ADL}$ are the means of two intralaboratory results obtained under repeatability conditions for ADF content and ADL content, respectively, expressed as percentage mass fractions.

Equations (7) and (8) have been calculated for an ADF mass fraction between 3,5 % and 73 % and for an ADL content between 1,5 % and 20 %.

11.3 Reproducibility

The absolute difference between two single test results, obtained using the same method on identical test material in different laboratories with different operators using different equipment, will in not more than 5 % of cases exceed the reproducibility limits calculated using Equations (9) and (10).

$$R_{ADF} = 0{,}077\,\overline{w}_{ADF} + 1{,}365 \quad (9)$$