SLOVENSKI STANDARD
SIST EN ISO 16472:2006

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Krma ï Doloļ evanje vlaknin z nevtralnim reagentom po predhodni obdelavi z amilazo (aNDF) (ISO 16472:2006)

Animal feeding stuffs - Determination of amylase-treated neutral detergent fibre content (aNDF) (ISO 16472:2006)

Futtermittel - Bestimmung des amylase-behandelten neutral gereinigten Fasergehalts (ISO 16472:2006)

Aliments des animaux - Détermination du contenu en fibre détergente neutre traitée a l'amylase (ISO 16472:2006)

Ta slovenski standard je istoveten z: **EN ISO 16472:2006**

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Animal feeding stuffs - Determination of amylase-treated neutral detergent fibre content (aNDF) (ISO 16472:2006)

Aliments des animaux - Détermination du contenu en fibre détergente neutre traitée à l'amylase (ISO 16472:2006)

Futtermittel - Bestimmung des amylase-behandelten neutral gereinigten Fasergehalts (ISO 16472:2006)

This European Standard was approved by CEN on 23 March 2006.

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This European Standard exists in three official versions (English, French, German). A version in any other language made by translation under the responsibility of a CEN member into its own language and notified to the Central Secretariat has the same status as the official versions.

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Foreword

This document (EN ISO 16472:2006) has been prepared by Technical Committee ISO/TC 34 "Agricultural food products" in collaboration with Technical Committee CEN/TC 327 "Animal feeding stuffs - Methods of sampling and analysis", the secretariat of which is held by NEN.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by October 2006, and conflicting national standards shall be withdrawn at the latest by October 2006.

According to the CEN/CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, Switzerland and United Kingdom.

Endorsement notice

The text of ISO 16472:2006 has been approved by CEN as EN ISO 16472:2006 without any modifications.

INTERNATIONAL STANDARD

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Animal feeding stuffs — Determination of amylase-treated neutral detergent fibre content (aNDF)

Aliments des animaux — Détermination du contenu en fibre détergente neutre traitée à l'amylase

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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

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ISO 16472 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 10, *Animal feeding stuffs*.

Animal feeding stuffs — Determination of amylase-treated neutral detergent fibre content (aNDF)

WARNING — The use of this International Standard may involve the use of hazardous materials, operations and equipment. This International Standard does not purport to address all the 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 methods for the determination of amylase-treated neutral detergent insoluble fibrous residue content in all types of animal feed.

It includes a gravimetric routine method and a reference method.

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
amylase-treated neutral detergent fibre content
aNDF content
mass fraction of insoluble fibre residues determined by the procedure specified in this International Standard

NOTE The aNDF content is expressed as a percentage by mass.

4 Principle

Neutral detergent (ND) solution and heat-stable alpha-amylase are used to dissolve the easily digestible proteins, lipids, sugars, starches and pectins in feeds, leaving an insoluble fibrous residue that is primarily cell wall components of plant materials (cellulose, hemicellulose and lignin) and indigestible nitrogenous matter in animal products.

5 Reagents

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

5.1 Sodium sulfite, anhydrous (Na_2SO_3).

5.2 Dried hominy corn (corn grits, raw), ground to pass through a 1 mm screen in a cutter mill.

5.3 Iodine solution, containing 2 g of potassium iodide and 1 g of iodine in 100 ml of water.

Store the solution in an amber or opaque bottle.

5.4 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).

EXAMPLE Termamyl 120 l from Novo Enzymes or equivalent.

Standardize the heat-stable alpha-amylase solution or enzyme powder extract so that two additions of 2 ml will remove starch from 0,5 g of raw corn starch (5.2). For a detailed procedure on standardizing heat-stable alpha-amylase solution, see Annex B.

5.5 Neutral-detergent (ND) solution

Pour between 400 ml and 500 ml of water into a 1 l flask. Add 4,0 g of sodium hydroxide (NaOH) 14,6 g of EDTA, 4,56 g of sodium hydrogen phosphate (Na_2HPO_4), and 6,81 g of sodium borate decahydrate ($Na_2B_4O_7 \cdot 10\ H_2O$) and mix until dissolved (heat if necessary). The NaOH and EDTA may be replaced with 18,6 g of disodium EDTA.

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 1 000 ml with water. If the pH is off the range by more than 0,5, discard the solution.

Store the ND solution at room temperature. If precipitation occurs, warm the solution to 25 °C and mix before use. Record the date the ND solution was prepared, the pH measurements and any adjustments in a reagent log book.

6 Apparatus

Usual laboratory apparatus and, in particular, the following.

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

6.2 Cyclone mill with 2 mm screen, or **cutter mill** with 1 mm screen, capable of grinding samples to obtain a geometric mean particle size of 220 µm to 260 µm

6.3 Refluxing apparatus, with individual heating units and cold water condensers that fit 600 ml flasks.

Any conventional apparatus suitable for crude fibre determinations is acceptable. Calibrate the heating unit settings so that 50 ml of water boils within 4 min to 5 min when using cold water condensers. A Fibertec type apparatus may be used and should boil 50 ml of water within 10 min.

6.4 Fritted-disc Gooch crucibles, coarse porosity (pore size 40 µm to 60 µm) crucibles, high-form, 40 ml to 50 ml capacity, or P2 (pore size 40 µm to 100 µm), 26 ml to 28 ml capacity.

Clean new crucibles and ash at 500 °C for 1 h. Clean crucibles after each use by ashing at 500 °C for 3 h, removing ash, 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. Fit the top of each crucible with a rubber stopper fitted with a port that is connected to a trap and vacuum line. Back-flush each crucible with water, by repeatedly plunging and removing the bottom of the crucible into water to create a vigorous rinsing action.

Occasionally check the filtration rate as follows. Fill each crucible with 50 ml of distilled water (25 ml for Fibertec P2 crucibles) and record the time required to drain completely without vacuum (should be 180 s ± 60 s for Gooch or 75 s ± 30 s for P2). If the drain time is < 100 s (or < 30 s for P2), discard the crucible. If it is < 120 s (or < 45 s for P2), check for cracks in the fritted disc. If the filtration takes > 240 s (or > 105 s for P2), clean the crucible with acid or alkaline cleaning solution (see Reference [1]). If cleaning does not improve the filtration rate, discard the crucible.

Instead of P2 crucibles, stainless-steel metal crucibles with a 90 µm aperture stainless-steel metal sieve may also be used.

6.5 Vacuum filter manifold (e.g. Fibertec type), that allows adequate soaking of fibrous residues.

The manifold should provide a vacuum-tight seal with the crucible to reduce foam formation in vacuum lines. Use thick-walled vacuum tubing to connect the manifold to a trap (4 l to 18 l) and vacuum source. A vacuum reservoir (18 l) between the trap and vacuum source is recommended to ensure adequate vacuum capacity to remove the foam.

6.6 Boiling water supply

Use a continuous boiling water generator as described in Reference [1] or a suitable alternative. The apparatus shall be capable of supplying boiling water (> 95 °C) in a quantity sufficient for all samples being washed at one time, through a nozzle producing a fine stream (flow rate of 35 ml to 40 ml per 10 s; a 2,5 ml disposable plastic pipette tip makes an acceptable nozzle). A fine nozzle minimizes the water needed to transfer particles to the crucible, but provides the water pressure needed to remove residues attached to the side of the flask. It is critical that water is boiling when added to the crucibles, especially for samples containing starches, pectic substances, mucilages or glyco-proteins. For Fibertec type apparatus, use a syringe with a cone-spray nozzle to rinse the condensers and a 60 ml disposable syringe with 12 gauge needle that is 10 cm in length to dislodge any residues adhering to the condensers.

7 Sampling

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

It is important that the laboratory receive a sample which is truly representative and has not been damaged or changed during transport or storage.

8 Preparation of test sample

Prepare the test samples in accordance with ISO 6498.

For sample storage and ease of grinding, samples should be air-dry (about 90 % dry matter)

Dry wet samples at < 60 °C to prevent creation of artefact fibre. The amount of residue after extraction is affected by the particle size of the sample. Grind representative samples to obtain a geometric mean particle size of 220 µm to 260 µm (see 6.2).

Grinding segregates the sample, with highest fibre content material passing out of the grinder last. Do not discard material in the grinder, combine it with material in the grinder receptacle. Mix the ground sample by placing it on a square sheet of paper (approximately 40 cm × 40 cm) creased along both diagonals. Lift two