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IS 14825 (2000): Animal Feeding Stuff - Determination of Nitrogen Content and Calculation of Crude Protein Content - Kjeldahl Method [FAD 5: Livestock Feeds, Equipment and Systems]



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पशु आहार — नाईट्रोजन की मात्रा ज्ञात करना एवं
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Indian Standard

**ANIMAL FEEDING STUFF — DETERMINATION OF
NITROGEN CONTENT AND CALCULATION OF
CRUDE PROTEIN CONTENT —
KJELDAHL METHOD**

ICS 65.120

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NATIONAL FOREWORD

This Indian Standard which is identical with ISO 5983 : 1997 'Animal feeding stuffs — Determination of nitrogen content and calculation of crude protein content — Kjeldahl method' issued by the International Organization for Standardization (ISO) was adopted by the Bureau of Indian Standards on the recommendation of the Livestock Feeds Sectional Committee and approval of the Food and Agriculture Division Council.

Method of test for determination of nitrogen content and calculation of crude protein content — Kjeldahl method, was covered in 5 of IS 7874 (Part 1); after publication of this Indian Standard the above referred clause of IS 7874 (Part 1) will be deleted through amendment.

In this adopted standard, certain terminology and conventions are not identical to those used in Indian Standards. Attention is drawn specially to the following:

- a) Wherever the words 'International Standard' appear referring to this standard, they should be read as 'Indian Standard'.
- b) Comma (,) has been used as a decimal marker while in Indian Standards, the current practice is to use a point (.) as the decimal marker.

The technical committee responsible for the preparation of this standard has reviewed the provisions of the following ISO standards and has decided that they are acceptable for use in conjunction with this standard:

ISO 1871 Agricultural food products — General directions for the determination of nitrogen by the Kjeldahl method

ISO 5725 Precision of test methods — Determination of repeatability and reproducibility for a standard test method by inter-laboratory tests (now withdrawn)

ISO 5725-1 Accuracy (trueness and precision) of measurement methods and results — Part 1: General principles and definitions

ISO 5725-2 Accuracy (trueness and precision) of measurement methods and results — Part 2: Basic method for the determination of repeatability and reproducibility of a standard measurement method

In reporting the results of a test or analysis made in accordance with this standard, if the final value, observed or calculated, is to be rounded off, it shall be done in accordance with IS 2 : 1960 'Rules for rounding off numerical values (*revised*)'.

Indian Standard

**ANIMAL FEEDING STUFF — DETERMINATION OF
NITROGEN CONTENT AND CALCULATION OF
CRUDE PROTEIN CONTENT —
KJELDAHL METHOD**

1 Scope

This International Standard specifies a method for the determination of the nitrogen content of animal feeding stuffs by the Kjeldahl process, and a method for the calculation of the crude protein content.

This method does not distinguish between protein nitrogen and non-protein nitrogen. If it is important to determine the content of non-protein nitrogen, an appropriate method should be used.

NOTE — Under certain circumstances, full recovery of nitrogen in nitrates and nitrites is not possible by this method.

2 Normative reference

The following standard contains provisions which, through reference in this text, constitute provisions of this International Standard. At the time of publication, the edition indicated was valid. All standards are subject to revision, and parties to agreements based on this International Standard are encouraged to investigate the possibility of applying the most recent edition of the standard indicated below. Members of IEC and ISO maintain registers of currently valid International Standards.

ISO 6498:1983, *Animal feeding stuffs — Preparation of test samples.*

3 Principle

Digestion of organic matter by sulfuric acid in the presence of a catalyst. Rendering of the reaction product alkaline, then distillation and titration of the liberated ammonia. Calculation of the nitrogen content. Multiplication of the result by the conventional factor 6,25 to obtain the crude protein content.

4 Reagents and materials

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

The reagents (except the standard materials (4.6)) shall be practically free from nitrogenous compounds.

4.1 Potassium sulfate.

4.2 Catalyst, either 4.2.1 or 4.2.2.

4.2.1 Copper(II) oxide (CuO).

4.2.2 Copper(II) sulfate pentahydrate (CuSO₄·5H₂O).

4.3 Sulfuric acid, $c(\text{H}_2\text{SO}_4) = 18 \text{ mol/l}$, $\rho_{20}(\text{H}_2\text{SO}_4) = 1,84 \text{ g/ml}$.

4.4 Paraffin wax.

4.5 Saccharose.

4.6 Standard materials, either 4.6.1 or 4.6.2.

4.6.1 Acetanilide, with melting point 114 °C; nitrogen (N) content 103,6 g/kg.

4.6.2 Tryptophan, with melting point 282 °C; nitrogen (N) content 137,2 g/kg.
Before use, dry the tryptophan.

4.7 Sodium hydroxide solution, $w(\text{NaOH}) = 33 \% (m/m)$.

4.8 Collecting liquid, either 4.8.1 or 4.8.2.

4.8.1 Sulfuric acid, standard volumetric dilution, $c(\text{H}_2\text{SO}_4) = 0,05 \text{ mol/l}$ or $c(\text{H}_2\text{SO}_4) = 0,125 \text{ mol/l}$.

4.8.2 Boric acid, $\rho(\text{H}_3\text{BO}_3) = 40 \text{ g/l}$.

4.9 Solution for titration, either 4.9.1 or 4.9.2.

4.9.1 Sodium hydroxide, standard volumetric solution, $c(\text{NaOH}) = 0,1 \text{ mol/l}$ or $c(\text{NaOH}) = 0,25 \text{ mol/l}$.

4.9.2 Sulfuric acid, standard volumetric dilution, $c(\text{H}_2\text{SO}_4) = 0,05 \text{ mol/l}$ or $c(\text{H}_2\text{SO}_4) = 0,125 \text{ mol/l}$.

4.10 Mixed indicator, neutral point at pH 4,4 to 5,8.

Dissolve 2 g of Methyl red and 1 g of Methylene blue in 1000 ml of ethanol [$w(\text{C}_2\text{H}_5\text{OH}) = 95 \% (V/V)$].

4.11 Litmus paper.

4.12 Boiling aids, such as granulated pumice stone, or glass beads of diameter 5 mm to 7 mm, or carborundum chips, washed in hydrochloric acid and in distilled water, and ashed.

5 Apparatus

Usual laboratory apparatus and, in particular, the following.

5.1 Analytical balance.

5.2 Digestion, distillation and titration apparatus.

6 Sampling

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

Sampling is not part of the method specified in this International Standard. A recommended sampling method is given in ISO 6497⁽⁵⁾

Store the sample in such a way that deterioration and change in its composition are prevented.

7 Preparation of test sample

Prepare the test sample in accordance with ISO 6498.

8 Procedure

NOTE — For general directions on the application of the Kjeldahl method, see ISO 1871¹⁾.

8.1 Test portion

Weigh, to the nearest 1 mg, a mass of the test sample chosen according to the expected nitrogen content so that the test portion contains between 0,005 g and 0,2 g of nitrogen and, preferably, more than 0,02 g.

NOTE — The mass of the test portion of homogeneous air-dry samples should be between 0,5 g and 2,0 g. The mass of the test portion of wet and/or inhomogeneous samples should be between 2,5 g and 5,0 g.

8.2 Determination

WARNING - The following operations should be carried out under a well-ventilated hood or in a fume cupboard which is resistant to sulfuric acid.

8.2.1 Digestion of organic matter

Transfer the test portion quantitatively into a Kjeldahl digestion flask of suitable size (usually 800 ml).

Add 15 g of potassium sulfate (4.1).

Add an appropriate quantity of catalyst as follows: 0,3 g of copper(II) oxide (4.2.1) or 0,9 g to 1,2 g of copper(II) sulfate pentahydrate (4.2.2).

Add 25 ml of sulfuric acid (4.3) for the first gram of dry matter of the test portion and 6 ml to 12 ml for each additional gram of dry matter. Mix thoroughly, ensuring complete wetting of the test portion.

Support the flask so that its axis is inclined at an angle of 30° to 45° to the vertical. Maintain the flask in this position throughout heating.

Heat the flask moderately at first to prevent foam from rising into the neck of the flask or escaping from the flask.

NOTES

1 It may be advisable to add an anti-foaming agent such as paraffin wax (4.4).

2 A destruction block is a suitable digestion apparatus for ensuring that the destruction mixture is heated steadily.

Heat moderately, swirling from time to time, until the mass has carbonized and the foam has disappeared. Then heat more intensively until the liquid is boiling steadily.

NOTE 3 Heating is adequate if the boiling acid condenses towards the middle of the neck of the Kjeldahl flask.

Avoid overheating of the walls of the flask not in contact with liquid.

NOTE 4 If a naked flame is used, such overheating can be prevented by placing the flask on a sheet of heat-resistant material with an aperture of diameter slightly less than that of the flask at the liquid level.

After the liquid has become clear with a light green-blue colour, heat for 2 h.

Leave to cool. If the digest starts to solidify, add some water and mix by swirling.

8.2.2 Distillation of ammonia

8.2.2.1 Carefully add 250 ml to 350 ml of water to dissolve the sulfates completely. If necessary, facilitate dissolving by heating the flask in warm water. Mix by swirling and allow to cool.

Add a few boiling aids (4.12).

NOTE — For some specific samples, the sulfates may not completely dissolve in the water. In that case, it is recommended to repeat the digestion with a reduced mass of potassium sulfate (4.1).

8.2.2.2 Pipette into the collecting flask of the distillation apparatus 25 ml of the sulfuric acid (4.8.1), choosing the concentration according to the expected nitrogen content of the test portion. Add 100 ml to 150 ml of water. Add a few drops of the mixed indicator (4.10). Proceed in accordance with 8.2.2.4.

8.2.2.3 Alternatively, transfer into the collecting flask 100 ml to 250 ml of boric acid (4.8.2). Add a few drops of mixed indicator (4.10).

NOTE — Simultaneous titration of the ammonia (see 8.2.3.2) during distillation is recommended since it facilitates verification of the end of distillation.

8.2.2.4 Immerse the end of the condenser in the liquid contained in the collecting flask, to a depth of at least 1 cm.

Slowly pour 100 ml of sodium hydroxide solution (4.7) into the digestion flask along the wall.

Immediately connect the flask to the distillation apparatus.

Heat the flask in such a manner that approximately 150 ml of distillate is collected in 30 min. At the end of this time, check the pH of the distillate at the tip of the condenser using litmus paper (4.11). If the reaction is alkaline, continue distillation.

IMPORTANT - Lift the condenser from the liquid just before the end of the distillation, to prevent backflow.

If, during distillation using sulfuric acid as collecting liquid, the contents of the collecting flask become alkaline, recommence the determination, making appropriate adjustments.

8.2.3 Titration

NOTE — Titration with automatic endpoint indication using a pH-meter is recommended. Otherwise, the endpoint is indicated by the change in colour of the mixed indicator (4.10) added in 8.2.2.

8.2.3.1 If sulfuric acid is used as the collecting liquid, titrate, in the collecting flask, the excess sulfuric acid with sodium hydroxide solution (4.9.1), $c(\text{NaOH}) = 0,1 \text{ mol/l}$ or $c(\text{NaOH}) = 0,25 \text{ mol/l}$ as appropriate, until the endpoint is indicated by the pH-meter or until the colour changes from violet to green.

8.2.3.2 If boric acid is used as the collecting liquid, titrate the ammonia with sulfuric acid (4.9.2), $c(\text{H}_2\text{SO}_4) = 0,05 \text{ mol/l}$ or $c(\text{H}_2\text{SO}_4) = 0,125 \text{ mol/l}$ as appropriate, until the endpoint is indicated by the pH-meter or the colour changes from green to violet.

If simultaneous titration is not possible (see the note in 8.2.2.3), the titration should be carried out as soon as possible after the distillation is complete, ensuring that the temperature of the distillate does not exceed 25 °C. Under these conditions, losses of ammonia are avoided.

8.3 Blank test

Perform a blank test using about 1 g of saccharose (4.5) in place of the test portion.

8.4 Check test

Perform a check test by determining the nitrogen content of acetanilide (4.6.1) or tryptophan (4.6.2) after addition of 1 g of saccharose (4.5).

The choice of the substance for the check test should be related to the digestibility of the samples to be analysed. Acetanilide is easily digested, whereas the digestion of tryptophan is more difficult.

The recovery of nitrogen from acetanilide or tryptophan should be at least 99,5 % for acetanilide and at least 99,0 % for tryptophan.

The maximum difference between determinations carried out simultaneously should not exceed 3 g per kilogram of dry matter content.

9 Calculation and expression of results

9.1 Calculation of nitrogen content

9.1.1 Distillate collected in sulfuric acid

Provided that the volumes of sulfuric acid used to collect the ammonia for the determination (8.2) and for the blank test (8.3) are equal, calculate the nitrogen content of the test sample by the equation:

$$w_{N1} = \frac{(V_0 - V_1) \times c_1 \times M}{m}$$

where

- w_{N1} is the nitrogen content, in grams per kilogram, of the test sample;
- V_0 is the volume, in millilitres, of the sodium hydroxide solution (4.9.1) required for the blank test;
- V_1 is the volume, in millilitres, of the sodium hydroxide solution (4.9.1) required for the determination;
- c_1 is the concentration, in moles per litre, of the sodium hydroxide solution (4.9.1) used for the titrations;
- M is the molar mass, in grams per mole, of nitrogen ($M = 14$ g/mol);
- m is the mass, in grams, of the test portion.

Report the result to the nearest 0,01 g/kg.

9.1.2 Distillate collected in boric acid

Calculate the nitrogen content of the test sample by the equation:

$$w_{N2} = \frac{2(V_3 - V_2) \times c_2 \times M}{m}$$

where

- w_{N2} is the nitrogen content, in grams per kilogram, of the test sample;
- V_2 is the volume, in millilitres, of the sulfuric acid (4.9.2) required for the blank test;
- V_3 is the volume, in millilitres, of the sulfuric acid (4.9.2) required for the determination;
- M is the molar mass, in grams per mole, of nitrogen ($M = 14$ g/mol);
- c_2 is the concentration, in moles per litre, of the sulfuric acid (4.9.2) used for the titrations;
- m is the mass, in grams, of the test portion.

Report the result to the nearest 0,01 g/kg.

9.2 Calculation of crude protein content

Calculate the crude protein content of the test sample by the equation:

$$w_p = 6,25 w_N$$

where

w_p is the crude protein content, in grams per kilogram, of the test sample;
 w_N is the nitrogen content, in grams per kilogram, of the test sample (either w_{N1} or w_{N2});

Report the result to the nearest 1 g/kg.

10 Precision

10.1 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 may not be applicable to concentration ranges and matrices other than those given.

10.2 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 limit (r) derived from the equation:

$$r = 2,28 \text{ g/kg} + 0,0147 \bar{w}_p$$

where

r is the repeatability limit, in grams per kilogram;
 \bar{w}_p is the mean of the two single test results for crude protein content, in grams per kilogram.

10.3 Reproducibility

The absolute difference between two single test results obtained using the same method on identical test material in different laboratories by different operators using different equipment, will in not more than 5 % of cases exceed the reproducibility limit (R) derived from the equation:

$$R = 12,8 \text{ g/kg} + 0,0361 \bar{w}_p$$

where

R is the reproducibility limit, in grams per kilogram;
 \bar{w}_p is the mean of the two single test results for crude protein content, in grams per kilogram.

11 Test report

The test report shall specify:

- all information necessary for the complete identification of the sample;
- the method with which sampling was carried out, if known;
- the method used;
- the test result obtained, either the nitrogen content, or the crude protein content combined with the conversion factor used (i.e. 6,25);
- if the repeatability has been checked, the final quoted result obtained;
- all operating details not specified in this International Standard, or regarded as optional, together with details of any incidents occurred when performing the method, which may have influenced the test result(s).

Annex A
(informative)

Results of interlaboratory test

An interlaboratory test was organized by ISO/TC 34/SC 10, *Animal feeding stuffs*, in 1987 and carried out in accordance with ISO 5725:1986^[2]. The final statistical analysis was carried out in accordance with ISO 5725-2^[4]. In this test 25 laboratories participated; samples of corn gluten feed, finished mixed feed stuff, fish meal, mixed feed stuff concentrate (2 types), premixed feed stuff and yeast were investigated.

Table A.1 - Statistical results of interlaboratory test

Parameter	Sample ¹⁾						
	1	2	3	4	5	6	7
Number of laboratories retained after eliminating outliers	25	25	25	25	25	25	25
Mean crude protein content, g/kg ²⁾	704	810	456	29,8	394	474	252
Repeatability standard deviation (s_r), g/kg	4,6	4,5	3,2	0,85	2,9	2,3	3,2
Repeatability relative standard deviation, %	0,65	0,55	0,71	2,85	0,75	0,50	1,26
Repeatability limit (r) [$r = 2,8 \times s_r$], g/kg	12,88	12,60	8,96	2,38	8,12	6,44	8,96
Reproducibility standard deviation (s_R), g/kg	10,6	12,9	12,5	4,3	12,4	10,3	8,2
Reproducibility relative standard deviation, %	1,51	1,59	2,75	14,5	3,16	2,17	3,24
Reproducibility limit (R) [$R = 2,8 \times s_R$], g/kg	29,68	36,12	35,00	12,04	34,72	28,84	22,96
1) Sample 1: fish meal; Sample 2: corn gluten feed; Sample 3: yeast; Sample 4: premixed feed stuff; Sample 5: mixed feed stuff concentrate; Sample 6: mixed feed stuff concentrate; Sample 7: finished mixed feed stuff. 2) Based on dry matter.							

Annex B
(informative)

Bibliography

- [1] ISO 1871:1975, *Agricultural food products — General directions for the determination of nitrogen by the Kjeldahl method*
- [2] ISO 5725:1986, *Precision of test methods — Determination of repeatability and reproducibility for a standard test method by inter-laboratory tests* (now withdrawn).
- [3] ISO 5725-1:1994, *Accuracy (trueness and precision) of measurement methods and results — Part 1: General principles and definitions*
- [4] ISO 5725-2:1994, *Accuracy (trueness and precision) of measurement methods and results — Part 2: Basic method for the determination of repeatability and reproducibility of a standard measurement method*
- [5] ISO 6497: —¹, *Animal feeding stuffs — Sampling*

¹ To be published.

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Amendments are issued to standards as the need arises on the basis of comments. Standards are also reviewed periodically; a standard along with amendments is reaffirmed when such review indicates that no changes are needed; if the review indicates that changes are needed, it is taken up for revision. Users of Indian Standards should ascertain that they are in possession of the latest amendments or edition by referring to the latest issue of 'BIS Catalogue' and 'Standards: Monthly Additions'.

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