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# International Standard



# 5983

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## Animal feeding stuffs — Determination of nitrogen content and calculation of crude protein content

*Aliments des animaux — Détermination de la teneur en azote en vue du calcul de la teneur en protéines brutes*

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## FOREWORD

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Draft International Standards adopted by the technical committees are circulated to the member bodies for approval before their acceptance as International Standards by the ISO Council.

International Standard ISO 5983 was developed by Technical Committee ISO/TC 34, *Agricultural food products*, and was circulated to the member bodies in November 1977.

It has been approved by the member bodies of the following countries :

Australia	Iran	Romania
Bulgaria	Ireland	South Africa, Rep. of
Canada	Israel	Spain
Czechoslovakia	Mexico	Thailand
Egypt, Arab Rep. of	Netherlands	Turkey
Ethiopia	New Zealand	United Kingdom
France	Philippines	USA
Hungary	Poland	USSR
India	Portugal	Yugoslavia

No member body expressed disapproval of the document.

# Animal feeding stuffs — Determination of nitrogen content and calculation of crude protein content

## 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.

For general directions on the application of the Kjeldahl method, see ISO 1871.

## 2 FIELD OF APPLICATION

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

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

## 3 REFERENCE

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

## 4 PRINCIPLE

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

## 5 REAGENTS

All reagents shall be of recognized analytical quality. The water used shall be distilled water or water of at least equivalent purity.

All reagents [except the standards (5.6)] shall be practically free from nitrogenous compounds.

**5.1 Potassium sulphate.**

**5.2 Catalyst :**

**WARNING — Attention is drawn to the toxic hazards of mercury compounds. All necessary handling precautions**

should be taken. Solutions containing mercury should not be discarded directly into drainage systems but should be collected for subsequent treatment.

**5.2.1 Mercury; or**

**5.2.2 Mercury(II) oxide (HgO); or**

**5.2.3 Copper(II) oxide (CuO); or**

**5.2.4 Copper(II) sulphate pentahydrate (CuSO<sub>4</sub>·5H<sub>2</sub>O).**

**5.3 Sulphuric acid,  $\rho_{20}$  1,84 g/ml.**

**5.4 Paraffin wax.**

**5.5 Sucrose.**

**5.6 Standards :**

**5.6.1 Acetanilide** [melting point 114 °C; nitrogen (N) content 10,37 % (m/m)].

**5.6.2 Tryptophan** [melting point 282 °C; nitrogen (N) content 13,37 % (m/m)].

**5.7 Sodium hydroxide solution, 33 % (m/m).**

**5.8 Mercury precipitation reagent :**

**5.8.1 Sodium thiosulphate solution**, prepared by dissolving 80 g of sodium thiosulphate pentahydrate (Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>·5H<sub>2</sub>O) in 1 000 ml of water; or

**5.8.2 Sodium or potassium hypophosphite.**

**5.9 Collecting liquid :**

**5.9.1 Sulphuric acid, 0,1 N or 0,25 N standard volumetric solution; or**

**5.9.2 Boric acid solution, 40 g/l.**

**5.10 Solution for titration :**

**5.10.1 Sodium hydroxide, 0,1 N or 0,25 N standard volumetric solution; or**

**5.10.2 Sulphuric acid, 0,1 N or 0,25 N standard volumetric solution.**

**5.11 Mixed indicator.**

Dissolve 2 g of methyl red and 1 g of methylene blue in 1 000 ml of 95 % (V/V) ethanol.

**5.12 Litmus paper.**

**5.13 Boiling aid :** granulated pumice stone, or glass beads of diameter 5 to 7 mm, or carborundum chips, washed in hydrochloric acid and calcined.

**6 APPARATUS**

Usual laboratory apparatus not otherwise specified, and in particular :

**6.1 Analytical balance.**

**6.2 Digestion, distillation and titration apparatus.**

**7 SAMPLE**

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

**8 PROCEDURE**

**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 and 0,08 g of nitrogen and, preferably, more than 0,02 g. The mass of the test portion should be between 0,5 and 2,0 g (preferably 1,0 g).

NOTE — When the inhomogeneity of the sample makes it necessary to take an amount greater than that prescribed, and hence the expected amount of nitrogen may exceed 0,08 g, increase proportionally the amount of sulphuric acid in the collecting flask (see 8.2.2), if sulphuric acid is used as the collecting liquid.

**8.2 Determination**

**WARNING — The following operations should be carried out under a well ventilated hood or in a fume cupboard.**

**8.2.1 Digestion of organic matter**

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

Add potassium sulphate (5.1); if mercury or mercury(II) oxide is used as catalyst, a quantity of 10 g of potassium sulphate is sufficient; with copper(II) oxide or copper(II) sulphate pentahydrate as catalyst, 15 g of potassium sulphate is required.

Add an appropriate quantity of catalyst : 0,65 g (1 drop) of mercury (5.2.1) or 0,7 g of mercury(II) oxide (5.2.2) can be used for all products; 0,3 g of copper(II) oxide (5.2.3) or 0,9 to 1,2 g of copper(II) sulphate pentahydrate (5.2.4) may be used instead, but it should be recognized that for complete nitrogen recovery a longer digestion time will then be needed.

NOTE — Mercury is preferred as the catalyst when high protein contents have to be determined.

Add 25 ml of the sulphuric acid (5.3) for the first gram of dry matter of the test portion and 6 to 12 ml for each additional gram of dry matter (starch and fat digestion require about 6 and 12 ml respectively). Mix thoroughly, ensuring complete wetting of the test portion.

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

NOTE — It may be advisable to add an anti-foaming agent such as paraffin wax (5.4).

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. 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; if a naked flame is used, such over-heating can be prevented by placing the flask on a sheet of asbestos with a hole of diameter slightly less than that of the flask at the liquid level.

Throughout the heating, the flask should be supported so that its axis is inclined at an angle of 30 to 45° to the vertical.

After the liquid has become clear, continue heating for 1 h in the case of a mercury catalyst, or for 2 h in the case of a copper catalyst.

Leave to cool. If the cold digest solidifies, recommence the determination using a larger amount of sulphuric acid than that specified above.

**8.2.2 Distillation of ammonia**

Carefully add 250 to 350 ml of water to dissolve the sulphates completely, mix by swirling and allow to cool.

Add a few boiling aids (5.13).

Pipette into the collecting flask of the distillation apparatus 25 ml of 0,1 N or 0,25 N sulphuric acid solution (5.9.1), choosing the normality according to the expected nitrogen content of the test portion (see the note to 8.1), and add 100 to 150 ml of water and a few drops of mixed indicator (5.11).

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 (5.7) into the digestion flask along the wall.

NOTE — If a larger amount of sulphuric acid (5.3) than that specified is used (see 8.2.1, final paragraph), the amount of sodium hydroxide shall be increased proportionally.

If a mercury-based catalyst has been used, the sodium hydroxide solution shall be mixed with 25 ml of sodium thiosulphate solution (5.8.1) before addition to the flask.

NOTE — If added separately, thiosulphate might react with the acid in the flask to produce hydrogen sulphide, leading to incorrect results. Instead of thiosulphate, hypophosphite (5.8.2) may be used, in which case there is no risk of hydrogen sulphide production;

1 g of the hypophosphite added in solid form after the dilution with water and before the addition of alkali, suffices for the precipitation of up to 1 g of mercury.

Immediately connect the flask to the distillation apparatus.

Heat the flask in such a manner that 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 (5.12). If the reaction is alkaline, continue distillation.

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

If, during distillation, the contents of the collecting flask become alkaline, recommence the determination, making appropriate adjustments.

Alternatively, the distillate may be collected in 100 to 250 ml of the boric acid solution (5.9.2).

### 8.2.3 Titration

If sulphuric acid is used as the collecting liquid, titrate, in the collecting flask, the excess sulphuric acid with 0,1 N or 0,25 N sodium hydroxide solution (5.10.1), as appropriate until the colour changes from violet to green.

If boric acid solution is used as the collecting liquid, titrate the ammonia with 0,1 N or 0,25 N sulphuric acid solution (5.10.2), as appropriate, until the colour changes from green to violet. Simultaneous titration of the ammonia during distillation is recommended since it facilitates verification of the end of distillation. The end point is indicated by the change of colour of the mixed indicator (5.11).

If simultaneous titration is not possible, 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 Number of determinations

Carry out two determinations on the same test sample.

### 8.4 Blank test

Perform a blank test using about 1 g of sucrose (5.5) as the test portion.

### 8.5 Check test

Perform a check test by determining the nitrogen content of the acetanilide (5.6.1) or the tryptophan (5.6.2) and adding 1 g of sucrose (5.5).

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. Tryptophan should be dried before use.

## 9 EXPRESSION OF RESULTS

### 9.1 Calculation of nitrogen content

#### 9.1.1 Method of calculation and formulae

##### 9.1.1.1 DISTILLATE COLLECTED IN SULPHURIC ACID

Provided that the quantities of sulphuric acid used to collect the ammonia for the determination (8.2) and for the blank test (8.4) are equal, then the nitrogen content, expressed as a percentage by mass of the product, is equal to

$$\frac{(V_0 - V_1) \times T \times 0,014 \times 100}{m}$$

$$= \frac{1,4 (V_0 - V_1) \times T}{m}$$

where

$V_0$  is the volume, in millilitres, of the sodium hydroxide solution (5.10.1) required for the blank test;

$V_1$  is the volume, in millilitres, of the sodium hydroxide solution (5.10.1) required for the determination;

$T$  is the normality of the sodium hydroxide solution (5.10.1) used for the titrations;

$m$  is the mass, in grams, of the test portion.

##### 9.1.1.2 DISTILLATE COLLECTED IN BORIC ACID

The nitrogen content, expressed as a percentage by mass of the product, is equal to

$$\frac{(V_1 - V_0) \times T \times 0,014 \times 100}{m}$$

$$= \frac{1,4 (V_1 - V_0) \times T}{m}$$

where

$V_0$  is the volume, in millilitres, of the sulphuric acid solution (5.10.2) required for the blank test;

$V_1$  is the volume, in millilitres, of the sulphuric acid solution (5.10.2) required for the determination;

$T$  is the normality of the sulphuric acid solution (5.10.2) used for the titrations;

$m$  is the mass, in grams, of the test portion.

##### 9.1.1.3 RESULT

Take as the result the arithmetic mean of the two determinations, provided that the requirement for repeatability (see 9.1.2) is satisfied. Report the result to the nearest 0,01 % ( $m/m$ ).

**9.1.2 Repeatability**

The difference between the results of two determinations carried out simultaneously or in rapid succession by the same analyst should not exceed :

0,03 absolute value for nitrogen contents of less than 3 % (m/m);

1 % relative to the mean value for nitrogen contents from 3 to 6 % (m/m);

0,06 absolute value for nitrogen contents above 6 % (m/m).

**9.2 Calculation of crude protein content**

Calculate the crude protein content of the product by multiplying the nitrogen content by the factor 6,25.

Report the result to the nearest 0,1 % (m/m).

**10 TEST REPORT**

The test report shall show the method used and the result obtained, expressed as nitrogen or crude protein. It shall also mention the conversion factor used (i.e. 6,25), if the result is expressed as crude protein, and any operating conditions not specified in this International Standard or regarded as optional, as well as any circumstances that may have influenced the result.

The test report shall include all details required for complete identification of the sample.

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