



**iTeh STANDARD PREVIEW**  
**(standards.iteh.ai)**

SIST ISO 5983:2001

<https://standards.iteh.ai/catalog/standards/sist/1cd61aa2-7cce-4d7f-9683-30b1d014a9ca/sist-iso-5983-2001>

# INTERNATIONAL STANDARD

**ISO  
5983**

Second edition  
1997-11-01

---

---

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

*Aliments des animaux — Détermination de la teneur en azote et calcul de la  
teneur en protéines brutes — Méthode Kjeldahl*

**iTeh STANDARD PREVIEW**  
**(standards.iteh.ai)**

[SIST ISO 5983:2001](#)

<https://standards.iteh.ai/catalog/standards/sist/1cd61aa2-7cce-4d7f-9683-30b1d014a9ca/sist-iso-5983-2001>



Reference number  
ISO 5983:1997(E)

**ISO 5983:1997(E)****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.

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.

International Standard ISO 5983 was prepared by Technical Committee ISO/TC 34, *Agricultural food products*, Subcommittee SC 10, *Animal feeding stuffs*.

This second edition cancels and replaces the first edition (ISO 5983:1979), the procedure of which has been technically revised to exclude the use of mercury as a catalyst.

Annexes A and B of this International Standard are for information only.

© ISO 1997

All rights reserved. Unless otherwise specified, no part of this publication may be reproduced or utilized in any form or by any means, electronic or mechanical, including photocopying and microfilm, without permission in writing from the publisher.

International Organization for Standardization  
Case postale 56 • CH-1211 Genève 20 • Switzerland  
Internet central@iso.ch  
X.400 c=ch; a=400net; p=iso; o=isocs; s=central

Printed in Switzerland

# Animal feeding stuffs — 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*.  
<https://standards.iteh.ai/catalog/standards/sist/1cd61aa2-7cce-4d7f-9683-30b1d014a9ca/sist-iso-5983-2001>

## 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 (CuSO<sub>4</sub>·5H<sub>2</sub>O).

### 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 <sup>[5]</sup>.

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 1871 <sup>(1)</sup>.

### 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.