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

Part 2: Block digestion and steam distillation method

Aliments des animaux — Dosage de l'azote et calcul de la teneur en protéines brutes —

Partie 2: Méthode de digestion en bloc et distillation à la vapeur

Reference number
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Foreword

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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.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

ISO 5983-2 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 10, *Animal feeding stuffs*.

This second edition cancels and replaces the first edition (ISO 5983-2:2005), which has been technically revised.

ISO 5983 consists of the following parts, under the general title *Animal feeding stuffs — Determination of nitrogen content and calculation of crude protein content*:

— *Part 1: Kjeldahl method*

— *Part 2: Block digestion and steam distillation method*

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

Part 2:
Block digestion and steam distillation method

WARNING — The use of this method may involve the use of hazardous materials, operations and equipment. This part of ISO 5983 does not purport to address all the safety risks associated with its use. It is the responsibility of the user of this method to establish appropriate health and safety practices and determine the applicability of local regulatory limitations prior to use.

1 Scope

This part of ISO 5983 specifies a method for the determination of nitrogen content of animal feeding stuffs according to the Kjeldahl method, and a method for the calculation of the crude protein content.

It is suitable for use as a semi-micro rapid routine method using block digestion, copper catalyst, and steam distillation into boric acid.

The method is applicable to the determination of greater than 0,5 % mass fraction Kjeldahl nitrogen in animal feeding stuffs, pet foods, and their raw materials.

The method does not measure oxidized forms of nitrogen nor heterocyclic nitrogen compounds.

The method does not distinguish between protein nitrogen and non-protein nitrogen.

NOTE If it is of importance to determine the content of non-protein nitrogen, an appropriate method can be 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 referred document (including any amendments) applies.

ISO 1871, *Food and feed products — General guidelines for the determination of nitrogen by the Kjeldahl method*

ISO 6498, *Animal feeding stuffs — Guidelines for sample preparation* [1)]

1) To be published. (Revision of ISO 6498:1998)

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3 Terms and definitions

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

3.1
nitrogen content
mass fraction of nitrogen determined by the procedure specified in this part of ISO 5983

NOTE The nitrogen content is expressed as a percentage mass fraction or in grams per kilogram.

3.2
crude protein content
nitrogen content (3.1) as a mass fraction multiplied by the factor 6,25

NOTE The crude protein content is expressed as a percentage mass fraction or in grams per kilogram.

4 Principle

The test portion is digested using a block digestion or equivalent apparatus. Concentrated sulfuric acid is used to convert protein nitrogen to ammonium sulfate at a boiling point elevated by the addition of potassium sulfate. A copper catalyst is used to enhance the reaction rate. An excess of sodium hydroxide is added to the cooled digest to liberate ammonia.

The liberated ammonia is distilled, using a manual, semi-automatic or fully automatic steam distillation unit. In the case of manual or semi-automatic steam distillation, distillation of the ammonia into an excess of boric acid solution is followed by titration with hydrochloric acid solution to a colorimetric endpoint. Where a fully automatic system is employed, automatic titration of the ammonia is carried out simultaneously with the distillation and the endpoint of the titration can also be detected by means of a potentiometric pH system.

The nitrogen content is calculated from the amount of ammonia produced. The crude protein content is obtained by multiplying the result by the conventional conversion factor of 6,25.

NOTE In principle, sulfuric acid can also be used for the titration.

5 Reagents

During the analysis, unless otherwise stated, use only reagents of recognized analytical grade and distilled or demineralized water or water of equivalent purity.

5.1 Kjeldahl catalyst tablets, comprising 3,5 g of potassium sulfate and 0,4 g of copper(II) sulfate pentahydrate per tablet.

These tablets are commercially available.

Other types of tablet may be used provided that:

a) they contain a quantity of potassium sulfate such that 7 g of potassium sulfate and 0,8 g of copper(II) sulfate pentahydrate can be dispensed using an integral number of whole tablets; and

b) they do not contain salts of toxic metals such as selenium or mercury.

5.2 Sulfuric acid (H_2SO_4), at least 98 % mass fraction, nitrogen-free (ρ_{20} ≈ 1,84 g/ml).

5.3 Hydrogen peroxide solution, containing approximately 30 g of H_2O_2 per 100 ml.

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5.4 Antifoaming agent. A silicone preparation is recommended, e.g. with a mass fraction of 30 % aqueous emulsion.

5.5 Sodium hydroxide (NaOH) solution, approximately 40 % mass fraction, nitrogen-free (< 5 µg of nitrogen per gram).

5.6 Indicator solutions.

5.6.1 Methyl red solution. Dissolve 100 mg of methyl red ($C_{15}H_{15}N_3O_2$) in 100 ml of ethanol or methanol.

5.6.2 Bromocresol green solution. Dissolve 100 mg of bromocresol green ($C_{21}H_{14}Br_4O_5S$) in 100 ml of ethanol or methanol.

5.7 Concentrated boric acid solution, $c(H_3BO_3) = 40{,}0$ g/l.

Dissolve 400 g of boric acid in about 5 l to 6 l of hot water. Mix and add more hot water to a volume of about 9 l. Allow to cool to room temperature. Add 70 ml of the methyl red solution (5.6.1) and 100 ml of the bromocresol green solution (5.6.2) and mix. Dilute to a final volume of 10 l with water and mix well. Depending on the water used, the pH of the boric acid solution can differ from batch to batch. Often an adjustment with a small volume of alkali is necessary to obtain a positive blank (0,05 ml to 0,15 ml of titrant). The colour should turn green when 100 ml of water are added to 25 ml of the boric acid solution. If still red, titrate with 0,1 mol/l NaOH until “neutral grey” and calculate the amount of alkali needed for the 10 l batch.

Store the solution, which is red in colour, at room temperature and protect the solution from light and sources of ammonia fumes during storage.

5.8 Dilute boric acid solution, $c(H_3BO_3) = 10{,}0$ g/l (optional trapping solution for titrators that automatically begin titration when distillation begins).

Dissolve 100 g of boric acid in about 5 l to 6 l of hot water, mix and add more hot water to a volume of about 9 l. Allow to cool to room temperature. Add 70 ml of the methyl red solution (5.6.1) and 100 ml of the bromocresol green solution (5.6.2) and mix. Dilute to a final volume of 10 l with water and mix well. Depending on the water used, the pH of the boric acid solution can differ from batch to batch. Often an adjustment with a small volume of alkali is necessary to obtain a positive blank (0,05 ml to 0,15 ml of titrant). The colour should turn green when 100 ml of water are added to 25 ml of the boric acid solution. If still red, titrate with 0,1 mol/l NaOH until “neutral grey” and calculate the amount of alkali needed for the 10 l batch.

Store the solution, which is light green in colour, at room temperature and protect the solution from light and sources of ammonia fumes during storage.

NOTE The addition of about 3 ml to 4 ml of 0,1 mol/l NaOH into 1 l of 1 % mass fraction boric acid usually gives good adjustments.

5.9 Hydrochloric acid standard volumetric solution, $c(HCl) = 0{,}100\ 0$ mol/l.

Other concentrations of HCl or sulfuric acid may be used if this is corrected for in the calculations. The concentrations should always be expressed to four decimal places.

5.10 Ammonium sulfate [$(NH_4)_2SO_4$], min. 99,5 % mass fraction, with certified purity. Dry ammonium sulfate at 102 °C ± 2 °C for 4 h and store in a desiccator.

The percentage mass fraction of nitrogen in ammonium sulfate (at 99,5 % mass fraction purity) is 21,09.

5.11 Ammonium iron(II) sulfate [$(NH_4)_2Fe(SO_4)_2{\cdot}6H_2O$], with certified purity.

The percentage mass fraction of nitrogen in ammonium iron(II) sulfate (at 100 % mass fraction purity) is 7,145.

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5.12 Standard materials.

One of 5.12.1 and 5.12.2 may be used.

In addition to the standard materials listed in 5.12.1 and 5.12.2, suitable reference materials with certified values for Kjeldahl nitrogen and crude protein should be used whenever possible.

NOTE The moisture content can be checked on a separate portion.

5.12.1 Tryptophan ($C_{11}H_{12}N_2O_2$), with melting point 282 °C; nitrogen content 137,2 g/kg. Dry the tryptophan before use.

5.12.2 Acetanilide (C_8H_9NO), minimum assay 99 % mass fraction, nitrogen content 103,6 g/kg. Do not dry in an oven before use.

5.13 Sucrose ($C_{12}H_{22}O_{11}$), with a nitrogen content of not more than 0,002 % mass fraction. Do not dry in an oven before use.

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 Digestion block, aluminium alloy block or equivalent block, fitted with an adjustable temperature control and device for measuring block temperature, capable of being maintained at 420 °C ± 5 °C.

6.3 Digestion tubes, of capacity 250 ml, suitable for use with the digestion block (6.2).

6.4 Exhaust manifold, suitable for use with the digestion tubes (6.3).

6.5 Centrifugal scrubber apparatus, filter pump or aspirator, constructed of acid-resistant material, for use with mains water supply.

6.6 Automatic pipettes (**dispensers**), capable of delivering portions of up to 25 ml, ISO 8655-2 [6] (ISO 8655-5 [8]).

6.7 Graduated measuring cylinders, capacity 50 ml.

6.8 Distillation unit, capable of steam distilling, manual or semi-automatic, suitable to accommodate the digestion tubes (6.3) and the conical flasks (6.9), or capable of steam distillation and autotitration.

6.9 Conical flasks, of capacity 250 ml.

6.10 Burette, capacity 25 ml or other suitable capacity, with at least a readability of 0,05 ml, ISO 385 [1] class A.

Alternatively, an automatic burette, ISO 8655-3 [7], fulfilling the same requirements, may be used.

6.11 Automatic titrator, with a **pH meter** calibrated in the range pH 4 to pH 7.

7 Sampling

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

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Sampling is not part of the method specified in this part of ISO 5983. A recommended sampling method is given in ISO 6497 [5].

8 Preparation of test sample

Prepare the test sample in accordance with ISO 6498.

9 Procedure

9.1 General

Usually, test samples should be analysed in batches according to the procedure specified. For general requirements on the application of the Kjeldahl method, see ISO 1871.

9.2 Test portion

As the test portion, weigh, to the nearest 0,1 mg:

a) approximately 1,0 g for materials with 3 % to 30 % protein mass fraction;

b) approximately 0,5 g for materials with 30 % to 80 % protein mass fraction;

c) approximately 0,3 g for materials with more than 80 % protein mass fraction.

Do not exceed 1,2 g.

Always perform quality control and standards as well as a reagent blank with each batch.

9.3 Determination

9.3.1 Digestion

Transfer the test portion (9.2) to the digestion tube (6.3) and add two catalyst tablets (5.1) to each tube. Using a pipetting dispenser (6.6), add 12 ml of sulfuric acid (5,2) to each tube. Use 15 ml for high fat materials (> 10 % mass fraction fat). It is possible to stop at this point and continue work the following day.

If foaming is a problem, slowly add 3 ml to 5 ml of hydrogen peroxide (5.3). Swirl gently and let the reaction subside. Alternatively, a few drops of antifoaming agent (5.4) may be used.

Attach the heat side shields to the tube rack. Place the exhaust manifold (6.4) tightly on the tubes and turn the water aspirator or scrubber (6.5) on completely. Place the rack of tubes in the digestion block pre-heated to 420 °C (6.2).

After 10 min, turn the water aspirator down until the acid fumes are just contained within the exhaust hood. A condensation zone should be maintained within the tube. After the bulk of the fumes of sulfur oxides have been produced during the initial stages of digestion, reduce the vacuum source to prevent loss of sulfuric acid.

Digest for an additional 50 min. The total digestion time should be approximately 60 min.

Turn the digestor off. Remove the rack of tubes with the exhaust still in place and put it in the stand to cool for 10 min to 20 min. When fuming has stopped, remove the manifold and shut off the aspirator. Remove the side shields.

Allow the tubes to cool. Manual predilution of samples is recommended prior to distilling. Wearing gloves and eye protection, carefully add a few millilitres of water to each tube. If spattering occurs, this means that the

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tubes are still too hot. Allow to cool for a few more minutes. Add water to each tube to a total volume of approximately 80 ml.

If the sample solidifies, place the tube containing the diluted digest in the block digester and carefully warm with occasional swirling until salts dissolve, or distil for a further 30 s to 60 s.

NOTE 1 Some instruments perform the addition of water automatically. The predilution before placing the tube in the instrument is only required if very solid cakes form.

NOTE 2 Some distillation instruments start with the addition of steam before the addition of alkali, which leads to a dissolution of salt cakes and a less violent reaction during alkali addition. Crystallization during digestion can cause nitrogen losses.

9.3.2 Distillation

Transfer the digestion tube (see 9.3.1) to the distillation unit (6.8).

Where titration of the ammonia content of the distillate is performed manually, the procedure mentioned below applies. Where the distillation unit is fully automated to include titration of the ammonia content of the distillate, follow the manufacturer's instructions for operation of the distillation unit.

Place a conical flask (6.9) containing 25 ml to 30 ml of the concentrated boric acid solution (5.7) under the outlet of the condenser in such a way that the delivery tube is below the surface of the excess boric acid solution. Adjust the distillation unit to dispense 50 ml of sodium hydroxide solution (5.5). Operate the distillation unit in accordance with the manufacturer's instructions and distil off the ammonia liberated by the addition of the sodium hydroxide solution. Collect the distillate in the boric acid receiving solution. The amount of distillate (time of steam distillation) depends on the amount of nitrogen in the sample. Follow the manufacturer's instructions.

NOTE In a semi-automatic distillation unit, the addition of excess sodium hydroxide and the steam distillation are performed automatically.

9.3.3 Titration

9.3.3.1 Colorimetric. Titrate the contents of the conical flask (6.9) with the hydrochloric acid standard volumetric solution (5.9) using a burette (6.10) and read the amount of titrant used. The endpoint is reached at the first trace of pink colour in the contents. Estimate the burette reading to the nearest 0,05 ml. An illuminated magnetic stirrer plate or a photometric detector may aid visualization of the endpoint.

This can be done automatically using a steam distiller with automatic titration.

9.3.3.2 Potentiometric. Titrate the contents of the conical flask (see 6.9) with the hydrochloric acid standard volumetric solution (5.9) using a properly calibrated automatic titrator provided with a pH-meter (6.11). The pH endpoint of the titration is reached at pH 4,6, being the steepest point in the titration curve (inflection point). Read the amount of titrant used from the automatic titrator.

Follow the manufacturer's instructions for operation of the specific distiller or combined distiller and titrator.

When an automatic titration system is used, titration begins immediately after distillation starts and dilute boric acid solution (5.8) should be used.

9.4 Blank test

Carry out a blank test following the procedure specified in 9.1 to 9.3.3 taking 2 ml of water and about 0,7 g of sucrose (5.13) instead of the test portion. Keep a record of blank values. If blank values change, identify the cause.

The amount of titrant used in the blank test should always be greater than 0,0 ml. Blanks within the same laboratory should be consistent over time.

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9.5 Recovery tests

9.5.1 General

Recovery tests to be run regularly to check the accuracy of the procedure and equipment are specified in 9.5.2 to 9.5.4.

9.5.2 Nitrogen loss

Use 0,12 g of ammonium sulfate (5.10) and 0,67 g of sucrose (5.13) per flask. Add all other reagents as stated in 9.3. Digest and distil under the same conditions as for the sample. Recoveries shall be $\geqslant$ 99 % mass fraction.

9.5.3 Digestion efficiency

Use a test portion of at least 0,15 g of tryptophan (5.12.1) or acetanilide (5.12.2), weighed to the nearest 0,1 mg, and with addition of about 0,7 g of sucrose (5.13). Determine the nitrogen content according to the procedure described in 9.1 to 9.3.3. The recoveries should be $\geqslant$ 99,5 % mass fraction for acetanilide and $\geqslant$ 98,5 % mass fraction for tryptophan (Reference [9]).

9.5.4 Distillation and titration efficiency

Weigh, to the nearest 0,000 1 g, 0,10 g to 0,15 g of ammonium sulfate (5.10), or, to the nearest 0,000 1 g, 0,3 g to 0,5 g of ammonium iron(II) sulfate (5.11) into a tube. Add 80 ml of water and proceed according to 9.3.2 and 9.3.3. The recovery shall be $\geqslant$ 99,5 % mass fraction.

9.5.5 Limits

Recoveries less than those specified or more than 101,0 % mass fraction in any of the above recovery tests indicate failures in the procedures and/or inaccurate concentration of the standard volumetric hydrochloric acid solution (5.9).

10 Calculation and expression of results

10.1 Calculation

10.1.1 Calculation of nitrogen content

Calculate the nitrogen content, w_N, as a percentage mass fraction of the sample

$$w_N = \frac{1{,}400\ 7\left(V_s - V_b\right)c_s}{m} \qquad (1)$$

where

V_s is the numerical value of the volume, in millilitres, expressed to the nearest 0,05 ml, of the hydrochloric acid standard volumetric solution (5.9) used in the determination (9.3);

V_b is the numerical value of the volume, in millilitres, expressed to the nearest 0,05 ml, of the hydrochloric acid standard volumetric solution (5.9) used in the blank test (9.4);

c_s is the numerical value of the exact concentration, in moles per litre, expressed to four decimal places, of the hydrochloric acid standard volumetric solution (5.9);

m is the numerical value of the mass, in grams, of the test portion (9.2).

For reporting the result in grams per kilogram, a factor of 14,007 may be used in Equation (1), instead of 1,400 7.

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10.1.2 Calculation of recovery for ammonium salts

Calculate the recovery for ammonium sulfate with 99,5 % mass fraction purity level, w_1, as follows:

$$w_1 = \frac{w_{N,r}}{21,09} \times 100 \quad (2)$$

where $w_{N,r}$ is the recovery of nitrogen, as a percentage mass fraction.

Calculate the recovery for ammonium iron(II) sulfate with 100 % mass fraction purity level, w_2, as follows:

$$w_2 = \frac{w_{N,r}}{7,145} \times 100 \quad (3)$$

Adjust the denominators in Equations (2) and (3) if other purity levels of ammonium salts are used.

10.2 Calculation of crude protein content

Calculate the crude protein content, w_p, as a percentage mass fraction, using Equation (4):

$$w_p = w_N \, f_K \quad (4)$$

where

w_N is the nitrogen content, expressed as a percentage mass fraction to four decimal places (10.1), of the sample;

f_K is a conversion factor for Kjeldahl nitrogen to protein — for feedstuffs, $f_K = 6,25$.

To report crude protein content in grams per kilogram, multiply the right-hand side of Equation (4) by 10.

10.3 Expression of crude protein content results

Express the results to four decimal places if needed for further calculations. For end results, express those obtained for the nitrogen content to three decimal places, and for the protein content to two decimal places.

The results should not be rounded further until the final use of the test value is made. This is particularly true when the values are to be used for further calculations.

EXAMPLE 1 When the individual test values obtained from the analysis of many sample materials are used to calculate method performance statistics for within and between laboratory variation.

EXAMPLE 2 When the values are used as a reference for instrument calibration (e.g. infrared analyser), where the values from many samples are used in a simple or multiple regression calculation before they are used for further calculations.

11 Precision

11.1 Interlaboratory tests

Details of interlaboratory tests, using colorimetric endpoint detection, on the precision of the method are summarized in Annex A. The values derived from these tests may not be applicable to concentration ranges and matrices other than those given.

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Details of a proficiency test where a comparison was made showing the equivalence of the colorimetric and potentiometric endpoint determination of the titration are summarized in Annex B.

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, shall in not more than 5 % of cases exceed the repeatability limit, r, as a protein percentage mass fraction, derived from Equation (5):

$$r = 0{,}234 + 0{,}005\overline{w}_{\mathrm{p}} \tag{5}$$

where $\overline{w}_{\mathrm{p}}$ is the mean of the two single test results for crude protein content, expressed as a percentage mass fraction.

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, shall in not more than 5 % of cases be greater than the reproducibility limit, R, as a protein percentage mass fraction, derived from Equation (6):

$$R = 0{,}193 + 0{,}029\overline{w}_{\mathrm{p}} \tag{6}$$

12 Test report

The test report shall contain at least the following information:

a) all information required for the complete identification of the sample;

b) the sampling method used, if known;

c) details of the method used, with reference to this part of ISO 5983;

d) all operating details not specified in this part of ISO 5983, or regarded as optional, together with details of any incident which may have influenced the results;

e) the test results obtained, either the nitrogen content, expressed as a percentage mass fraction or in grams per kilogram, or the crude protein content, expressed as a percentage mass fraction or in grams per kilogram, quoted with the conversion factor, 6,25;

f) if the repeatability has been checked, the final result obtained;

g) if the recovery has been checked, the final result obtained.

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Annex A
(informative)

Results of interlaboratory tests

A first interlaboratory test using the block digestion and steam distillation method with colorimetric endpoint detection was organized by the AOAC International in 2001 and carried out in accordance with ISO 5725-2 [3]. In this test, 13 laboratories in North America and Europe participated. Investigated were 14 blind samples, including meat and bone meal, dog food, chinchilla food, bird seed, soybeans, corn silage, green chop, grass hay, alfalfa hay, milk replacer, albumin, swine pellets, sunflower seeds, protein block (with urea) and fish meal. For results, see Table A.1.

The recovery of nitrogen from standard compounds was 100,1 % for acetanilide and 98,8 % for tryptophan.

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Table A.1 — Results of first interlaboratory test

Parameter	Sample													
	1 Protein block	2 Swine pellets	3 Corn silage	4 Grass hay	5 Fish meal	6 Dog food	7 Chinchilla feed	8 Albumin	9 Bird seed	10 Meat and bone meal	11 Milk replacer	12 Soybeans	13 Sunflower seed	14 Legume hay
Number of laboratories retained after elimination of outliers	11	11	13	12	12	12	10	11	12	12	12	11	12	12
Mean crude protein content, $\bar{w}_p$, % (on as-is basis)	40,20	37,00	7,10	7,10	64,60	24,50	18,10	79,10	13,50	50,10	20,80	38,80	17,40	18,80
Repeatability standard deviation, s_r, % crude protein	0,20	0,20	0,20	0,10	0,50	0,20	0,20	0,30	0,10	0,90	0,30	0,40	0,40	0,30
Coefficient of variation of repeatability, %	0,40	0,50	1,90	1,90	0,70	0,80	0,80	0,40	0,80	1,80	1,30	0,90	2,30	1,40
Repeatability limit, r (= 2,8 s_r), % crude protein	0,50	0,50	0,40	0,40	1,30	0,60	0,40	0,90	0,30	2,60	0,80	1,00	1,10	0,70
Horwitz ratio, HorRat [a]	0,3	0,3	1,0	1,0	0,5	0,5	0,5	0,3	0,5	1,3	0,8	0,6	1,3	0,8
Reproducibility standard deviation, s_R, % crude protein	0,30	0,20	0,20	0,10	0,70	0,20	0,20	0,40	0,20	0,90	0,30	0,40	0,40	0,30
Coefficient of variation of reproducibility, %	0,70	0,60	2,70	1,90	1,00	0,90	0,80	0,50	1,30	1,80	1,30	1,00	2,30	1,40
Reproducibility limit, R (= 2,8s_R), % crude protein	0,80	0,60	0,50	0,40	1,90	0,60	0,40	1,00	0,50	2,60	0,80	1,00	1,10	0,70
Horwitz ratio, HorRat [a]	0,3	0,2	0,9	0,6	0,5	0,4	0,3	0,2	0,5	0,8	0,5	0,4	0,9	0,5

[a] A value of 1 usually indicates satisfactory precision, while a value > 2 indicates unsatisfactory precision, i.e. too variable for most analytical purposes or where the variation obtained is greater than expected for the type of method employed (References [9], [10]).

A second interlaboratory test using the colorimetric endpoint detection was organized in Thailand in 2004, involving 26 laboratories from government and public sectors and following the ISO 5725-2[3] protocol. Seven samples were tested. For results, see Table A.2.

Table A.2 — Results of second interlaboratory test

Parameter	Sample [a]						
	1 Fish feed, small floating pellet (extruded)	2 Fish feed, large floating pellet (extruded)	3 Shrimp feed, crumble	4 Shrimp feed, large floating pellet	5 Shrimp feed, small floating pellet	6 Larvae feed, flake	7 Wheat grain
Number of laboratories retained after elimination of outliers	24	24	24	25	26	26	25
Mean crude protein content, $\bar{w}_p$, % (on the dry basis)	33,65	29,18	40,48	37,73	38,04	51,07	15,76
Repeatability standard deviation, s_r, % crude protein	0,20	0,13	0,10	0,17	0,14	0,18	0,10
Coefficient of variation of repeatability, %	0,58	0,44	0,24	0,44	0,37	0,35	0,63
Repeatability limit, r (= 2,8s_r), % crude protein	0,55	0,36	0,28	0,46	0,39	0,50	0,28
Horwitz ratio, HorRat	0,38	0,28	0,16	0,29	0,24	0,24	0,36
Reproducibility standard deviation, s_R, % crude protein	0,40	0,38	0,42	0,49	0,45	0,64	0,25
Coefficient of variation of reproducibility, %	1,18	1,31	1,03	1,31	1,18	1,26	1,59
Reproducibility limit, R (= 2,8s_R), % crude protein	1,11	1,07	1,17	1,38	1,26	1,80	0,70
Horwitz ratio, HorRat	0,50	0,54	0,45	0,56	0,51	0,57	0,60

[a] Sampling method: ISO 6497[5].

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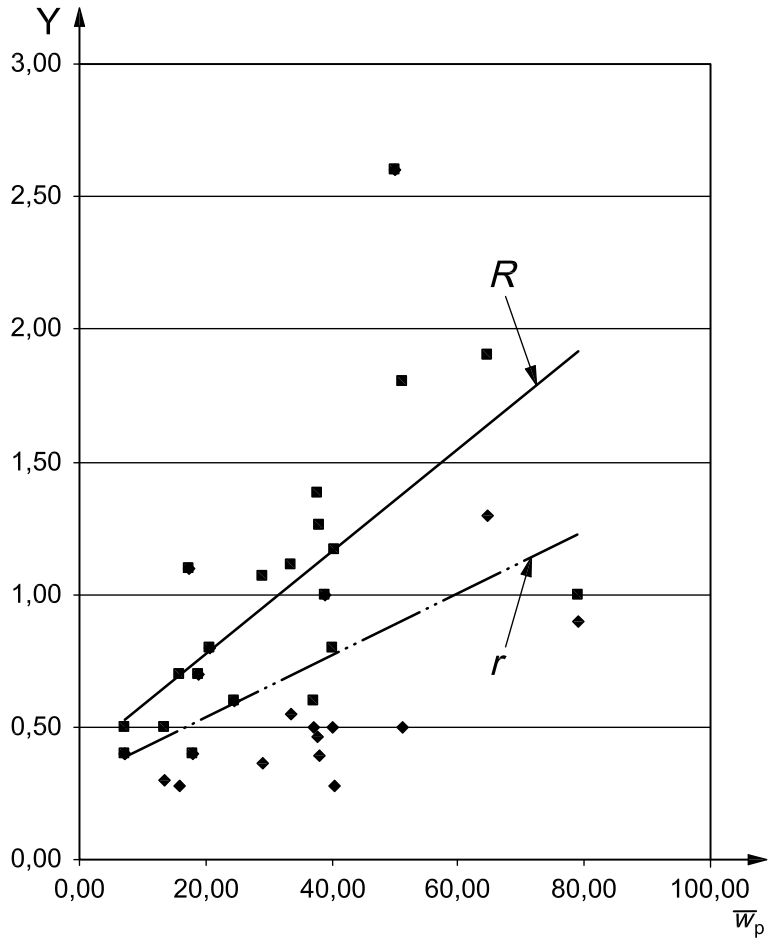

Key

$\overline{w}_p$ mean crude protein content, % mass fraction

Y precision values, % mass fraction

◆ r repeatability limit, $r = 0{,}005\ \overline{w}_p + 0{,}234$

■ R reproducibility limit, $R = 0{,}029\ \overline{w}_p + 0{,}193$

Figure A.1 — Relationship between precision values (r, R) and the mean crude protein content, $\overline{w}_p$

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Annex B
(informative)

Results of a profiency test; comparison of the colorimetric and potentiometric endpoint determination of the titration

In a Dutch proficiency testing programme, organized by the Kwaliteitsdienst Landbouwkundige Laboratoria (KDLL), both the potentiometric and colorimetric endpoint determination for the titration were applied to different types of feed. The results presented in Table B.1 show that both methods of endpoint detection yield equivalent results.

Table B.1 — Statistical evaluation of a proficiency test

Parameter	Sample					
	1 Cat feed	2 Cat feed	3 Maize	4 Maize	5 Soya expellers	6 Pig feed
Colorimetric determination						
Mean crude protein content, $\overline{w}_{p,col}$, % mass fraction	29,8	29,7	9,2	9,2	42,2	16,0
Standard deviation, s_{col}, % mass fraction	0,38	0,33	0,17	0,13	0,63	0,24
No. labs, N_{col}	24	24	25	25	25	22
Potentiometric determination						
Mean crude protein content, $\overline{w}_{p,pot}$, % mass fraction	29,9	29,9	9,3	9,2	42,3	16,0
Standard deviation, s_{pot}, % mass fraction	0,25	0,43	0,13	0,15	0,60	0,21
No. labs, N_{pot}	14	14	14	14	15	15
F**-test for equality of variances**						
F	2,28	1,65	1,76	1,30	1,11	1,39
F_{crit}	2,43	2,18	2,42	2,15	2,35	2,38
Conclusion	no significant difference	no significant difference	no significant difference	no significant difference	no significant difference	no significant difference
t**-test for equality of means (two-tail)**						
t_{stat}	0,94	1,40	0,48	0,85	0,55	0,12
t_{crit}	2,03	2,03	2,03	2,03	2,02	2,03
Conclusion	no significant difference	no significant difference	no significant difference	no significant difference	no significant difference	no significant difference

NOTE Significance level = 5 %; $F < F_{crit}$ = no significant difference; $t_{stat} < t_{crit}$ = no significant difference.

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