INTERNATIONAL STANDARD

ISO 6654

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Animal feeding stuffs — Determination of urea content

Aliments des animaux — Détermination de la teneur en urée

Reference number
ISO 6654:1991(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.

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Animal feeding stuffs — Determination of urea content

1 Scope

This International Standard specifies a spectrometric method for the determination of the urea content of animal feeding stuffs.

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 Definition

For the purposes of this International Standard, the following definition applies.

urea content: The mass fraction of substances determined using the procedure specified in this International Standard.

It is expressed as a percentage by mass.

4 Principle

Suspension in water of the test portion in the presence of a decolorant. Stirring of the suspension, then filtration. Addition to the filtrate of 4-dimethyl-amino-benzaldehyde (4-DMAB) and spectrometric measurement at 420 nm of the absorbance of the solution thus obtained.

5 Reagents

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

5.1 Activated carbon, which does not adsorb urea.

5.2 4-dimethyl-amino-benzaldehyde (4-DMAB), solution prepared as follows.

Dissolve 1,6 g of 4-DMAB in 100 ml of 96 % (*V*/*V*) ethanol, add 10 ml of concentrated hydrochloric acid (ρ_{20} = 1,19 g/ml) and mix.

This solution may be stored for a maximum of 2 weeks.

5.3 Carrez I solution.

Dissolve in water 24 g of zinc acetate dihydrate [$Zn(CH_3COO)_2 \cdot 2H_2O$] and 3 g of glacial acetic acid. Make up to 100 ml with water and mix.

5.4 Carrez II solution.

Dissolve in water 10,6 g of potassium hexacyanoferrate(II) trihydrate (potassium ferrocyanide trihydrate) {$K_4[Fe(CN)_6] \cdot 3H_2O$}. Make up to 100 ml with water and mix.

5.5 Urea, standard solution corresponding to 1 g of urea per litre.

6 Apparatus

Usual laboratory apparatus and, in particular, the following.

6.1 Rotary shaker, capable of being operated at a rotational frequency of about 30 min^{-1} to 40 min^{-1}.

6.2 Spectrometer, suitable for measuring absorbance at a wavelength of 420 nm, and equipped with cells of thickness 10 mm.

6.3 Test tubes, 160 mm × 16 mm, fitted with ground glass stoppers.

6.4 Volumetric flasks, of 100 ml and 500 ml capacity.

6.5 Water-bath, capable of being controlled at 20 °C.

7 Sampling

Sampling will be the subject of a future International Standard.

8 Preparation of the test sample

Prepare the test sample in accordance with ISO 6498.

9 Procedure

9.1 Test portion

Weigh, to the nearest 1 mg, about 2 g of the test sample (clause 8).

For urea contents greater than 3 % (*m*/*m*), reduce the test portion to 1 g or dilute the test solution (see 9.2) so that it does not exceed a concentration of 50 mg of urea per 500 ml.

For low urea contents, the test portion may be increased provided that the filtrate remains clear and colourless.

9.2 Preparation of the test solution

9.2.1 Transfer the test portion (9.1) together with 1 g of the activated carbon (5.1) into a 500 ml volumetric flask (6.4). Add 400 ml of water, 5 ml of the Carrez I solution (5.3) and 5 ml of the Carrez II solution (5.4). Mix for 30 min using a rotary shaker (6.1). Make up to the mark with water, mix and filter on a dense, slow, qualitative filter paper.

9.2.2 If the filtrate is coloured, prepare a fresh test solution in accordance with 9.2.1 but increase the quantity of activated carbon added.

9.3 Colour development

Transfer, by means of a pipette, 5 ml of the clear colourless filtrate (9.2) into a test tube (6.3) and add, by means of a pipette, 5 ml of the 4-DMAB solution (5.2).

Mix and leave to stand for 15 min in a water-bath (6.5) controlled at 20 °C.

9.4 Blank test

Carry out a blank test in parallel with the determination using the same procedure and the same quantities of all reagents, but omitting the test portion.

9.5 Preparation of the calibration graph

9.5.1 Pipette into a series of five 100 ml volumetric flasks (6.4), 1 ml, 2 ml, 4 ml, 5 ml and 10 ml of the urea standard solution (5.5). Make each flask up to the mark with water. One millilitre of the standard solutions contains 10 µg, 20 µg, 40 µg, 50 µg and 100 µg of urea respectively.

9.5.2 Pipette into a series of five test tubes (6.3) 5 ml of each of these solutions (9.5.1) (one dilution per test tube). Add to each test tube, by means of a pipette, 5 ml of the 4-DMAB solution (5.2) and mix. Transfer the solutions to spectrometer cells and measure their absorbances at 420 nm, using the spectrometer (6.2), against a compensation solution containing 5 ml of 4-DMAB and 5 ml of water.

9.5.3 Plot the calibration graph, with the absorbance values on the ordinate and the corresponding concentrations of urea, in micrograms per millilitre, on the abscissa.

9.6 Spectrometric measurement

Transfer the solution obtained in 9.3 to a spectrometer cell and measure its absorbance at 420 nm, using the spectrometer (6.2), against the blank test (9.4).

NOTE 1 If the sample contains simple nitrogenous compounds, such as amino acids, carry out the measurement of absorbance at a wavelength of 435 nm.

9.7 Number of determinations

Carry out two determinations on test portions taken from the same test sample.

10 Expression of results

The urea content, expressed as a percentage by mass, of the sample is equal to

$$\frac{c}{20 \times m}$$

where

c is the urea content, in micrograms per millilitre, of the filtrate of the test solution, determined from the calibration graph (9.5.3);