L.N. 252 of 2005

VETERINARY SERVICES ACT (CAP. 437)

Community Methods for the Analysis and Official Control of Feedingstuffs Rules, 2005

IN exercise of the powers conferred by article 10 of the Veterinary Services Act, the Minister for Rural Affairs and the Environment has, after consultation with the Minister of Health, the Elderly and Community Care, made the following rules:–

Title and scope.

1. (1) The title of these rules is the Community Methods for the Analysis and Official Control of Feedingstuffs Rules, 2005.

(2) The scope of these rules is to implement the provisions found under European Union Council Directive 73/46/EEC regarding official controls of feedingstuffs as regards moisture contents of animal and vegetable fats and oils and magnesium and crude fibre contents of feeding stuffs in accordance with the methods described in the Schedule to these rules and in accordance with article 22 (c) of the Veterinary Services Act.

SCHEDULE

(Rule 2)

Chapter I

Determination of Moisture in Animal and Vegetable Fats and Oils

1. **Purpose and Scope**

This method makes it possible to determine the water and volatile substances content of animal and vegetable fats and oils.

2. **Principle**

The sample is dried to constant weight at 103 ºC. The loss in mass is determined by weighing.

3. **Apparatus**

3.1. Flat-bottomed dish, of a corrosion-resistant material, 8 to 9 cm in diameter and approximately 3 cm high.

3.2. Mercury thermometer with a strengthened bulb and expansion tube at the top end, graduated from approximately 80 ºC to at least 110 ºC, and approximately 10 cm in length.

3.3. Sand bath or electric hot-plate.

3.4. Desiccator, containing an efficient drying agent.

3.5. Analytical balance.

4. **Procedure**

Weigh out to the nearest mg approximately 20 g of the homogenized sample into the dry, weighed dish (3.1) containing the thermometer (3.2). Heat on the sand bath or hot-plate (3.3), stirring continuously with the thermometer, so that the temperature reaches 90 ºC in about 7 minutes.

Reduce the heat, watching the frequency with which bubbles rise from the bottom of the dish. The temperature must not exceed 105 ºC. Continue to stir, scraping the bottom of the dish, until bubbles stop forming.

In order to ensure complete elimination of moisture, reheat several times to 103 ºC ± 2 ºC, cooling to 93 ºC between successive heatings. Then leave to cool to room temperature in the desiccator (3.4) and weigh. Repeat this operation until the loss in mass between two successive weightings no longer exceeds 2 mg.

N.B. An increase in the mass of the sample after repeated heating indicates an oxidation of the fat, in which case calculate the result from the weighing carried out immediately before the mass began to increase.

5. **Calculation of results**

The moisture content, as a percentage of the sample, is given by the following formula:

$$(M1 - M2) \cdot \frac{100}{Mo}$$

where:

Mo = mass, in grammes, of the test sample;

M1 = mass, in grammes, of the dish with its contents before heating;

M2 = mass, in grammes, of the dish with its contents after heating.

Results lower than 0·05 % must be recorded as 'lower than 0·05 %'.

Repeatability

The difference in moisture between the results of two parallel determinations carried out on the same sample must not exceed 0·05 %, in absolute value.

Chapter II

Determination of Magnesium

— by atomic absorption spectrophotometry —

1. **Purpose and Scope**

This method makes it possible to determine the quantity of magnesium in feeding stuffs. It is particularly appropriate for determining magnesium contents lower than 5 %.

2. **Principle**

The sample is ashed and dissolved in dilute hydrochloric acid. If it contains no organic substances, it is dissolved directly in dilute hydrochloric acid. The solution is diluted and the magnesium content determined by atomic absorption spectrophotometry at 285·2 nm, by comparison with standard solutions.

3. **Reagents**

3.1. Hydrochloric acid a.p. d: 1·16.

3.2. Concentrated hydrochloric acid a.p. d: 1·19.

3.3. Magnesium ribbon or wire, or magnesium sulphate heptahydrate, dried at room temperature.

3.4. Strontium salt solution (chloride or nitrate) at 2·5 % (w/v) strontium

(= 76·08 g SrC12 · 6H2O a.p., or 60·38 g Sr (NO3)2 a.p.).

3.5. Standard magnesium solution: weigh out to the nearest mg 1 g magnesium (3.3) which has previously had its oxide coating carefully removed, or the corresponding quantity (10·143 g) of magnesium sulphate heptahydrate (3.3). Place ina 1 000 ml graduated flask, add 80 ml hydrochloric acid (3.1), leave to dissolve and make up to 1 000 ml with water. 1 ml of this solution contains 1·000 mg magnesium.

4. **Apparatus**

4.1. Platinum, silica or porcelain ashing crucibles.

4.2. Thermostatically controlled electric muffle furnace.

4.3. Atomic absorption spectrophotometer.

5. **Procedure**

5.1. *Preparation of the sample solution*

5.1.1. *Feeding stuffs composed exclusively of mineral substances*

Weigh out to the nearest mg 5 g of the sample into a 500 ml graduated flask with 250 to 300 ml water. Add 40 ml hydrochloric acid (3.1), bring to the boil and keep the liquid gently boiling for 30 minutes. Leave to cool, make up to volume with water, mix and filter into a dry beaker through a dry pleated filter. Discard the first 30 ml of the filtrate. In the presence of silica, treat 5 g of sample with a sufficient quantity (15—30 ml) of hydrochloric acid (3.2), evaporate to dryness on a water bath and transfer to an oven at 105 ºC for one hour. Proceed as from the third sentence of 5.1.2.

5.1.2. *Feeding stuffs composed predominantly of mineral substances*

Weigh out to the nearest mg 5 g of the sample into a crucible and ash at 550 ºC in the muffle furnace until an ash which is free from carbonaceous particles is obtained, and leave to cool. In order to eliminate silica, add to the ash a sufficient quantity (15—30 ml) of hydrochloric acid (3.2), evaporate to dryness on a water bath and transfer to an oven at 105 ºC for one hour. Treat the residue with 10 ml hydrochloric acid (3.1) and transfer to a 500 ml graduated flask using warm water. Leave to cool and make to volume with water. Mix and filter into a dry beaker through a dry pleated filter. Discard the first 30 ml of the filtrate.

5.1.3. *Feedingstuffs composed predominantly of organic substances*

Weigh out to the nearest mg 5 g of the sample into a crucible and ash at 550 ºC in the muffle furnace until an ash which is free from carbonaceous particles is obtained. Treat the ash with 5 ml hydrochloric acid (3.2), evaporate to dryness on a water bath and then dry for one hour in the oven at 105 ºC in order to render the silica insoluble. Treat the ash with 5 ml hydrochloric acid (3.1), transfer to a 250 ml graduated flask using warm water, bring to the boil, leave to cool and make up to volume with water. Mix and filter into a dry beaker through a dry pleated filter. Discard the first 30 ml of the filtrate.

5.2. *Measurement by atomic absorption*

By diluting the standard solution (3.5) with water, prepare at least 5 reference solutions of increasing concentration, corresponding to the optimal measuring range of the spectrophotometer. Add to each solution 10 ml strontium salt solution (3.4) and then make up the volume to 100 ml with water. Dilute with water one aliquot part of the filtrate obtained from 5.1.1, 5.1.2 or 5.1.3, so as to obtain a magnesium concentration which is within the limits of concentration of the reference solutions. The hydrochloric acid concentration of this solution must not exceed 0·4 N. Add 10 ml strontium salt solution (3.4) and then make up the volume to 100 ml with water. Measure the absorption of the solution to be determined and of the reference solutions at 285·2 nm.

6. **Calculation of results**

Calculate the quantity of magnesium in the sample by relation to the reference solutions. Express the result as a percentage of the sample.

Repeatability

The difference between the results of two parallel determinations carried out on the same sample must not exceed 5 %, in relative value.

Chapter III

Determination of Crude Fibre

1. Purpose and scope

This method makes it possible to determine fat-free organic substances in feeding stuffs which are insoluble in acid and alkaline media and are conventionally described as crude fibre.

2. Principle

The sample, defatted where necessary, is treated successively with boiling solutions of sulphuric acid and potassium hydroxide of specified concentrations. The residue is separated by filtration on a sintered-glass filter washed, dried, weighed and ashed within a range of 475 to 500 ºC. The loss of weight resulting from ashing corresponds to the crude fibre present in the test sample.

3. **Reagents**

3.1. Sulphuric acid, c = 0,13 mol/l.

3.2. Anti-foaming agent (e.g. n-octanol).

3.3. Filter aid (Celite 545 or equivalent), heated at 500 ºC for four hours

(8.6).

3.4. Acetone.

3.5. Light petroleum boiling-range 40 to 60 ºC.

3.6. Hydrochloric acid, c = 0,5 mol/l.

3.7. Potassium hydroxide solution, c = 0,23 mol/l.

4. Apparatus

4.1. Heating unit for digestion with sulphuric acid or potassium hydroxide solution, equipped with a support for the filter crucible (4.2) and provided with an outlet tube with a tap to the liquid outlet and vacuum, possibly with compressed air. Before use each day preheat the unit with boiling water for five minutes.

4.2. Glass filter crucible with fused sintered glass filter plate pore size 40-90 µm. Before first use, heat to 500 ºC for a few minutes and cool (8.6).

4.3. Cylinder of at least 270 ml with a reflux condenser, suitable for boiling.

4.4. Drying oven with thermostat.

4.5. Muffle furnace with thermostat.

4.6. Extraction unit consisting of a support plate for the filter crucible (4.2.) and with a discharge pipe with a tap to the vacuum and liquid outlet.

4.7. Connecting rings to assemble the heating unit (4.1), crucible (4.2) and cylinder (4.3) and to connect the cold extraction unit (4.6) and crucible.

5. Procedure

Weigh out to the nearest 0,001 g, 1 g of the prepared sample and place it in the crucible (4.2), (see observations 8.1, 8.2 and 8.3) and add 1 g of filter aid (3.3).

Assemble the heating unit (4.1) and the filter crucible (4.2), then attach the cylinder (4.3) to the crucible. Pour 150 ml of boiling sulphuric acid (3.1) into the assembled cylinder and crucible and if necessary add a few drops of antifoaming agent (3.2).

Bring the liquid to the boil within 5 ± 2 minutes and boil vigorously for exactly 30 minutes. Open the tap to the discharge pipe (4.1) and, under vacuum, filter the sulphuric acid through the filter crucible and wash the residue with three consecutive 30 ml portions of boiling water, ensuring that the residue is filtered dry after each washing. Close the outlet tap and pour 150 ml boiling potassium hydroxide solution (3.7) to the assembled cylinder and crucible and add a few drops of antifoaming agent (3.2). Bring the liquid to boiling point within 5 ± 2 minutes and boil vigorously for exactly 30 minutes. Filter and repeat the washing procedure used for the sulphuric acid step. After the final washing and drying, disconnect the crucible and its contents and reconnect it to the cold extraction unit (4.6). Apply the vacuum and wash the residue in the crucible with three consecutive 25 ml portions of acetone (3.4) ensuring that the residue is filtered dry after each washing.

Dry the crucible to constant weight in the oven at 130 ºC. After each drying cool in the desiccator and weigh rapidly. Place the crucible in a muffle furnace and ash to constant weight at 475 ºC to 500 ºC for at least 30

minutes. After each heating cool first in the furnace and then in the desiccator before weighing.

Carry out a blank test without the sample. Loss of weight resulting from ashing must not exceed 4 mg.

6. Calculation of results

The crude fibre content as a percentage of the sample is given by the expression:

$$\frac{(b-c) \times 100}{a}$$

where

a = mass of sample in g;

b = loss of mass after ashing during the determination, in g;

c = loss of mass after ashing during the blank test, in g.

7. Repeatability

The difference between two parallel determinations carried out on the same sample must not exceed:

— 0,3 in absolute value for crude fibre contents lower than 10 %,

— 3 % relative to the higher result, for crude fibre contents equal to or greater than10 %.

8. Observations

8.1. Feedingstuffs containing more than 10 % crude fat must be defatted prior to analysis with light petroleum (3.5). Connect the filter crucible (4.2) and its contents to the cold extraction unit (4.6) and apply vacuum and wash the residue with three consecutive 30 ml portions of light petroleum, ensuring that the residue is dry. Connect the crucible and its contents to the heating unit (4.1) and continue as described under 5.

8.2. Feedingstuffs containing fats which cannot be extracted directly with light petroleum (3.5) must be defatted as shown in 8.1 and defatted once more after boiling with acid. After boiling with acid and the subsequent washing connect the crucible and its contents to the cold extraction unit (4.6) and wash three times with 30 ml acetone followed by three further washings with 30 ml portions of light petroleum. Filter under vacuum until dry and continue the analysis as described under 5, beginning with potassium hydroxide treatment.

8.3. If the feeding stuffs contain over 5 % of carbonates, expressed as calcium carbonate, connect the crucible (4.2) with the weighed sample to the heating unit (4.1). Wash the sample three times with 30 ml hydrochloric acid (3.6). After each addition let the sample stand for about one minute before filtering. Wash once with 30 ml water and then continue as described under 5.

8.4. If an apparatus in the form of a stand is used (several crucibles attached to the same heating unit) no two individual determinations on the same sample for analysis may be carried out in the same series.

8.5. If after boiling it is difficult to filter the acidic and basic solutions, use compressed air through the discharge pipe of the heating unit and then continue filtering.

8.6. The temperature for ashing should not be higher than 500 ºC in order to extend the lifetime of the glass filter crucibles. Care must be taken to avoid excessive thermal shock during heating and cooling cycles.

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