SRI LANKA STANDARD 626:1983
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METHODS OF TEST FOR
ANIMAL FEEDS

BUREAU OF CEYLON STANDARDS

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SLS 626:1983

Gr. 8

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Sri Lanka.

SRI LANKA STANDARD

METHODS OF TEST FOR ANIMAL FEEDS

FOREWORD

This Sri Lanka Standard was authorized for adoption and publication by the Council of the Bureau of Ceylon Standards on 1983-12-20, after the draft, finalized by the Drafting Committee on Animal Feeds had been approved by the Agricultural and Food Products Divisional Committee.

This standard is intended for the introduction of uniform methods of analysis of Animal Feeds.

All values given in this standard are in SI units.

In reporting results of a test or analysis made in accordance with this standard, if the final value observed or calculated, is to be rounded off, it shall be done in accordance with CS 102.

In the preparation of this standard, the assistance obtained from publications of the International Organization for Standardization, Association of Official Analytical Chemists, Indian Standards Institution and Veterinary Research Institute of Sri Lanka is gratefully acknowledged.

1 SCOPE

This standard prescribes the methods for the determination of the following characteristics of animal feeds:

a) Particle size;

b) Moisture;

c) Crude protein;

d) Crude fat;

e) Crude fibre;

f) Total ash;

g) Acid-insoluble ash;

h) Calcium;

j) Phosphorus; and

k) Sodium chloride.

2 REFERENCES

CS 102 Presentation of numerical values

CS 124 Test sieves.

3 PREPARATION OF SAMPLE

3.1 If the sample is in finely divided form and passes through a standard sieve of nominal aperture size 1 mm, mix thoroughly a portion not less than 100 g and place it in an airtight container.

3.2 If the sample does not wholly pass through a standard sieve of nominal aperture size 1 mm, but passes through a standard sieve having nominal aperture size 2.3 mm, mix it thoroughly, from this sample, draw a portion for moisture determination at once.

3.3 If the sample is in coarse form, as for example, pieces of broken cake, pulverise until the whole sample passes through a standard sieve having nominal aperture 2.3 mm. Mix the sample thoroughly. Draw a portion of the sample for moisture determination at once.

3.4 From the mixed sample as in 3.2 or from the coarsely crushed sample as in 3.3, weigh not less than 100 g, powder it, pass through a sieve of aperture size 1 mm. Place the sample so prepared in an air-tight bottle. Weigh and take adequate quantities for analysis.

3.5 If the original sample is appreciably moist, or if for any reason the operations of pulverisation and mixing are likely to result in loss or gain of moisture, determine the moisture of the sample prepared according to 3.4, as well as in 3.2 or 3.3. Use these moisture values for correction of results in 6.5.1, 6.5.2, 11.3, and 12.4.

3.6 Materials which cannot be conveniently pulverised or passed through a sieve shall be thoroughly mixed by the most suitable means.

4 DETERMINATION OF PARTICLE SIZE

4.1 Apparatus

Make a nest of *two test sieves*, the upper sieve of aperture size 699 µm and the lower sieve of aperture size 211 µm, both conforming to CS 124.

4.2 Procedure

Weigh to the nearest mg about 100 g of the material of the unground sample and transfer this quantity to the top sieve. Cover the top sieve. Place the nest of sieves in a suitable mechanically driven shaker and shake it continuously for five minutes. Brush the fractions of the material from each of the two sieve separately into weighing dishes and weigh the fractions to the nearest milligram. Express percentage by mass of the material retained in each sieve.

5 DETERMINATION OF MOISTURE

5.1 Apparatus

5.1.1 *Aluminium dish*, having a diameter of at least 50 mm and a depth not exceeding 40 mm.

5.1.2 *Oven*, capable of maintaining a temperature of 103 ± 2 °C.

5.2 Procedure

Weigh to the nearest mg about 5 g of the material in a dish and place the dish in the oven, pre-heated at 103 °C. Place the lid of the dish beneath or beside the dish. Not more than one dish per litre of oven volume shall be placed in the oven.

Leave to dry for 4 h measured from the moment when the oven temperature has returned to 103 °C. Place the lid on the dish remove the dish from the oven, leave to cool for 20 min. in the desiccator and weigh to the nearest mg.

NOTE - Preserve the material in the dried condition for the determination of crude fat (see **7***) crude fibre (see* **8***) and total ash (see* **9***).*

5.3 Calculation

Calculate as follows :

$$\text{Moisture, per cent by mass} = \frac{100\ (m_1 - m_2)}{m_1 - m}$$

where,

m_1 is the mass, in g, of the dish with the material before drying;

m_2 is the mass, in g, of the dish with the material after drying; and

m is the mass, in g, of the empty dish.

6 DETERMINATION OF CRUDE PROTEIN

6.1 Method

6.1.1 The percentage of crude protein is ascertained by multiplying the percentage of total nitrogen other than ammoniacal nitrogen by 6.25, the quantity of ammoniacal nitrogen (see 6.4.2) is separately determined and deducted from total nitrogen (see 6.4.1).

6.2 Apparatus

6.2.1 *Kjeldahl flask*, 500 ml capacity.

6.2.2 *Distillation assembly*

The assembly consists of a round-bottom flask of 1 000 ml capacity fitted with a rubber stopper through which passes one end of the connected bulb tube. The other end of the bulb tube is connected to the condenser which is attached by means of a rubber tube to a dip tube which dips into a known quantity of standard sulphuric acid contained in a conical flask of 500 ml capacity to which 3 to 4 drops of methyl red indicator solution have been added.

6.3 Reagents

6.3.1 *Potassium sulphate* or *anhydrous sodium sulphate*.

6.3.2 *Copper sulphate*.

6.3.3 *Sulphuric acid*, concentrated, sp. gr. 1.84.

6.3.4 *Sodium hydroxide solution*. Dissolve about 450 g of sodium hydroxide in 100 ml of water.

6.3.5 *Sulphuric acid*, standard 0.1 N.

6.3.6 *Sodium hydroxide solution*, standard 0.1 N.

6.3.7 *Methyl red indicator solution*. Dissolve one gram of methyl red in 200 ml of rectified spirit (95 per cent by volume).

6.3.8 *Magnesium oxide* (carbonate free) - freshly ignited.

6.4 Procedure

6.4.1 *For total nitrogen*

Transfer carefully 0.75 to 1.25 g of the material, weighed to the nearest 1 mg, to the Kjeldahl flask. Add about 10 g of potassium sulphate or anhydrous sodium sulphate, about 0.5 g of copper sulphate and 25 ml or more, if necessary, of concentrated sulphated sulphuric acid. Place the flask in an inclined position, and heat below the boiling point of the acid until frothing ceases. Increase heat until the acid boils vigorously and digest for about 1 h, after the mixture

turn clear. Cool the contents of the flask. Transfer quantitatively to the round bottom flask with water, the total quantity of water used being about 200 ml. Add a few pieces of pumice stone to prevent bumping. Add sodium hydroxide solution in quantity sufficient to make the solution alkaline adding it carefully along the side of the flask so that it does not mix at once with the acid solution but forms a layer below the acid layer. Assemble the apparatus taking care that the tip of the dip tube extends below the surface of the standard sulphuric acid (25 ml) in the receiver. Mix the contents of the flask by shaking and distil for about 5 min until all ammonia has passed over into the standard sulphuric acid. Titrate the excess of acid with the standard sodium hydroxide solution.

6.4.1.1 Carry out a blank determination using all reagents in the same quantities but without the material to be tested.

6.4.2 *For ammoniacal nitrogen*

Weigh to the nearest mg, 2 to 4 g of the material. Shake it well with water and filter. Wash the residue thoroughly with water. Transfer the filtrate to the distillation flask and dilute to about 200 ml with water. Add about 5 g of magnesium oxide. Connect the flask to the condenser by means of the connecting bulb tube and distil about 100 ml of liquid into the receiver containing standard sulphuric acid and methyl red indicator solution. Titrate the contents of the receiver with the standard sodium hydroxide solution.

6.4.2.1 Carry out a blank determination using all reagents in the same quantities but without the material to be tested.

6.5 Calculation

6.5.1 Total nitrogen, per cent by mass $= \dfrac{1.4\ (V_1 - V_2)\ N}{m_1}$

where,

V_1 is volume, in ml, of the standard sodium hydroxide solution used to neutralize the acid in blank determination (see 6.4.1.1).

V_2 is volume, in ml, of the standard sodium hydroxide solution used to neutralize the excess acid in the test with the material (see 6.4.1).

N is normality of the standard sodium hydroxide solution.

m_1 is mass, in g, of the material taken for the test (6.4.1), corrected for loss or gain of moisture (see 3.5).

6.5.2 Ammoniacal nitrogen, per cent by mass $= \frac{1.4\ (V_1 - V_2)\ N}{m_2}$

where,

V_1 is volume, in ml, of the standard sodium hydroxide solution used to neutralize the acid in blank determination (see 6.4.2.1).

V_2 is volume, in ml, of the standard sodium hydroxide solution used to neutralize the excess acid in the test with the material (see 6.4.2).

N is normality of the standard sodium hydroxide solution.

m_2 is mass, in g, of the material taken for the test (see 6.4.2), corrected for loss or gain of moisture (see 3.5).

6.5.3 Crude protein, per cent by mass $= 6.25\ (X - Y)$

where,

X is per cent by mass of total nitrogen (see 6.5.1); and

Y is per cent by mass of ammoniacal nitrogen (see 6.5.2).

7 DETERMINATION OF CRUDE FAT

7.1 Apparatus

7.1.1 *Soxhlet extractor*, of about 100 ml capacity with appropriate ground glass joints to fit an efficient condensor above and an extraction flask below.

7.1.2 *Extraction thimble*, suitable size.

7.1.3 *Efficient condenser.*

7.1.4 *Extraction flask*, 250-ml.

7.1.5 *Heating mantle.*

7.1.6 *Steam bath.*

7.1.7 *Oven*, capable of maintaining a temperature of 100 ± 2 °C.

7.2 Reagents

Petroleum ether, boiling range 60 °C to 80 °C.

7.3 Procedure

7.3.1 Weigh to the nearest mg about 5 g of the material dried as described in **5.2**. Extract the material with 150 ml petroleum ether for 6 h in a Soxhlet extractor using a heating mantle. The extraction period may from 4 h at a condensation rate of 5 to 6 drops per second to 10 h at 2 to 3 drops per second. Distil off the solvent using a steam bath. Dry the flask containing the extracted fat, at 100 ± 2 °C. Cool in a desiccator and weigh.

NOTE - If necessary, preserve the fat-free material in a desiccator for the determination of crude fibre; and use the extracted fat for the determination of free fatty acids.

7.4 Calculation

7.4.1 Crude fat (on a moisture, free basis), per cent by mass $= \frac{100\ (m_2 - m_1)}{m}$

where,

m_1 is the mass, in g, of the extraction flask;

m_2 is the mass, in g, of the extraction flask with the dried crude fat; and

m is the mass, in g, of the dried material taken for the test.

8 DETERMINATION OF CRUDE FIBRE

8.1 Reagents

8.1.1 *Sulphuric acid*, dilute 0.255 N, that is, 1.25 per cent (*m/V*).

8.1.2 *Sodium hydroxide solution*, 0.313 N, that is, 1.25 per cent (*m/V*).

8.2 Procedure

Weigh to the nearest mg about 2 g of the dried material (see note under **5.2**) and extract the fat for about 8 hours with petroleum ether using a Soxhlet or other suitable extractor. Transfer the fat-free dry residue to a one litre conical flask. Take 200 ml of dilute sulphuric acid in a beaker and bring to the boil. Transfer the whole of the boiling acid to the flask containing the fat free material and immediately connect the flask to a reflux water condenser and heat so that the contents of the flask begin to boil within one minute. Rotate the flask frequently, taking care to keep the material from remaining on the sides of the flask but of contact with the acid. Continue boiling for exactly 30 minutes. Remove the flask and filter through fine linen (about 10 threads to the centimetre) held in a funnel, and wash with boiling water until the washings are no longer acid to litmus. Boil an adequate quantity of sodium hydroxide solution under a reflux condenser. Wash the residue on the linen into the flask with 200 ml of the boiling sodium hydroxide solution.

Immediately connect the flask to the reflux condenser and boil for exactly 30 minutes.

Remove the flask and immediately filter through the filtering cloth. Thoroughly wash the residue with boiling water and transfer to a Gooch crucible prepared with a thin but compact layer of ignited asbestos of micro-analytical reagent grade. Wash the residue thoroughly first with hot water and then with about 15 ml of 95 per cent *(m/V)* ethyl alcohol. Dry the Gooch crucible and contents at 105 ± 1 °C in the air oven to constant mass. Cool and weigh. Incinerate the contents of the Gooch crucible at 600 ± 20 °C in a muffle furnace until all the carbonaceous matter is burnt. Cool the Gooch crucible containing the ash in a desiccator and weigh.

NOTE - Alternatively, instead of the conical flask and the reflux condenser, use a tallform spoutless beaker of 600 ml to 800 ml capacity and cover it with a round bottom flask filled with cold water, which acts as a condenser. If the water in the flask becomes hot, it may be replaced by another flask containing cold water.

8.3 Calculation

$$\text{Crude fibre (on moisture free basis) per cent by mass} = \frac{100\ (m_1 - m_2)}{m}$$

where,

m_1 is mass, in g, of Gooch crucible and contents before ashing;

m_2 is mass, in g, of Gooch crucible containing asbestos and ash; and

m is mass, in g, of the dried material taken for the test.

9 DETERMINATION OF TOTAL ASH

9.1 Apparatus

9.1.1 *Silica dish.*

9.1.2 *Muffle furnace,* operating at 550 ± 25 °C.

9.2 Procedure

Transfer about 2 g (weighed to the nearest mg) of the dried material (see Note under **5.2**) to a tared silica dish and weigh. Incinerate the material at low heat not exceeding dull redness until free from all carbonaceous matter (the mass ceases to glow) and the ash is white or greyish white. Cool in a desiccator and weigh.

NOTE - Preserve the ash for the determination of acid insoluble ash.

9.3 Calculation

9.3.1 Total ash (on moisture-free basis), per cent by mass

$$\frac{m_2 - m}{m_1 - m} \times 100$$

where,

m is mass, in g, of the empty dish;

m_1 is mass, in g, of the dish with the dried material taken for the test; and

m_2 is mass, in g, of the dish with the ash.

10 DETERMINATION OF ACID INSOLUBLE ASH

10.1 Apparatus

10.1.1 *Silica dish*

10.1.2 *Watch glass*

10.1.3 *Water bath*

10.1.4 *Whatman ashless filter paper*, No. 42 or equivalent.

10.1.5 *Oven*, operating at a temperature of 100 ± 2 °C.

10.1.6 *Muffle furnace*, operating at a temperature of 550 ± 25 °C.

10.2 Reagent

Hydrochloric acid, approximately 5 N.

10.3 Procedure

To the ash contained in the silica dish (see **9.2**) add 25 ml of hydrochloric acid, cover with a watch glass and heat on a water bath for 10 minutes. Allow to cool and filter the contents of the dish through the ashless filter paper. Wash the residue with water until the washings are free from acid. Return the filter paper and the residue to the dish. Dry it in the air oven for about three hours. Ignite in the muffle furnace for three hours. Cool the dish in a desiccator and weigh.

10.4 Calculation

$$\text{Acid-insoluble ash (moisture free basis) per cent by mass} = \frac{m_2 - m}{m_1 - m} \times 100$$

where,

m is mass, in g, of the empty dish;

m_1 is mass, in g, of the dish with the dried material taken for the determination of total ash; and

m_2 is mass, in g, of the dish with the acid-insoluble ash.

11 DETERMINATION OF CALCIUM

11.1 Reagents

11.1.1 *Hydrochloric acid*, 25 per cent (*V/V*).

11.1.2 *Methyl red indicator solution*, dissolve one gram of methyl red in 200 ml of rectified spirit 95 per cent by volume.

11.1.3 *Ammonium hydroxide solution*, 50 per cent (*V/V*).

11.1.4 *Ammonium hydroxide solution*, dilute, 2 per cent (*V/V*).

11.1.5 *Ammonium oxalate solution*, saturated.

11.1.6 *Sulphuric acid*, concentrated, sp. gr. 1.84.

11.1.7 *Potassium permanganate solution*, standard 0.1 N.

11.2 Procedure

Weigh to the nearest mg about 2 g of the material in a vitreosil basin and ignite at 600 ± 2 °C in a muffle furnace to carbon free ash. Boil the ash in 40 ml of hydrochloric acid and a few drops of nitric acid. Transfer to a 250-ml measuring flask. Cool, dilute to mark and mix thoroughly. Transfer 25 ml of this solution to a 400-ml beaker, dilute to about 100 ml with water and add two drops of methyl red indicator solution. Add ammonium hydroxide solution dropwise until a brownish orange colour is obtained (pH 5.6). Add two drops of hydrochloric acid so that the colour of the solution is pink (pH 2.5 to 3.0). Dilute to about 150 ml, bring to the boil and add slowly with constant stirring 10 ml of hot ammonium oxalate solution. If the red colour of the solution changes to orange or yellow, add hydrochloric acid dropwise until the colour again changes to pink. Leave overnight to allow the precipitate to settle. Filter the supernatant liquid through ashless filter paper and wash the precipitate thoroughly with dilute ammonium hydroxide solution. Place the paper in a mixture of 125 ml of water and 5 ml of concentrated sulphuric acid. Heat to 70 °C and titrate with the standard potassium permanganate solution until the first slight pink colour is obtained.

11.3 Calculation

$$\text{Calcium, per cent by mass} = \frac{20 \times V \times N}{m}$$

where,

V is volume, in ml, of the standard potassium permanganate solution required in the titration;

N is normality of the standard potassium permanganate solution, and

m is mass, in g, of the material taken for the test. (corrected for loss or gain of moisture (see 3.5).

12 DETERMINATION OF TOTAL PHOSPHORUS

12.1 Reagents

12.1.1 *Vanadate-molybdate composite reagent*. Dissolve 20 g ammonium molybdate in 400 ml of warm water (50 °C) and cool. Dissolve 1.0 g ammonium vanadate in 300 ml of boiling water, cool and add 140 ml of conc. nitric acid gradually with stirring. Then add the molybdate solution gradually to the acid vanadate solution with stirring and dilute to 1 litre with water.

12.1.2 *Standard phosphate solution*. Prepare a stock solution containing 3.834 g of potassium dihydrogen phosphate (KH_2PO_4) per litre. Dilute 25 ml to 250 ml (1 ml = 0.2 mg P_2O_5).

12.2 Preparation of standard graph

To a series of 100-ml volumetric flasks, add 0, 2.5, 10, 20, 30, 40 and 50 ml of the standard phosphate solution (0 - 10 mg P_2O_5) and dilute each to 50 - 60 ml with water. Add a few drops of 0.88 N ammonia solution and make just acedic with nitric acid (1:2). Add 25 ml of the vanadate-molybdate reagent, dilute to the mark and mix. Allow to stand for 10 min and measure the optical density in a 2.5 or 10 mm cell at 4 700 nm.

12.3 Procedure

12.3.1 *Preparation of sample solution*

Weigh to the nearest mg about 3 g of the material into a silica dish. Char carefully and continue the ashing an a muffle furnace at a temperature not above 450 °C until the ash is white or almost so. Cool the ash, moisten with a few millilitre of water and add 3 ml to 5 ml of concentrated hydrochloric acid drop by drop. Evaporate to dryness on a water bath and continue heating on the water bath for 1 h to render silica insoluble. Moisten the residue with 20 ml water and add about 2 to 3 ml of conc. hydrochloric acid. Heat on a water bath for a few minutes and filter through medium filter paper into 250-ml volumetric flask. Wash the filter paper thoroughly with hot water, cool the filtrate, make up to volume and shake thoroughly.

12.3.2 *Determination of phosphorus*

Transfer a suitable volume of solution of the sample containing 0.5 mg to 10 mg P_2O_5 to a 100-ml volumetric flask. About 10 ml to 25 ml of sample solution may be adequate for this purpose. Neutralize by the dropwise addition of 'ammonia' solution (0.88 N) and then proceed as for the standard graph (12.2).

12.4 Calculation

Using the standard graph (12.2), estimate phosphorus as P_2O_5 corresponding to the test solution.

$$\text{Phosphorus per cent by mass} = \frac{43.66 \times 250 \times w}{V \times m}$$

where,

w is estimated value of phosphorus as P_2O_5;

V is volume, in ml, of the sample solution taken for the test; and

m is mass, in g, of the sample used to prepare the sample solution.

13 DETERMINATION OF SODIUM CHLORIDE

13.1 Reagents

13.1.1 *Silver nitrate solution*, 0.1 N.

13.1.2 *Thiocyanate solution*, 0.1 N, dissolve either 7.613 g of ammonium thiocyanate (chloride-free) or 0.719 g of potassium thiocyanate (chloride-free) into 1 litre of solution.

Acidify 5.0 ml of standardized silver nitrate solution in a conical flask with 5 ml of nitric acid. Add 2 ml indicator solution (see 13.1.3) and titrate with thiocyanate solution until a persistent pale-rose colour is obtained. Calculate the correction factor for the 0.1 N thiocyanate solution.

13.1.3 *Indicator solution*

Make a saturated solution of ferric alum - $(NH_4)_2SO_4.Fe_2(SO_4)_3.24H_2O$ in water.

13.1.4 *Nitric acid*, concentrated.

13.2 Procedure

Introduce a known mass of the material into a 250-ml conical flask. If necessary, disperse the sample with a little water. Add a known volume, of the silver nitrate solution (so as to give an excess of

silver nitrate) and 20 ml of concentrated nitric acid. Boil gently on a sand bath until all solids except silver chloride dissolve and the supernatant liquid is clear. Wash down the sides of the flask with 50 ml of water. Add 5 ml of indicator solution. Titrate with the thiocyanate solution until a permanent reddish brown solution is obtained.

13.3 Calculation

Calculate the amount of sodium chloride in the material.
1 ml 0.1 N silver nitrate ≡ 5.845 mg NaCl.

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