

13.4 Determination of total nitrogen in SRL

Principle

When sample containing nitrogen is digested with H_2SO_4 , the total nitrogen converts into ammonium sulphate $(\text{NH}_4)_2\text{SO}_4$. In the presence of alkali, ammonia is released from the ammonium sulphate and released ammonia is distilled and trapped in a known volume of standard acid, which is then back titrated with the standard alkali. The whole process is completed in three steps. The reactions during estimation are as follows:

Digestion: Organic nitrogen + Conc. $\text{H}_2\text{SO}_4 = (\text{NH}_4)_2\text{SO}_4$

Distillation: $(\text{NH}_4)_2\text{SO}_4 + 2\text{NaOH} = \text{Na}_2\text{SO}_4 + 2\text{NH}_3 + 2\text{H}_2\text{O}$

Trapping: $2\text{NH}_3 + \text{H}_2\text{SO}_4 = (\text{NH}_4)_2\text{SO}_4$

Apparatus

- Balance
- Micro kjeldahl distillation assembly
- Digestion bench
- Kjeldahl flasks
- Pipette
- Conical flask
- Beaker
- Burette
- Volumetric flask

Reagents

- Digestion mixture ($\text{K}_2\text{SO}_4 + \text{CuSO}_4$ in the ratio of 9:1): 90 g potassium sulphate and

10 g copper sulphate^{2 4 4} mixed together.

- Concentrated H₂SO₄
- 40% NaOH solution

- 0.01 N NaOH
- 0.01 N H₂SO₄
- Methyl red indicator: Dissolve 0.1 g methyl red indicator in 60 ml ethanol and add distilled water to make the volume 100 ml.

Procedure

Digestion

- Take 5 ml strained rumen liquor in a Kjeldahl flask.
- Add 10 ml concentrated H₂SO₄.
- Add 2-3 g digestion mixture.
- Keep the flask on digestion bench and allow gentle boiling. Bumping should be avoided.
- When the solution becomes clear blue, then remove the flask from the digestion bench and cool it.
- Add 5 to 10 ml distilled water to the Kjeldahl flask. Transfer the whole material in 100 ml volumetric flask with repeated washings of distilled water. Make the volume to 100 ml.

Distillation

- Set the Kjeldahl distillation assembly.
- Take 10 ml 0.01 N H₂SO₄ in a conical flask and add 2-3 drops of indicator
- Keep the flask under the condenser in such a way that the tip of the condenser should be dipped in acid, to avoid ammonia loss during distillation.
- Take 10 ml aliquot of digested sample and transfer it into the kjeldahl assembly.
- Add 15-20 ml 40% NaOH to make the aliquot contents alkaline and put the stopper immediately.
- Allow distillation for 15 min.

Titration

- Remove the flask after washing tip of the condenser with distilled water.
- Titrate the contents of the flask with standard 0.01 N NaOH till the pink color develops (end point).
- Record the volume of alkali used for titration on the burette.
- Run a blank using all reagents but no sample and following the whole procedure to estimate the nitrogen contents of the reagents, if any.

Calculation

1 ml 0.01 N H₂SO₄ = 0.00014 g nitrogen

$$\text{Nitrogen/100 ml rumen liquor} = \frac{V \times 0.00014 \times D \times 100}{v \times A}$$

Where,

$$V = A - B$$

D = Dilution (Volume made in volumetric flask)

v = Initial volume of rumen liquor taken for the digestion

A = Aliquot taken (10 ml)

Total nitrogen of sample = Total nitrogen of sample – Total nitrogen of blank

Reference: Laboratory manual of animal nutrition. IVRI, Izatnagar, U.P. – 243 122.