**Nitrogen and Calculation of Crude Protein – Kjeldahl**

**1. Principle**

For determination of nitrogen the sample is digested using sulphuric acid in the presence of a catalyst to convert sample nitrogen to ammonium sulphate. The acid solution is made alkaline with sodium hydroxide solution. The ammonia is distilled and collected in an excess of boric acid solution, followed by titration with Hydrochloric acid standard volumetric solution. For determination of crude protein nitrogen is multiplied by a factor, 6.25 .

**2. Scope**

The method described is applicable for determination of nitrogen in feeds.

**3. Equipment**

1. Analytical balance.

2. Digestion tubes fitted for the Kjeldahl digestion unit.

3. Kjeldahl digestion unit with fume removal manifold.

4. Kjeldahl distillation apparatus.

5. Titration unit .

**4. Reagents**

1. Sulphuric acid, concentrated, 95–98% .

2. Kjeldahl catalyst tablets .

3. Boric acid, 10 g/litre.

4. NaOH solution, 40% .

5. Indicator solution: Methyl red indicator, dissolve 1 g methyl red (sodium salt) in 100 ml methanol or ethanol.

6. Hydrochloric acid standard volumetric solution, 0.1 M .

**5. Procedure**

**A- Digestion**

1. Weigh approximately 2 g sample transfer to the digestion tube .

2. Add two Kjeldahl tablets and 20 ml sulphuric acid . If fuming is a problem, add a few drops of anti-foaming agent.

3. Place the tubes in a digestion unit and connect to the fume removal manifold.

4. Digest the sample at least 1 hour at 420 ± 20 °C.

5. Turn the digestion off, remove the tubes and allow to cool for 10–20 minutes.

6. Add distilled water to each tube to a total volume of approximately 80 ml.

**B- Distillation and titration**

1. Place a conical flask containing 25–30 ml of the concentrated boric acid, under the outlet of the condenser of the distillation unit in such a way that the delivery tube is below the surface of the boric acid solution.

2. Add 50 ml NaOH and distill the ammonium by following the instructions of the manufacturer.

3. Titrate the content of the conical flask with hydrochloric acid standard solution using a titration unit and read the amount of titrant used. The endpoint is reached at the first trace of pink colour in the contents.

4. Record the amount of acid used .

**1.Digestion 1. الهضم**

**a-** Organic nitrogen + H2SO4 SO4 + NH4+ + CO2 + H2O

**b-** NH4\* + H2SO4 (NH4)2SO4

**2.Neutralization 2.تحرير الامونيا**

(NH4)2SO4 + 2NaOH 2NH3 + Na2SO4 + 2H2O

**3. Absorption by boric acid 3.امتصاص الامونيا بواسطة حامض البوريك**

2NH3 + 4H3BO3 (NH4)2B4O7 + 5H2O

**4. Titration by strong acid 4.معايرة الامونيا بواسطة حامض قوي**

(NH4)2B4O7 + 5H2O + 2HCl 2NH4Cl + 4H3BO3

**6. Calculation**

Percent Nitrogen (% N)

**% N = ML(HCl) x M(HCl) x 14.007 x 100**

**W**

where,

**ML(HCl)** = ml HCl needed to titrate sample,

**M(HCl)** = molarity of HCl,

**14.007** = molecular weight of N,

**100** = conversion from mg/g to %

**W** = weight of the sample (g).

Calculation percent Crude Protein (% CP):

**% CP = % N x F**

where,

**F** = 6.25 for all forages, feeds and mixed feeds,

**F** = 5.70 for wheat grains, and

**F** = 6.38 for milk and milk products.