Nitrogen and Calculation of Crude Protein – Kjeldahl

What Is Protein?

Protein is found throughout the body in muscle, bone, skin, hair, and virtually every other body part or tissue. It makes up the enzymes that power many chemical reactions and the hemoglobin that carries oxygen in your blood.

Protein is made from twenty-plus basic building blocks called **amino acids**. Because we don't store amino acids, our bodies make them in two different ways: either from scratch, or by modifying others.

Nine **essential amino acids** are very important (the human body cannot make them on their own) (histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine) and the rest are **non-essential amino acids** (the body can make them inside the body).

Protein sources for ruminants feeding

1. **True proteins**: Vegetarian and animals
2. **Non-Protective Nitrogen** (NPN) : urea and ammonium salts

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 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

a- Organic nitrogen + H_2SO_4 $\longrightarrow$ $SO_4 + NH_4^+ + \uparrow CO_2 + H_2O$

b- $NH_4^* + H_2SO_4 \longrightarrow (NH_4)_2SO_4$

2.Neutralization

$(NH_4)_2SO_4 + 2NaOH \longrightarrow 2NH_3\uparrow + Na_2SO_4 + 2H_2O$

3. Absorption by boric acid

$2NH_3 + 4H_3BO_3 \longrightarrow (NH_4)_2B_4O_7 + 5H_2O$

4. Titration by strong acid

$(NH4)_2B_4O_7 + 5H_2O + 2HCl \longrightarrow 2NH_4Cl + 4H_3BO_3$

6. Calculation

Percent Nitrogen (% N)

$$\% N = \frac{ML(HCl) \times M(HCl) \times 14.007}{W} \times 100$$

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):

$$\% CP = \% N \times F$$

where,

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

F = 5.70 for grains

F = 6.38 for milk and milk products.