Animal nutrition practical \master

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Protein and determination of nitrogen

Definitions:

Crude protein is a mixture of *real protein* and *unreal protein*, and it is termed that every **100 gm** of crude protein contains **16 gm** of nitrogen. Accordingly, every **1 g** of nitrogen is found in **6.25** grams of protein. If the nitrogen is estimated in a sample and multiplied **by 6.25**, the result is the crude protein present.

Protein function:-

- Protein is found in every cell of the body, and it has a role in the most important biological and cellular processes in the body, including:
- **Immune system response**, as some antibodies that are one of the body's defenses against pathogens are proteins made by the immune system.
- **Building bon**es, muscles, cartilage, skin, nails, and hair, in addition to contributing to the repair of damaged tissues.
- Carrying oxygen and distributing it to various parts of the body, through the protein hemoglobin contained in red blood cells.
- Build hormones, such as insulin and growth hormone. In addition to regulating the action of hormones, especially during the stages of growth, puberty and cell development in children, adolescents and pregnant women.
- Making enzymes, including the enzymes responsible for digesting food and forming new cells. The building of enzymes in the body will consume about half of the amount of protein ingested daily

It is agreed, after practical analyzes, that the crude protein contains 16% nitrogen, and this percentage is approximately the same as the percentage of nitrogen in animal protein

- **animal protein contains 16.67% nitrogen,**
- **milk casein contains 15,15% nitrogen).**
- **vegetable proteins, they contain a higher percentage of nitrogen and range between 16.38% - 18.73%)**

Feed materials contain raw protein:

Animal remains contain high amounts of protein, for example, blood meal contains 80% protein, fish meal contains 50% protein, as well as meat and bone meal. The flax seed meal contains 35%, the coconut meal contains 21%, the unhulled cotton seed meal contains 24%, the grains of leguminous plants contain 20-25% - the cereal seeds contain 10-12% and the wheat bran according to the degree of sieving from 10-17 % and hay from 6-15% and hay of leguminous plants is the one that contains a high amount of protein, unlike alfalfa hay.

The hay has 3-5% protein, and the legume hay has a crude protein amount ranging between 11-14%.

The stem and tuber roots such as potatoes and beets contain 1-2% crude protein.

- Amino acids:

Proteins are hydrolyzed by enzymes, acids or alkalis into amino acids - amino acids are characterized by containing an amino group (NH2) and a carboxyl group (-COOH). Most of the amino acids naturally found in proteins are α-type. Some Acids contain a second group of amino and another section contains a second group of carboxyl.

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

- ❖ Analytical balance.
- ❖ . Digestion tubes fitted for the Kjeldahl digestion unit.
- ❖ . Kjeldahl digestion unit with fume removal manifold.
- ❖ Kjeldahl distillation apparatus.
- ❖ Titration unit .

4. Reagents

- Sulphuric acid, concentrated, 95–98% .
- . Kjeldahl catalyst tablets .
- . Boric acid, 10 g/litre.
- . 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

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

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

2.Neutralization

$(NH_4)_2SO_4 + 2NaOH$ $2NH_3 + Na_2SO_4 + 2H_2O$

3. Absorption by boric acid

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

4. Titration by strong acid

$(NH_4)_2B_4O_7 + 5H_2O + 2HCl$ $2NH_4Cl + 4H_3BO_3$

5. Calculation

Percent Nitrogen (% N)

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

W sample

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.