LECTUER -6 Determination of nitrogen and Crude protein

Principle the kjeldahl method is the standard method of nitrogen determination. This method is applicable for the determination of nitrogen (N) in all types of forages and feeds.

The “block” method consists of three basic steps as in original kjeldahl method:

1. Digestion (boiling) of the sample in concentrated sulfuric acid with a catalyst (copper or selenium K2So4, cuso4) which results in removes all OM and conversion of nitrogen to ammonia sulfate.
2. NaOH is then added to convert the NH4SO4 to ammonia NH3 which is distillates into a trapping solution (weak acid, boric acid H3Bo3.
3. Quantification of the ammonia in boric acid by titration with a standard acid solution HCl acid . the amount of acid neutralized by the ammonia is an estimate of the amount of nitrogen in the sample. Crude protein is estimated by multiply the nitrogen with a constant factor.
4. Digestion

CHOSN+H2SO4+Catalyst (NH4)2SO4+CO2+H2O+SO2

1. Distillation

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

2NH4OH

NH4OH+ H3BO3 NH4H2BO3+H2O

1. Titration

NH4H2BO3+ HCl NH4Cl+H3BO3

Equipment :

* Block digester, capable of maintaining 420c and digesting 20 sample at time of 20 – 45 min in 250ml tubes constricted at top.
* Fume hood.
* Weighing paper, nitrogen – free
* Electronic analytical balance, sensitive to 0.1mg
* Steam distillation apparatus – digestion tube connected to distillation trap (by rubber stopper), which is connected to condenser.

Reagents

* Digestion sulfuric acid (95-98%) reagent grade.
* Catalyst tablets (potassium sulfate K2SO4+either copper sulfate or selenium)
* Sodium hydroxide,40%w/w solution. dissolve 2kg low NaOH and distilled water containing 70ml 0.1%alcoholic solution of methy red and 100 ml 0.1% alcoholic solution of bromocresol green dilute to 10L with distilled water.
* Standard hydrochloric acid(HCl)solution (titrating sol.)0.1-0.2N for boric acid trapping solution it by diluting 85-170ml 36.5to38%HClto 10L with distilled water and then standardize it againt standard alkali such asNa2CO3 or NaOH.

Procedure:

Digestion

1. Weigh ground sample into digestion tube, recording weight(W)to nearest 0.1 mg weight range should depend on protein content of sample (0.8-1.5g) for (4-50%CP).
2. Add sufficient catalyst tablets and then add 10-12ml concentrated sulphuric acid.
3. Place tubes set on bock digester preheated to 420C (Digester must be equipped with an exhaust and /or placed in an fume hood), digest about 45min.
4. Remove tube and let cool about 10 min in a fume hood (time will depend upon airflow around tubes), then add directly about 25-30ml of distilled water to the bottom of each tube to dissolve acid digest completely).

Distillation / titration

1. Place NaOH in alkali of steam distillation unit. Make sure that sufficient NaOH is dispensed from unit to neutralize all acid in tube and excess (about 50- 55ml) before conducting distillation.
2. Place 250ml titration flask containing trapping solution (about25ml 4% boric acid containing indicator)on the receiving platform with tube the condenser extending below the surface of the trapping solution.
3. Attach digestion tube containing diluted, cooled digest to distillation unit.
4. Dispense appropriate volum of distilled water (50ml) and the base (NaoH) solution (50ml).
5. Steam distillation until 100- 125ml distillate collects in titration flask.
6. Remove titrating flask from unit, rinsing condenser tip with water.
7. Titrate trapping solution containing ammonia (N)with 0.1 or 0.2 N HCl to neutral gray endpoint, record volum of acid VA required to nearest 0.01ml , and then titrate reagent blank (VB) similarly. Colour change is from pink to green to gray to purple.

Calculation:

N% (as fed basis) = (VA-VB1)\*NHCl\*0.014/W \*100

N% (as DM basis) = N % (as fed basis)\* 100/DM%

* VA= volume, in ml, of standard HCl required for sample
* VB1=volume in ml of standard HCl required for blank
* NHCl= normality of acid standard
* 0.014= milliequivalent weight of N, in grams

W= weight of sample in grams

CP% (DM basis)= N%(DM basis)\*F

F= 6.25 for all forages and feeds except:

F=5.70 for wheat grains and F= 6.38 for milk and products.