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Crude Protein Determination in Feed and Forages
Macro-Kjeldahl Method

1. Application

This procedure is applicable for the determination of nitrogen (N) in forage. Crude protein is derived through a calculation using this nitrogen value.

2. Summary of Methods

The Kjeldahl method is the standard method of nitrogen determination. The procedure consists of three basic steps: 1) digestion of the sample in sulfuric acid with a catalyst, which results in conversation of nitrogen to ammonia; 2) distillation of the ammonia into a trapping solution; and 3) quantification of the ammonia by titration with a standard solution.

3. Safety

All chemicals should be considered a potential health hazard. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material handling data sheets should be made available to all personnel involved in the chemical analysis.

4. Interferences

Reagent proportions, heat input and digestion time are critical factors and any variations to these could result in improper analysis.

5. Sample Collection, Preservation, and Handling

All samples are dried at 55^{o}C in a cabinet-type forced air dryer for 12-16 hours. After drying the sample is ground to pass through a 1 mm mill.

6. Apparatus and Materials

6.1 #1 Whatman filter papers
6.2 Analytical balance, sensitive to 0.1 mg
6.3 Kjeldahl flasks, 800 ml
6.4 Kjeldahl packets (see reagents)
6.5 Boiling chips, selenized
6.6 Kjeldahl digestion unit with fume removal manifold
6.7 Kjeldahl rack

6.8 Rubber stoppers
6.9 Erlenmeyer flasks, 500 ml
6.10 Burettes

7. Reagents

7.1 Sodium Hydroxide
- 7.1.1 Add 5lbs each of sodium hydroxide flakes to two plastic 4000 ml beakers. Use a scale under a hood. Gently add 3500 ml distilled water, avoid splashing. Stir flakes until completely dissolved; approximately 5 minutes. Heat is emitted in this step so place in sink filled with cold water to level of the solution in beakers. Let beakers cool in water approximately 5 hours.
- 7.1.2 After 5 hours bring solution in each beaker to 4000 ml with distilled water. Add both beakers of solution to a 15 L carboy. Rinse beakers with 100 ml distilled water and add to carboy. Bring volume to total of 10 liters.
- 7.1.3 In a 4000 ml glass beaker, add 240 g sodium thiosulfate to approximately 2500 ml distilled water. Stir until dissolved or put on a stir plate. Bring to 3000 ml volume with distilled water. Add to carboy and rinse the beaker with 2000 ml distilled water. Total volume of 15 liters in carboy.

7.2 14 L Boric Acid

7.3 Boric Acid Indicator
- 7.3.1 0.40000g Bromocresol Green
- 7.3.2 0.0800g Methyl Red indicator
- 7.3.3 480 ml 95% Ethanol

7.4 0.1 N Sulfuric Acid

7.5 Kjeldahl Packets, per pack
- 7.5.1 10g potassium sulfate (K_2SO_4), anhydrous
- 7.5.2 0.3g copper sulfate ($CuSO_4$), anhydrous
- 7.5.3 0.1g pumice

7.6 Mossy zinc

8. Methods

Digestion:

8.1 Weigh out approximately1g forage samples on #1 Whatman filter paper circles. Record weight to nearest 0.1 mg. Fold filter paper around forage and put in Kjeldahl flasks.

8.2 Run one blank with each set. An laboratory standard forage sample should also be run to gauge acceptability of the run.

8.3 Put one Kjeldahl packet and 2-3 boiling chips in flask with sample.

8.4 Add 30 ml sulfuric acid.
8.5 Turn on water aspirator of the Kjeldahl unit. Also turn on ventilation fan for fume extraction.
8.6 Digest on Kjeldahl unit for 2 hours with burners on high. Turn flasks a half turn after one half hour of digesting.
8.7 After 2 hours, turn off burners and let flasks sit for 5-10 minutes. Remove flasks from burners and cap immediately with rubber stoppers and place on Kjeldahl rack. Turn off water aspirator.
8.8 Let flasks cool for at least 30 minutes.

Distillation:
8.9 Put 50 ml of boric acid in 500 ml Erlenmeyer flasks for each sample being run. Put these flasks on the Kjeldahl unit for distillation.
8.10 Make sure the sodium hydroxide and water burettes are filled and turn on water for condensing. Turn burners on high.
8.11 In the following order, add 250 ml distilled water, 3-4 chips of mossy zinc, and 100 ml sodium hydroxide slowly. Immediately attach to the condenser.
8.12 Turn off burners and move flasks off the condenser tubes when the liquid reaches 200 ml. Distillation takes about 30 minutes. Shut off water and wash Kjeldahl flasks when all done.

Titration:
8.13 Titrate the liquid in the Erlenmeyer flask using 0.1N sulfuric acid until the blue liquid returns to a purplish pink color. Be careful not to add too much sulfuric acid. Read ml of sulfuric acid used on burette and record number.

9. Calculations

9.1 % Crude Protein = {(ml titrated– blank)(.8756)/(sample wt in grams)(% lab DM)}*100
9.2 % Nitrogen = {(ml titrated – blank)(.1401)/(sample wt in grams)(% lab DM)}*100

10. Quality Control

A reagent blank and at least one sample of in-house standard are run as check of the correctness of the procedure. If digestion is not complete, make appropriate adjustments.

11. Reporting

Results are reported as crude protein (CP) as a % of Dry Matter.

12. References

12.1 Protein (Crude) Determination in Animal Feed: Copper Catalyst Kjeldahl Method. (984.13) Official Methods of Analysis. 1990. Association of Official Analytical Chemists. 15th Edition.

12.2 Protein (Crude) Determination in Animal Feed: $CuSO_4/TiO_2$ Mixed Catalyst Kjeldahl Method. (988.05) Official Methods of Analysis. 1990. Association of Official Analytical Chemists. 15th Edition.