7.14 Determination of urea in animal feed - Colorimetric method

Apparatus

Spectrophotometer – Instrument with maximum band width 2.4 nm and 420 nm, with 1 cm cells.

Reagents

- Di-methyl amino benzaldehyde (DMAB) solution – Dissolve 16.00 g in 1 L alcohol and add 100 ml HCl. Stable 1 month. Prepare new standard curve with each new batch of reagent.
- Zinc acetate solution – Dissolve 22.0 g $Zn(CH_3COO)_2 2H_2O$ in water add 3 ml CH_3COOH, and dilute to 100 ml.
- Potassium ferocyanide solution – Dissolve 10.6 g $K_4Fe(CN)_6 2H_2O$ in water and dilute to 100 ml.
- Vegetable charcoal
- Phosphate buffer solution – pH 7.0. Dissolve 3.403 g anhydrous KH_2PO_4 and 4.355 g anhydrous KH_2PO_4 separately in ca 100 ml portions freshly distilled water.
- Urea standard solutions – (1) Stock solution – 5 mg/ml. Dissolve 5.000 ± 0.001 g reagent grade urea in H_2O and dilute to 1 l with H_2O (2) Working solutions – 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8 and 2.0 mg urea/5 ml. Pipette 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20 ml stock solution into 250 ml volumetric flasks and dilute to volume with phosphate buffer. (3) Reference solution – Use standard solution containing 1.0 mg urea/ 5 ml as reference standard, store at < 24ºC. Stable 1 week.

Preparation of standard curve

Pipette 5 ml aliquots of working standard solutions into 20 x 150 mm (25 ml) test tubes and add 5 ml DMAB solution to each. Prepare reagent blank of 5 ml buffer solution and 5 ml DMAB solution. Shake tubes thoroughly and let stand 10 min in water bath at 25°C. Read A in 1 cm cell at 420 nm with reagent blank at zero A. Plot A against concentration urea. Plot should be straight line; if not, repeat, using new lot of DMAB.

Determination

Weigh 1.00 g ground sample into 500 ml volumetric flask. Add 1 g charcoal, ca 250 ml H_2O, 5 ml $Zn(CH_3COO)_2$ solution and 5 ml $K_4Fe(CN)_6$ solution. Shake mechanically 30 min and dilute to volume with water. Let stand until precipitate settles. Decant through Whatman No. 40 paper and collect clear filtrate. Pipette 5 ml filtrate into test tube, add 5 ml DMAB solution, and shake thoroughly. Include reference standard (5 ml solution and 5 ml DMAB solution) and reagent blank with each group of samples. Let stand 10 min in water bath at 25ºC. Read A at 420 nm against reagent blank.

$$\% \text{ Urea} = (1.0 \times A_{\text{sample}} \times 100) / (A_{\text{standard}} \times \text{mg sample in aliquot})$$

Reference: AOAC Official Method 967.07, 16th Edition.