NONPROTEIN NITROGEN OF FEEDSTUFFS
Updated September 2013

I. Reference:
Waldo, D.R. and H.K. Goering. 1979. J. Animal. Sci. 49:1560.

II. Personal Protective Equipment:
A. Lab coat
B. Safety glasses/goggles
C. Latex gloves

III. Reagents:
A. 0.15 M Sodium Chloride (NaCl) (8.77 g NaCl/L).
B. 10% (w/v) TCA ($C_2HCl_3O_2$)

III. Procedure:
A. Weigh in duplicate or triplicate 2 to 5 g of sample into 250 ml Erlenmeyer flasks, include blanks.
B. Add 100 to 200 ml .15 M NaCl.
C. Place on shaking hot plate. Heat to 40^{o}C and shake for 1 hour.
D. Filter into 250 ml volumetric flasks through S&S Whatman 410 filter paper (unless soluble N is desired) and rinse several times and q.s. with 0.15 M NaCl.
E. Take 20 ml filtrate and place in 50 ml centrifuge tube containing 20 ml 10% TCA.
F. Swirl and place in cooler overnight.
G. Centrifuge at 14,000 x g for 15 minutes.
H. Digest 10 ml of supernatant via the Kjeldahl procedure.
I. Rinse digesta into 100 ml volumetric and q.s. with water.
J. Save a portion for NH_3-N determination on auto analyzer.

IV. Calculations:

$$\% \text{ NPN} = \frac{(\text{NH3-N, mg/ml} - \text{blank})\ (5000)}{(\text{Sample wt})\ (\text{DM})\ (1000)}$$

5000 = Dilution factor
1000 = Conversion from g to mg

$$\text{NPN, \% of total N} = \frac{\% \text{ NPN}}{\% \text{ Total N}}$$