PEPSIN INSOLUBLE NITROGEN (PIN)
Up-dated September 2013

I. Reference:

Goering, H.K. and P.J. Van Soest. 1970. Forage Fiber Analyses. USDA Handbook No. 379.

II. Personal Protective Equipment:

A. Lab coat
B. Safety glasses/goggles
C. Latex gloves

III. Reagents:

A. Pepsin - HCl solution
   1. Add 8.5 mL of 12 N HCl carefully to approximately 800 mL distilled water.
   2. Add 10 g pepsin, mix and make up to one liter.

IV. Procedure:

A. Samples must be freeze-dried to avoid heat damaging the nitrogen in the sample
B. Weigh 0.5 g dried ground sample into 100 mL in vitro tube
C. Add 50 ml pepsin-HCl solution
D. Incubate in water bath at 39 ^{o}C for 20 hours
E. Filter with hot water onto Whatman 541 filter paper. Wash thoroughly.
F. Run Kjeldahl of filter papers or scrape, mix, and weigh residue for LECO analysis

V. Calculation:

A. Determine g N in residue:

g N = %N * residue wt.

B. Determine PIN as a percent of DM:

%PIN = X 100

C. Determine N as a percent of DM:

%N = X 100

D. Express PIN a percent of total N

PIN, % total N = X 100

$$\frac{\text{g N in residue}}{\text{(Sample wt.)(DM)}}$$

$$\frac{\text{g N}}{\text{(Sample wt.) (DM)}}$$

$$\frac{\%\text{PIN}}{\%\text{N}}$$