Estimation of UIP by In Situ Neutral Detergent Insoluble Nitrogen (NDIN) Method
Updated September 2013

<u>References:</u>

Mass, Ryan, Lardy, Greg, Grant, Rick, and Klopfenstein, Terry.   Protocol for the
   Estimation of UIP by the In Situ Neutral Detergent Insoluble Nitrogen (NDIN) Method.
   Univ, of Nebraska. J. Animal Sci. 1999   77:1565-1571

<u>Personal Protective Equipment:</u>
1. Lab coat
2. Safety glasses/goggles
3. Latex gloves

<u>Procedure:</u>
Refer to: STANDARD IN SITU PROCEDURE FOR FORAGES for more detailed instruction
1. Samples are ground to pass through a 2mm Wiley mill screen
2. Samples (1.25g for a small Dacron bag, 5g for large Dacron bags) are placed in each bag.
   a.) Bags are either sealed by heating sealing or by wrapping the top around a rubber
      stopper with rubber bands
3. Sample bags are placed in a lingerie bag
   a.) Up to 50 small bags or 20 large bags can be included in each bag
4. Up so six lingerie bags are incubated in the ventral sac of the rumen of a large (>500 kg)
   fistulated bovine that is consuming 1.8% of its bodyweight in forage
5. After ruminal incubations, bags are rinsed in 39°C water for 15 minutes
   a.) this removes rumen contents from outside of the bags, no the microflora attached
      to forage residue
6. Excess water is allowed to drip off the bags and the bags are stored wet in a 4°C cooler
   until neutral detergent extractions can be conducted
   a.) Can be stored in this manner for as long as 3 days
   **Note: If small bags are used, continue with step 7. If large bags are used,
   proceed to step nine**
7. Neutral detergent extractions are conducted directly on small bags and their residue
   using the Ankom Fiber Analyzer.
   a.) can reflux up to 24 bags at one time
8. After75 minutes of reflux, bags are then rinsed thoroughly in boiling distilled water, dried
   at 60°C overnight, air equilibrated for 2 hours, weighed to determine the amount of
   residual forage and then subsampled for N
9. Large bags must be dried at 60°C over night and then air equilibrated for 2 hours before
   weighing, this determines the pool of residual sample
   a.) A subsample is taken for NDIN analysis using the conventional Berzelius
      Beaker method
10. The NDIN concentration of the residue is then calculated (g NDIN/ g of dry sample) for
   each bag
   a.) values from the 96-h bags are subtracted from the other times (2, 12, etc) in order
      that the rate of digestion is based on the **potentially rumen-degradable pool of
      NDIN**.
   b.) If a 96-h bag is not analyzed for each sample, a subset of samples (10-20%)
      should be analyzed for 96-h NDIN and the rate of NDIN digestion for those
      samples is calculated both with and without the subtraction of the 96-h values

from the other time points
  c.) A regression equation can then be calculated that uses the uncorrected rate of
      NDIN digestion on the x-axis and the corrected rate on the y-axis. This equation
      can then be used to correct all the rates
11. A rate of digestion is a first order rate. Therefore, the natural logarithm of each NDIN
    concentration must be calculated and those numbers are used to calculate rate of
    digestion. The slope of the regression equation is the rate and the y-intercept is the
    original pool of NDIN.

Sample	Original N (%DM)	K_d	K_p	pool (%DM)	96h (%DM)	UIP (%DM)	UIP (%CP)
Standard bromegrass hay	2.0	.14	.05	1.0	.20	2.7	23.3
Standard alfalfa hay	3.5	.33	.05	1.2	.35	3.2	14.3
Prairie hay	.09	.08	.02	.5	.10	1.6	28.0

Calculations:

$$\{[(k_p / (k_p + k_d)) * \text{digestible NDIN pool (\%DM)}] = \text{96-h NDIN (\%DM)}\} * 6.25$$

k_p = rate of passage
k_d = rate of NDIN digestion

Sample calculations for three forages: