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

References:

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

Procedure:
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.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Original N (%)DM</th>
<th>Kd</th>
<th>Kp</th>
<th>pool (%)DM</th>
<th>96h (%)DM</th>
<th>UIP (%)DM</th>
<th>UIP (%CP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard bromegrass hay</td>
<td>2.0</td>
<td>.14</td>
<td>.05</td>
<td>1.0</td>
<td>.20</td>
<td>2.7</td>
<td>23.3</td>
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<tr>
<td>Standard alfalfa hay</td>
<td>3.5</td>
<td>.33</td>
<td>.05</td>
<td>1.2</td>
<td>.35</td>
<td>3.2</td>
<td>14.3</td>
</tr>
<tr>
<td>Prairie hay</td>
<td>.09</td>
<td>.08</td>
<td>.02</td>
<td>.5</td>
<td>.10</td>
<td>1.6</td>
<td>28.0</td>
</tr>
</tbody>
</table>

Calculations:

\[
\left(\frac{k_p}{k_p + k_d}\right) \times \text{digestible NDIN pool (%DM)} = 96\text{-h NDIN (%DM)} \times 6.25
\]

\(k_p\) = rate of passage
\(k_d\) = rate of NDIN digestion

Sample calculations for three forages: