STANDARD IN SITU PROCEDURE FOR CONCENTRATES
Updated September 2013

I. References:

NRC. 2001. Nutrient Requirements of Dairy Cattle (7th Ed.). pp. 58-62. National Academy Press, Washington, D.C.

Vanzant, E.S., R.C. Cochran, and E.C. Titgemeyer. 1998. Standardization of in situ techniques for ruminant feedstuff evaluation. J. Animal. Sci. 76:2717-2729.

Whittet, K.M., C.W. Creighton, K.J. Vander Pol, G.E. Erickson, and T.J. Klopfenstein. 2002. Influence of rinsing technique and sample size on in situ protein degradation of protein sources. Abstract #1610. 2002 American Society of Animal Science Joint National Meeting. Vol. 80, Supp 1.

II. Personal Protective Equipment:

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

III. Supplies:

A. Ankom rumen in situ bags
    1. 5 cm X 10 cm (#R510), 50 µm pore size – small bags
    2. 10 cm X 20 cm (#R1020), 50 µm pore size – large bags
B. Ankom heat sealer - #1915 (used for all Ankom bags)

IV. Sample Preparation:

A. Concentrate samples
    1. Dry sample in 60°C forced air oven for 48h
    2. Leave samples un-ground or grind to pass through a 2-mm screen

V. Procedure:

A. Animal(s)
    1. Have animal on desired diet at least three days prior to starting any runs. If the previous diet is not similar, then allow seven days for adaptation.
        a. Mixed Diet
            (1) 70% Roughage
            (2) 30% Concentrate
    2. Feed at desired intake. Note intake (~1.8% of BW)
B. Sample Labeling
    1. Label in situ bag with simple number or code
    2. Use a black Sharpie (permanent marker)
    3. Label lower portion of the bag
C. Initial Weights
    1. Obtain weight of empty labeled bag
    2. Obtain weight of sample (as-is material)
        a. 1-2 g for small bags
        b. 5-20 g for large bags
D. Bag Sealing
    1. Seal bags completely with Ankom heat sealer

a. Place upper portion of bag on sealer strip leaving only 1-2 cm space
b. Hold down arm of heat sealer firmly so that red light illuminates
c. Once red light goes off, wait 3-6 seconds before releasing arm
d. Gently remove bag and repeat to **add a second seal** below the first

2. Check seals by gently pulling bag sides apart

E. Mesh Bag

1. 32 × 42 cm polyester with a nylon zipper
2. Weight bags down with two heavy bolt nuts
3. Tie off corner of bag to contain nuts with a cable tie
4. Place bags in mesh bag so that they are not stacked
5. Close zipper and ensure that it does not come open

F. Pre-incubation

1. Soak mesh bag(s) with sample bags in warm (39EC) water for 20 minutes
2. Allow excess water to drip before placing in the rumen

G. Incubation

1. Number of bags
   a. Up to 50 small bags or 20 large bags may be included in mesh bags
   b. Up to 6 mesh bags may be incubated in the ventral sac of the rumen of a large (>500kg) fistulated bovine
2. Time Points
   a. Incubations Times (0, 0.75 MRT, MRT, 48 or 72 hr)
      (1) Inverse of rate of passage = Mean Retention Time (MRT) (ex. 5%/hr (kp) = 20 hr MRT)
      (2) Incubation time should equal 0.75 MRT (ex. 15 hr)
      (3) Extent of digestion is needed for rate calculations – suggest 48 or 72 hr. Note: 0 hr sample is washed but not incubated.
   b. Complete exchange method - place bags in rumen at various times and remove all bags at once

H. Bag Rinsing

1. Remove all bags simultaneously from the rumen
2. Rinse bags in washing machine with 0.375 L of 39°C water / in situ bag
3. Rinse consists of five rinses of a one minute agitation and a two minute spin

I. Drying

1. Roll the bag top down to the residue exposing the label and gently squeeze the excess water out and place in wire rack
2. Rinse individual in situ bag from the top with dH_2O and rinse down.
3. Drain excess water from bag and roll with the label exposed.
4. Dry the washed bags in a 60EC forced-air oven overnight (12-**48** hours).
5. Place bags in a desiccator and get the dry weight of the bag.
6. After drying allow the bags to air equilibrate to room conditions for at least three hours before weighing
7. Once bags have equilibrated retrieve an air equilibrated weight from the bags.

J. Analysis

1. Nitrogen
   a. Determine the nitrogen concentration for a representative residue sub-sample (0.25g for LECO analysis)
   b. The nitrogen content of the non-incubated material is also needed
2. Dry matter
   a. Determine the dry matter content of the non-incubated material
   b. Create sub-sample of residues and determine the dry matter or instead of

air equilibrating samples, cool in a desiccator and weigh
(Necessary only if calculating DMD)

VI. Calculations:

A. $DMD = 1 - \frac{[(\text{Residue + in situ bag}) - \text{in situ bag}]}{(\text{Sample wt.})(DM)}$

B. $UIP\ (\%\ of\ CP) = 100 \times \frac{\text{Residue Wt.} * \text{Residue \%CP}}{\text{Sample Wt.} * \text{Sample \%CP}}$

$UIP\ (\%\ of\ DM) = UIP\ (\%\ of\ CP) * (\%\ CP/\%\ DM)$

or

$$\frac{\text{Residue Wt.} * \text{Residue \%CP (DM)}}{\text{Sample Wt. (DM)} * \text{Sample \%CP (DM)}}$$

C. Rate of degradation (kd)

1. Subtract extent (48 or 72 hr) values from other values (ex. 0 and 15 hr; units %CP/CP).
2. Divide value(s) by 0 hr value to obtain CP remaining as a number (not decimal). (ex. Divide 15 hr value by 0 hr value, each corrected for 48 or 72 hr values)
3. Take natural log (LN) of the percent remaining and subtract from 4.6 (LN of 100%).
4. Subtract LN (ex. 15 hr) from 4.6 and divide by incubation hours (ex. 15 hr).

D. UIP (from rates):

$$UIP = \frac{kp}{kp + kd}$$