**IN VITRO DRY MATTER DISAPPEARANCE**
**Updated September 2013**

I. References:

McDougall, E.I. 1948. Biochem. J. 43:99.

Tilley, J.M.A. and R.A. Terry. 1963. J. Br. Grassland Soc. 18:104.

Mertens, D.R. 1993. Rate and extent of digestion. pp 13-51 in Quantitative Aspects of Ruminant Digestion and Metabolism. Forbes and France, Eds. (AB International, Wallingford, United Kingdom).

II. Personal Protective Equipment:

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

III. Reagents:

A. 20% (v/v) HCl
B. 5% Pepsin Solution
   1. Add 5 g pepsin to 100 mL volumetric flask
   2. q.s. to 100 mL with dd$H_2O$
C. McDougall's Buffer:

| Compound | g/Liter | g/18 Liter | g/12 Liter | g/9 Liter |
|---|---|---|---|---|
| Sodium Bicarbonate ($NaHCO_3$) | 9.8 | 176.40 | 117.6 | 88.2 |
| Sodium Phosphate Dibasic ($Na_2HPO_4$) | 2.44 | 49.94 | 33.24 | 24.97 |
| Potassium Chloride (KCl) | 0.57 | 10.26 | 6.84 | 5.13 |
| Sodium Chloride (NaCl) | 0.47 | 8.46 | 5.64 | 4.23 |
| Magnesium Sulfate ($MgSO_47H_2O$) | 0.12 | 2.16 | 1.44 | 1.08 |
| Calcium Chloride ($CaCl_22H_2O$) | 0.16 | 2.90 | 1.92 | 1.45 |

***$CaCl_2$ must be added only after all others are completely in solution.**

- McDougall's Buffer is prepared in 18 liter batches and kept sealed in the walk-in **cooler**. Prior to use a volume is warmed to 39 $^{o}$C and reduced with a stream of $CO_2$.
- During the warming and reducing step, urea is added to the McDougall's at the rate of **1.0 gm/liter**.
  *For in vitro ammonia release studies urea is not used.

IV. Sample Preparation:

A. Weigh approximately 0.5000- 0.5040 g of feed (in triplicate) into 100 ml in vitro tube. Include at least 5 blanks and experiment # 0414 hay standards in triplicate or 3 tubes as

corncob standards.

V. Inoculum:

A. Obtain whole rumen contents from 2 fistulated steers, prior to feeding, being fed a 30% concentrate diet.

B. Squeeze through 4 layers of cheese cloth into a **pre-warmed thermos** (about 2.5 L rumen fluid per square of cheese cloth).

C. Pour filtered fluid into 500-1000 mL separatory funnels, gas with $CO_2$, stopper, and place in 39 $^{o}$C water bath until the particulate matter rises to the top.

D. Remove lower portion and mix with warmed, reduced McDougall's Buffer (typically 1:1 ratio) with 1 g urea/L of McDougall's buffer.

E. Maintain buffered inoculum at 39°C and constant $CO_2$ until used. The time lag before buffer fluid is dispensed should be kept as short as possible, usually no more than 10 minutes.

VI. In vitro Incubation:

A. When using dry feeds add 1 mL of $ddH_2O$ to each tube. Treat all tubes equally. Randomly distribute tubes to rack in the water bath

B. Dispense 50 ml of mixture into each tube.

C. Flush top of tube with $CO_2$ and place a stopper (No. 6 stopper which has had a 1/16 inch hole drilled through it. Care must be taken to insure that the 1/16 inch hole is not blocked) with a small hole in it in the tube.

D. Swirl tubes gently to avoid getting sample on the sides of the tube.

E. Place in water bath at 39 $^{o}$C for 48 hours, swirling tubes at least twice daily.

VII. Pepsin Incubation:

A. After 48 hours, remove stoppers and rinse any sample adhering to the stopper into the tube with a **minimum** of distilled water.

B. Using a repeater add 1 ml of 20% HCl, swirl tubes, add 1 ml of 20% HCl **let rack sit** for a minute add another 1mL of 20% HCl swirl tubes; and finally add 3 ml of 20% HCl swirl tubes (total HCl = 6 ml).

C. To avoid excess foam, wait until acid has been added to all tubes before swirling.

D. Add 2 ml 5% pepsin, swirl thoroughly, replace stoppers, and return to water bath.

E. Label (in pencil) and dry Whatman 541 filter papers (minimum 3 hrs in 100$^{o}$C oven) and weigh.

F. After 24-hour pepsin digestion, filter residue with suction through filter paper.

G. Wash tubes out thoroughly with hot distilled water.

H. Dry filter paper and residue at 60 $^{o}$C for 24 hrs or at 100$^{0}$C for at least 6 hours. Cool in desiccator for no longer than 2-4 minutes and weigh.

VIII. Calculation:

$$\% \text{ IVDMD} = 1 - \frac{[(\text{Residue + filter paper}) - \text{filter paper}] - \text{blank}}{(\text{Sample wt.})(\text{DM})}$$

Blank = (Blank residue + filter paper) – filter paper