I. References:


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 ddH₂O
C. McDougall’s Buffer:

<table>
<thead>
<tr>
<th>Compound</th>
<th>g/Liter</th>
<th>g/18 Liter</th>
<th>g/12 Liter</th>
<th>g/9 Liter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium Bicarbonate (NaHCO₃)</td>
<td>9.8</td>
<td>176.40</td>
<td>117.6</td>
<td>88.2</td>
</tr>
<tr>
<td>Sodium Phosphate Dibasic (Na₂HPO₄)</td>
<td>2.44</td>
<td>49.94</td>
<td>33.24</td>
<td>24.97</td>
</tr>
<tr>
<td>Potassium Chloride (KCl)</td>
<td>0.57</td>
<td>10.26</td>
<td>6.84</td>
<td>5.13</td>
</tr>
<tr>
<td>Sodium Chloride (NaCl)</td>
<td>0.47</td>
<td>8.46</td>
<td>5.64</td>
<td>4.23</td>
</tr>
<tr>
<td>Magnesium Sulfate (MgSO₄/7H₂O)</td>
<td>0.12</td>
<td>2.16</td>
<td>1.44</td>
<td>1.08</td>
</tr>
<tr>
<td>Calcium Chloride (CaCl₂/2H₂O)</td>
<td>0.16</td>
<td>2.90</td>
<td>1.92</td>
<td>1.45</td>
</tr>
</tbody>
</table>

*CaCl₂ 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 °C and reduced with a stream of CO₂.
- During the warming and reducing step, urea is added to the McDougall’s at the rate of 1.0 g/ 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 CO₂, stopper, and place in 39°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 CO₂ 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 ddH₂O to each tube. Treat all tubes equally.
B. Dispense 50 ml of mixture into each tube.
C. Flush top of tube with CO₂ 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°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°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°C for 24 hrs or at 100°C for at least 6 hours. Cool in desiccator for no longer than 2-4 minutes and weigh.

VIII. Calculation:

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

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