In Vitro Gas Production Procedure

Updated September 2013

I. Personal Protective Equipment
   a. Lab coat
   b. Safety glasses/goggles
   c. Latex gloves

II. Sample Preparation:
   a. Weigh approximately 1.000- 1.040 g of feed into 250 ml gas production bottle. Include 1 blank.

III. 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.
   c. Pour filtered fluid into 500-1000L separatory funnels, gas with CO2, stopper, and place in 39°C water bath until particulate matter floats to the top.
   d. Remove lower portion and mix with warmed, reduced McDougall’s Buffer (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.

IV. In vitro Gas Bottle Incubation:
   a. Dispense 100 ml of mixture into each bottle.
   b. Flush top of the bottle with CO2 and place the gas production module tightly onto the bottle.
   c. Swirl bottles gently to avoid getting sample on the sides of the bottle and on the gas production module.
   d. Place in water bath at 39°C for 48 hours, swirling bottles at least twice daily.