

## **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 CO<sub>2</sub>, 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 CO<sub>2</sub> 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 CO<sub>2</sub> 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.