In Vitro True Digestibility using the DAISY^{II} Incubator

ANKOM *Technology* - 1/24/17

A. Reagents

(a) Buffer Solution A:

	g/liter
KH_2PO_4	10.0
$MgSO_4 \cdot 7H_2O$	0.5
NaCl	0.5
$CaCl_2 \cdot 2H_2O$	0.1
Urea (reagent grade)	0.5

(b) Buffer Solution B:

Na_2CO_3	15.0
$Na_2S \cdot 9H_2O$	1.0

(c) Neutral Detergent Solution

B. Apparatus

(a) DAISY^{II} Incubator
(b) Filtration device - F57 Filter Bags.
(c) Impulse bag sealer - HS/HSi Heat Sealer.
(d) Thermos
(e) ANKOM$^{200/220}$ Fiber Analyzer

C. Procedure

Preparation of Filter Bags and Sample:

Pre-rinse F57 filter bags in acetone for three to five minutes and completely air-dry. The acetone rinse removes a surfactant that inhibits microbial digestion. Weigh each F57 filter bag and record weight (W_1). Zero the balance and weigh 0.25g of sample (W_2) **directly** into filter bag. NOTE: For 48 hr studies a sample size of 0.5 g is acceptable. Heat seal bag closed and place in the **Daisy^{II} Incubator** digestion jar (up to 25 samples per jar). Samples should be evenly distributed on both sides of the digestion jar divider. Include at least one sealed blank bag for correction factor (C_1).

Preparation of (combined) Buffer Solution: *(For each digestion jar)*

a) Pre-warm at 39°C both buffer solutions (A & B). In separate container add ~266 ml of solution B to 1330 ml of solution A (1:5 ratio). The exact amount of A to B should be adjusted to obtain a final pH of 6.8 at 39°C. No further adjustment of pH is necessary. Add 1600 ml of combined A/B mixture to each digestion jar.

b) Place the digestion jars with samples and buffer solution into **Daisy^{II} Incubator** and turn on heat and agitation switches. Allow temperature of digestion jars to equilibrate for at least twenty to thirty minutes.

Preparation of Inoculum and Incubation:

Maintain all glassware at 39°C

a) Preheat two 2L thermos bottles by filling with 39° C water. Empty heated water just prior to collection of rumen inoculum. Using the appropriate collection procedure, remove at least 2000 ml of rumen inoculum and place in thermos. Include approximately two "fistfuls" of the fibrous mat from the rumen with your collection in one thermos.

b) Preheat a blender by filling with 39^{o} C water. Empty the heated water just prior to pouring the rumen inoculum from the thermos into the blender. Purge the blender container with CO_2 gas and blend at a high speed for 30 seconds. The blending action serves to dislodge microbes that are attached to the mat and assure a representative microbial population for the *in vitro* fermentation. Filter the blended digesta through four layers of cheesecloth into a five-liter flask (pre-heated 39° C). Filter the remaining rumen fluid in the other thermos through four fresh layers of cheesecloth into the same five-liter flask. NOTE: Allow for extra cheesecloth around the edges to facilitate squeezing contents of filtered mat. The flask should be continually purged with CO_2 and continued during the transfer of the inoculum.

c) Remove one digestion jar from the **Daisy^{II} Incubator** and add the 400ml of inoculum to the buffer solution and samples. Purge the digestion jar with CO_2 gas for thirty seconds and secure lid.

d) Repeat process for all digestion jars to be used. NOTE: Do not allow CO_2 gas to bubble through the buffered inoculum, rather use the CO_2 to form a gaseous blanket over the contents of the jar.

e) Incubate for 48 hours. The **DAISY^{II} Incubator** will maintain a temperature of 39.5°C ± 0.5. If temperature of jars varies greater than one degree then move incubator to a warmer location or place blanket or similar insulator over incubator.

f) At completion of incubation, remove jars and drain fluid. Rinse bags thoroughly with cold tap water until water is clear. Use a minimum of mechanical agitation.

g) When determining True Digestibility it is necessary to remove microbial debris and any remaining soluble fractions using Neutral Detergent Solution. After rinsing the bags in water place them in the **ANKOM^{200} Fiber Analyzer** and follow the procedure for determining NDF. Record the post *in vitro* NDF weight as W_3. NOTE: Bags can be stored in the refrigerator or freezer until NDF determinations can be performed.

D. Calculate:

$$\textbf{\% IVTD} \text{ (as received basis)} = \frac{100 - (W_3 - (W_1 \times C_1))}{W_2} \times 100$$

$$\textbf{\% IVTD}_{\textbf{DM}} \text{ (DM basis)} = \frac{100 - (W_3 - (W_1 \times C_1))}{(W_2 \times DM)} \times 100$$

Where: W_1 = Bag tare weight
W_2 = Sample weight
W_3 = Final bag weight after In Vitro and sequential ND treatment
C_1 = Blank bag correction (final oven-dried weight/original blank bag weight)