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

Scope

This method is applicable to grains, feeds, forages, and all fiber-bearing material.

Definition

This method determines the true digestibility of forages and other feeds. It is based on the measurement of undigested cell wall constituents as neutral detergent fiber, using rumen fluid in an in vitro system.

Apparatus

a) ANKOM DAISY^{IIP} Incubator
b) Filtration Device – ANKOM F57 Filter Bags
c) Heat sealer – sufficient for sealing the filter bags to ensure complete closure (HS or HSi, ANKOM Technology).
d) Insulated container (e.g., Thermos) – for keeping rumen inoculum warm.
e) Blender – for distributing microbial load.
f) Cheesecloth
g) ANKOM Fiber Analyzer (ANKOM$^{200, A2000, or Delta}$)

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:

NaB_2COB_3	15.0
$NaB_2S \cdot 9HB_2O$	1.0

(c) Neutral Detergent Solution

Procedure *(see the NDF Analysis section of the Operator's Manual for more detail)*

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). Tare the balance and then weigh 0.25g sample (W_2) directly into the filter bag. NOTE: For 48 hr. studies a sample size of 0.5g is acceptable. Heat seal bag closed and placed 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 both buffer solutions (A & B) to 39°C. Add ~266ml of solution B to 1330 ml of solution A (1:5 ratio) in a separate container. The exact ratio 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 after this point. Add 1600ml of Solution A/B to each digestion jar.

b) Place the digestion jars with samples and buffer solution into the Daisy^{II} Incubator and turn on heat and agitation. Allow the 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 them with 39°C water. Empty heating-water just prior to collection of rumen inoculum. Using the appropriate collection procedure, remove at least 2000ml of rumen inoculum and place it in the Thermos bottles. Include approximately two "fistfuls" of the fibrous mat from the rumen with your collection in one thermos.

b) Preheat a blender by filling it with 39° C water. Empty the heated water just prior to pouring the rumen inoculum from the Thermos bottle into the blender. Purge the blender container with CO_2 gas and blend at 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 5L flask (pre-heated 39°C). Filter the remaining rumen fluid in the other Thermos bottle through four fresh layers of cheesecloth into the same 5L flask. NOTE: Allow for enough extra cheesecloth around the edges to facilitate squeezing contents of filtered mat. The flask should be continually purged with CO_2 during the transfer of the inoculum.

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

d) Repeat this 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 ± 0.5°C. If the temperature of the jars varies by more than 1°C, move the incubator to a warmer location or place a blanket or similar insulator over the incubator.

f) At completion of incubation, remove the jars and drain the fluid. Rinse bags about two times with cold tap water by pouring water into the jar, swirling, and then draining. Gently squeeze excess rinse water from the bags. The purpose of the rinse is to remove the bulk of the digestive solution and water-soluble compounds without excessive shaking or aggressively squeezing of the bags.

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 with water, place them in the ANKOM 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.

Calculations

$$\%\ IVTD\ (as\ received\ basis) = \frac{100 - \left(W3 - (W1\ x\ C1)\right)\ x\ 100}{W2}$$

$$\%\ IVTD\ (dry\ matter\ basis, DM) = \frac{100 - \left(W3 - (W1\ x\ C1)\right)\ x\ 100}{(W2\ x\ DM)}$$

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)