Appendix A

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

ANKOM TECHNOLOGY - 03/05

A. Reagents

a) **Buffer Solution A:**

	g/liter
KH_2PO_4	10.0
$MgSO_47H_2O$	0.5
NaCl	0.5
$CaCl_22H_2O$	0.1
Urea (reagent grade)	0.5

b) **Buffer Solution B:**

Na_2CO_3	15.0
Na_2S9H_2O	1.0

c) **Rumen fluid inoculum**

d) **Neutral Detergent Reagents** (see ANKOM Method for Determining Neutral Detergent Fiber)

B. Apparatus

a) **ANKOM Technology -** DAISY^{II} Incubator
b) Filtration device - **ANKOM Technology** - F57 Filter Bags
c) Impulse bag sealer- **ANKOM Technology -** 1915/1920 Heat Sealer
d) Graduated Cylinders – 1 L & 500 ml
e) Thermos – (2)
f) Cheese cloth for filtering
g) **ANKOM**$^{200/220}$ Fiber Analyzer

C. Procedure *(See attached illustration for additional detail)*

Preparation of Filter Bags and Sample:

a) Pre-rinse F57 filter bags in acetone for three to five minutes and completely air-dry. The acetone rinse removes a surfactant that may inhibit 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: A sample size of 0.5 g is acceptable for 48-hour digestion studies, however, recent studies suggest greater precision using 0.25 g. Heat seal each bag 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 weighed and sealed blank bag for correction factor (C_1).

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

a) Pre-warm (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 jar containing the sample bags.

b) Place digestion jars with samples and buffer solution into **Daisy^{II} Incubator** and activate heat and agitation switches (red lights in switches indicate power). Allow temperature of digestion jars to equilibrate for at least twenty to thirty minutes. This time could be used for collection and preparation of rumen inoculum.

Appendix A

Preparation of Inoculum and Incubation: (*See attached illustration for additional detail)*

Maintain all glassware at 39°C

a) Preheat two 2-liter 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 of the thermos'. The attached illustration pictures collection via a fistulated animal, however, collection of inoculum (without collection of the fibrous mat) by means of an esophageal tube is also possible.

b) Empty the rumen inoculum from the thermos into a 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) Measure 400ml of rumen inoculum in a graduated cylinder. 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 *(confirm that heat and agitation switches are on)* for 48 hours to determine the In Vitro True Digestibility result. The **DAISY^{II} Incubator** will maintain a temperature of 39.5°C ± 0.5.

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.

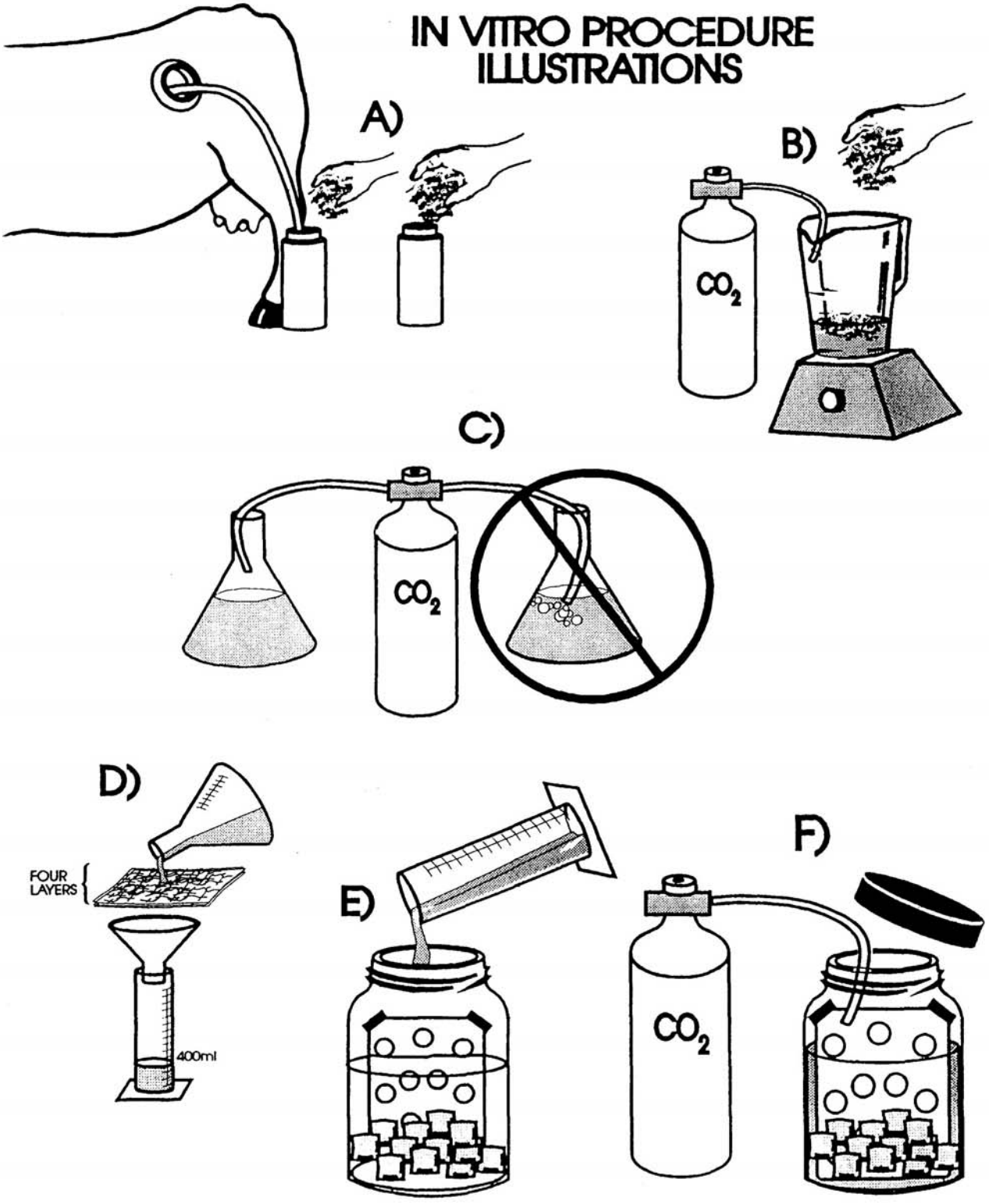
g) Place rinsed bags into the **ANKOM$^{200/220}$ Fiber Analyzer** and follow the procedure for determining NDF. Record the post *in vitro* NDF weight as W_3 for the formula below. The NDF analysis removes microbial debris and any remaining soluble fractions. **NOTE:** Bags can be stored in the refrigerator or freezer until NDF determinations can be performed.

D. Calculate: $\%\ IVTD_{\text{As-Received}} = 100 - ((W_3 - (W_1 \times C_1)) \times 100 / W_2)$

$$\%\ IVTD_{DM} = 100 - ((W_3 - (W_1 \times C_1)) \times 100 / (W_2 \times DM)$$

Where: W_1 = Bag tare weight
W_2 = Sample weight
W_3 = Final bag weight after In Vitro and sequential NDF determination
C_1 = Blank bag correction (final oven-dried weight/original blank bag weight).
DM = % dry matter (multiply by the decimal equivalent)

Appendix A


IN VITRO PROCEDURE
ILLUSTRATIONS
A)
B)
CO_2
C)
CO_2
D)
FOUR LAYERS
400ml
E)
F)
CO_2