Method for Determining Neutral Cellulase plus Gamanase (NCGD)
ANKOM TECHNOLOGY - 11/98

A. Reagents

(a) *Neutral Detergent Solution (ND)* -- Add 30.0 g sodium lauryl sulfate, USP; 18.61 g Ethylenediaminetetraacetic Disodium Salt, Dihydrate; 6.81 g sodium tetraborate decahydrate; 4.56 g sodium phosphate dibasic, anhydrous; and 10.0 ml triethylene glycol, in 1 L distilled H_2O (**ANKOM TECHNOLOGY, premixed chemical solution - FND20 or FND20C**). Agitate and heat to facilitate solubility. Check pH range to 6.9 to 7.1.

(b) *Alpha-amylase* -- Heat-stable bacterial alpha-amylase: activity = 340,000 Modified Wohlgemuth Units / ml (**ANKOM TECHNOLOGY - FAA**). One Modified Wohlgemuth Unit is that activity which will dextrinize 1.0 mg of soluble starch to a defined size dextrin in 30 minutes.

(c) *Sodium sulfite* -- Na_2SO_3, anhydrous (**ANKOM TECHNOLOGY - FSS**).

(d) *Acetone* -- Use grade that is free from color and leaves no residue upon evaporation (**ANKOM TECHNOLOGY - FACE**).

(e) *NCGD Enzyme Kit* -- Per M.A.F.F. "Prediction of the Energy Values of Compound Feeding Stuffs for Farm Animals" Appendix II, Biotal Limited is the only enzyme preparation currently approved. Dr. S.P. Mann/Mr. P. Vernon, c/o Biotal Limited, 5 Chiltern Close, Cardiff CF4 5DL.

(f) *Sodium Acetate* -- N.B. Analytical Grade

(g) *Glacial Acetic Acid* -- N.B. Analytical Grade

(h) *Chloramephenicol* -- N.B. Analytical Grade

B. Safety Precautions -- See MSDS provided with chemicals

(a) Rubber gloves and face shield should be worn when handling acid or basic solutions. When mixing always add sulfuric acid to water. If acid contacts skin wash with copious amounts of water. Follow any and all safety precautions listed on labels.

C. Apparatus

(a) Digestion apparatus -- ANKOM$^{200/220}$ FIBER ANALYZER

(b) Filtration device -- ANKOM TECHNOLOGY - F57 Filter Bags

(c) Impulse bag sealer - Requires high enough temperature to melt and seal polymer in filter bags. (**ANKOM TECHNOLOGY - 1915/1920**).

(d) Desiccator -- ANKOM TECHNOLOGY ***MoistureStop*** weigh pouch - F39

D. Procedure

(a) Follow steps D.a.1 through D.6.e of the ANKOM NDF procedure and set aside samples for preparation of NCGD steps.

(b) Prepare an Acetate buffer (pH 4.8) solution as follows: Dissolve 1.36g of sodium acetate in 500ml of distilled water. Add 0.6ml of glacial acetic acid and dilute to 1 litre. If necessary adjust pH to 4.8 with the addition of a sodium hydroxide solution. If storing the acetate buffer, check pH each day before use.

(c) Produce a buffered cellulase solution by weighing 20g of cellulase (from NCGD enzyme kit) and 0.1g of chloramephenicol into a digestion jar of the ANKOM Daisy^{II} Incubator. Add 1 litre of buffer solution (b), shake and place in the incubator. Switch the Heat and Agitate buttons to the on position and incubate for at least one hour. NOTE: Safety precautions must always be taken when handling enzymes of this nature. Check manufacturers instructions

(d) Mix gamanase thoroughly with buffered cellulase solution in the proportion of 9 volumes of cellulase solution to 1 volume of the gamanase preparation provided in the NCGD kit.. Filter the mixture through a glass microfibre filter (e.g. Whatman grade GF/A). Allow combined cellulase/gamanase solution to incubate until combined mixture is at 40.0 ° C.

(e) One at a time, remove jars from the incubator. Add samples from NDF preparatory step to each digestion jar of the Daisy^{II} Incubator at the rate of 1 sample per every 30ml of prepared solution. Divide samples equally between the digestion jar divider and return jar to incubator. ***Include at least one pre-weighed and sealed blank bag in every jar.*** Each Digestion jar is capable of holding up to 2000ml of solution.

(f) With Heat and Rotate buttons in the on position, set the timer for 40 hours, close and secure the incubator door and press the start button activating the timer. With the exception of periodic inspection to ensure the digestion jars are rotating and the controller is maintaining temperature, no other action is required.

(g) At the end of the 40-hour digestion period switch all buttons to the off position, open the incubator door and remove the digestion jars. Drain the enzyme solution from each jar and rinse samples thoroughly with hot (+90.0°C) water. Place rinsed samples in a small beaker (~24 samples per 250ml beaker) and cover with acetone.

(h) Spread bags out and allow acetone to evaporate. Complete drying in oven at 105° C for at least 2 hours. **WARNING: Do not place bags in the oven until acetone has completely evaporated.** Longer drying period may be required depending on oven and frequency of sample introduction into the oven. Remove bags from oven, place directly into ***MoistureStop*** weigh pouch and flatten to remove air. Cool to ambient temperature and weigh bags.

(i) Ignite samples at 500°C until ashing is complete (approx. 3 hours). Determine loss of weight on ignition. You will also need to determine the % total ash and dry matter on a separate sub-sample.

E. Calculate percent $NCGD_{OM}$ (DM basis):

$$100 - \frac{[(W_4 - (W_1 \times C_2))]}{W_2 \times DM} + \%\ Ash$$

Where: W_1 = Bag tare weight
W_2 = Sample weight
W_3 = Weight after extraction process
W_4 = Weight of Organic Matter (OM)(Loss of weight on ignition of bag and fiber residue)
C_2 = Ash corrected blank bag (Loss of wgt. on ignition of blank bag/original wgt.)
% Ash = % Ash determined from separate sub-sample