Determination of Neutral Cellulase Plus Gammanase Digestibility (NCGD)

Scope

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

Principle

The percentage of organic matter in a sample that is digested by neutral detergent solution and the enzymes (cellulase and gammanase) is denoted by the abbreviation 'NCGD'. The fat-free sample material is first extracted with neutral detergent solution to remove soluble cell contents. After NDF analysis, mostly cellulose and lignified cellulose will remain in the residue. The NCGD analysis uses enzymes to break down most remaining cellulose into glucose.

Apparatus

a) ANKOM Fiber Analyzer (ANKOM$^{200,\ 2000,\ or\ Delta}$)
b) ANKOM F57 Filter Bags (SKU: F57)
c) Bag heat sealer: Requires high enough temperature to melt and seal polymer in filter bags. (ANKOM Technology, SKU: HS and HSi).
d) Desiccator: MoistureStop weigh pouch (ANKOM Technology, SKU:X45)

Reagents

a) Neutral Detergent (ND) Solution: 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 (Lallemand Animal Nutrition UK Ltd.): *Lallemand Animal Nutrition UK Ltd (Spring Lane North, Malvern Link, Worcestershire, WR14 1BU (formerly Biotal Limited)) updated this enzyme kit (9-6-23). The MAFF sponsored Working Party on Metabolizable Energy has specified that kits from this source alone must be used in support of the prediction equation for ME of Ruminant Compound Feeds. Per APPENDIX II of the orange MAFF book "Prediction of the Energy Values of Compound Feeding Stuffs for Farm Animals". Updated 09-06-23 to revert Polysaccharase 161 (Cellulase) formulation.
f) Sodium Acetate: Analytical Grade
g) Glacial Acetic Acid: Analytical Grade
h) Chloramephenicol: Analytical Grade

Safety Precautions

Rubber gloves and face shield should be worn when handling acid or base solutions. When mixing, always add acid to water. If acid contacts skin, wash with copious amounts of water. Follow any and all safety precautions listed on labels. See MSDS provided with chemicals.

Procedure

a) First do an NDF analysis, and then retain bags for subsequent NCGD analysis. Make sure to run enough blank bags to later include at least one bag per Daisy digestion jar.
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 liter. If necessary, adjust pH to 4.8 with the addition of a sodium hydroxide solution. If the buffer is stored for longer than 24 hrs, check the pH each day before use.
c) Make a buffered cellulase solution: Weigh 20g cellulase and 0.1g of chloramphenicol in a digestion jar of the ANKOM DAISY^{II} Incubator. Add 1 liter of acetate buffer solution. Switch on the heat and agitate buttons and incubate for at least one hour at 40°C. Warning: Safety precautions must always be taken when handling enzymes of this nature. Check with the manufacturer (Lalllemand Animal Nutrition UK Ltd.) for specific instructions.
d) Cellulase/gammanase solution: Mix gammanase thoroughly with buffered cellulase solution in the proportion of 8 volumes of cellulase solution to 2 volumes of the new gammanase preparation provided in the NCGD kit. Filter the mixture through a glass microfiber filter (e.g., Whatman grade GF/A). Allow combined cellulase/gammanase solution to incubate until the solution is at 40°C.
e) Remove jars from the incubator one at a time. Add the bags from the NDF analysis to each digestion jar of the Daisy II Incubator at a ratio of 1 bag for every 33.75ml buffered cellulase/gammanase solution. Divide bags equally between the digestion jar divider and return the jar to incubator. Include at least one blank bag per digestion jar. These blank bags should also have gone through the NDF analysis. Each digestion jar can hold up to 2L of solution.
f) With heat and rotate buttons in the on position, set the timer for 40 hours. Close the incubator door and press the start button to activate the timer. Periodically inspect to ensure that the digestion jars are rotating, and the controller is maintaining temperature.
g) At the end of the 40-hr. digestion period, switch all buttons off. Open the incubator door and remove the digestion jars. Drain the enzyme solution from each jar and rinse bags thoroughly with hot (90°C) water. Place rinsed bags in a small beaker (~24 bags per 250ml beaker) and cover bags with acetone.
h) Remove bags from acetone and gently press excess acetone out. Lay bags flat 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 before all acetone has evaporated. A longer drying period may be required if the oven is opened during the drying time. After drying is completed, remove the bags from the oven, place it directly into a MoistureStop weigh pouch and flatten the bag to remove excess air. Cool bags in the pouch to ambient temperature and then weigh bags.
i) Ash bags at 500°C until complete (approx. 3 hours). Determine loss of weight of the bag and fiber residue (W4).
j) Determine ash and dry matter content on separate sub-samples.

Calculations

$$NCGD_{OM}\ (DM\ basis) = 100 - \left(\frac{(W4-(W1xC2))}{(W2xDM)} + Ash\right)$$

Where:
W1 = Empty bag weight (g)
W2 = Sample weight (g)
W4 = Weight loss on ignition of bag and fiber residue (g)
C2 = Ash corrected blank bag factor

$$= \frac{(weight\ loss\ on\ ashing\ of\ blank\ bag)}{(original\ weight\ of\ blank\ bag)}$$

Ash = Determined in separate analysis (%)
DM = Dry matter (DM) determined in separate analysis