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In Vitro Digestibility

1. Application

This procedure allows the determination of true digestibility and digestion kinetics of forages and other feeds based upon the measurement of undigested cell wall constituents as neutral detergent fiber using rumen fluid in an in vitro system.

2. Summary of Methods

Dried and ground samples are digested in an oxygen limiting environment using dairy rumen fluid, buffer, mineral and reducing solutions. Residual dry matter is determined and an NDF is run on residue if needed.

3. Safety

All chemicals should be considered a potential health hazard. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material handling data sheets should be made available to all personnel involved in the chemical analysis.

4. Interferences

This method is very sensitive to diet and temperature changes to donor animals.

5. Sample Collection, Preservation, and Handling

All samples are dried at 55^{o}C in a cabinet-type forced air dryer for 12-16 hours. After drying the sample is ground to pass through a 1 mm forage mill.

6. Apparatus and Materials

6.1 125 ml Erlenmeyer flask
6.2 Stoppers, with 2 inlet valves
6.3 Water Bath set to 39^{o}C
6.4 Canulated Cow
6.5 Automatic dispenser; 40 ml
6.6 Carbon Dioxide Tank
6.7 Auto dispensing syringe; 2ml and 10 ml
6.8 Rubber policemen
6.9 Incubation oven
6.10 Thermos

6.11 Rumen Fluid Pump
6.12 2 pails
6.13 Blender
6.14 Cheesecloth
6.15 2000 ml side arm flask

7. Reagents

7.1 In Vitro Media (pH 6.8) – makes 800 ml, adjust as needed for larger or smaller sample batches
- 7.1.1 2.0 g trypticase
- 7.1.2 400 ml distilled water
- 7.1.3 0.1 ml micromineral solution; see below
- 7.1.4 200 ml buffer solution; see below
- 7.1.5 200 ml macromineral solution; see below
- 7.1.6 1.0 ml resazurin (0.1% solution in distilled water; keep refrigerated)

7.2 Micromineral Solution
- 7.2.1 13.2 g $CaCl_2*2H_2O$
- 7.2.2 10.0 g $MnCl_2*4H_2O$
- 7.2.3 1.0 g $CoCl_2*6H_2O$
- 7.2.4 8.0 g $FeCl_3*6H_2O$
- 7.2.5 Bring to 100 ml with distilled water

7.3 Buffer Solution
- 7.3.1 18.0 L Distilled Water
- 7.3.2 72.0 g Ammonium Bicarbonate
- 7.3.3 630.0 g Sodium Bicarbonate

7.4 Macromineral Solution
- 7.4.1 18.0 L Distilled Water
- 7.4.2 102.6 g Na_2HPO_4, anhydrous
- 7.4.3 111.6 g KH_2PO_4, anhydrous
- 7.4.4 10.5 g $MgSO_4*7H_2O$

7.5 Reducing Solution
- 7.5.1 0.625 g cysteine hydrochloride
- 7.5.2 95 ml distilled water
- 7.5.3 0.625 g sodium sulfide nonahydrate

8. Methods

Sample processing:

8.1 Thoroughly mix sample and weigh out 0.5 gram of sample into 125 ml Erlenmeyer flask.

8.2 Concurrently weigh out a 2.0 gram sample for lab dry matter determination at 105^{o}C for 3 hrs. This will allow further calculations to be made on a dry matter basis.

8.3 In vitro blanks have no sample added to them, although all other reagents and rumen inoculum is added.

8.4 Run a neutral detergent fiber (NDF) on the original sample, to be used in calculating the NDFD after the in vitro is complete. (See NDF procedure).
8.5 Turn on water bath and allow the temperature to reach approximately 39°C. Be certain that the water level in the bath is above the 50 ml mark on the flasks so that the flask contents are maintained at 39°C.

Media preparation:
8.6 Calculate the amount of media and reducing solution needed based upon the number of samples being analyzed.
8.7 Add 40 ml of media per sample using automatic dispenser. Be careful not to splash sample out of flask or up on the sides.
8.8 Put stoppers in flasks and place in water bath, connect tubing, which will allow carbon dioxide to flow into flasks.

Reducing:
8.9 When all tubing and flasks are connected, turn on carbon dioxide. Adjust pressure to approximately 15-20 psi. Turn on gas flow with needle valve enough to feel gas escaping from all the flasks through inlet valve. The water manometer will cease bubbling. If "icing" of diaphragm and brass hose occurs, decrease gas flow rate by adjusting needle valve.
8.10 While flasks are being gassed for approximately 10 to 15 minutes, prepare the reducing solution.
8.11 Inject 2 ml of reducing solution into each flask with a syringe. Place rubber policeman onto each inlet valve as the solution is added.
8.12 When all flasks have had reducing solution added and are closed with a policeman, adjust CO_2 flow rate so that bubbling through water manometer is minimal.
8.13 Wait for media reduction to occur as evidenced by change in color from blue to pink to colorless (oxidized to reduced).

Collection and Preparation of Inoculum:
During processing of the rumen fluid these steps should be done as quickly as possible and effort should be taken to keep the fluid under CO_2 whenever possible.
8.15 Earlier in the day, place all equipment that will come in contact with rumen fluid in incubation ovens at approximately 39°C.
8.16 Put thermos and pump used for rumen fluid, in pails of hot water (to keep at body temperature). Fill thermos to the top with rumen fluid and seal immediately. Quickly return capped thermos to the warm water. Take immediately to the lab, noting collection time.
8.17 Discard approximately one-half of solids on top of fluid layer and pour contents into blender. Measure pH of rumen fluid and then blend for 60 seconds.
8.18 Filter through 4 layers of cheesecloth into 2000 ml side arm flask using a funnel.

Inoculating Flasks:
Time is of the essence with this procedure. The total time from collection to inoculation should not exceed 20-25 minutes.

8.19 While keeping rumen fluid stirring and under CO_2 quickly add 10 ml of fluid to each flask through the inlet valve using an auto dispensing syringe. Replace rubber policeman onto each inlet valve after inoculate is added. Swirl all flasks after inoculum addition, avoiding splashes up the sides.

8.20 Check all stoppers and policemen to be certain they are tight, and adjust CO_2 flow rate if necessary. Remember this system keeps all flasks under constant CO_2 pressure and only minimal bubbling of gas in the water manometer is necessary. If it takes excessive CO_2 pressure to bubble the manometer, check for loose stoppers, loose rubber policemen, or loose tubing causing a leak.

8.20 Keep track of incubation times. Record the time at the start of the digestion and when the flasks should be taken out, depending on the time point requested, and post it right on the water bath.

Fermentation Times:

8.21 Take flasks out of water bath at the time point you are testing (i.e. 48 hours) and rinse down the sides with distilled water to submerge all particles in liquid, stopper, and freeze immediately.

8.22 Alternatively, NDF on the residue can be tested immediately (see NDF procedure).

9. Calculations

9.1 Residue Weight = ((Crucible Weight + Fiber) – blank) – Crucible Weight w/o Fiber

9.2 Sample Weight Absolute = (Sample Weight * % Lab Dry Matter)

9.3 Indigestible Dry Matter % = Residue Weight / Sample Weight Absolute

9.4 IVTDMD = 1 - (Indigestible Dry Matter %)

9.5 IVNDFD = 1 – [(Indigestible Dry Matter % * 100) / % NDF]

10. Quality Control

At least one laboratory reagent blank (LRB) is analyzed with each batch of samples to gauge run acceptability.

11. Reporting

Results are reported as % NDFD on a dry matter basis.

12. References

12.1 Goering, H. K., and P. J. Van Soest. 1975. Forage Fiber Analyses. Agr. Handbook No. 379. Agricultural Research Service. USDA.

12.2 Mertens, D. R. 1992. Critical conditions in determining detergent fibers. Page C-1 in Proc. Natl. Forage Testing Assoc. Forage Anal. Workshop, Denver, CO. Natl. Forage Testing Assoc., Omaha, NE.