

IN VITRO STARCH DISAPPEARANCE (IVSD)
Updated September 2013

I. Reference:

Richards, C.J., J.F. Pedersen, R.A. Britton, R.A. Stock, and C.R. Krehbiel. 1995. In vitro starch disappearance procedure modifications. *Animal. Feed Sci. & Tech.* 55:35-45.

II. Personal Protective Equipment:

- A. Lab coat
- B. Safety glasses/goggles
- C. Latex gloves

III. Sample Preparation:

- A. Dry high moisture samples at 60°C for 48 hours or freeze dry. Do not dry samples with <15% moisture.
- B. Grind samples through 1 mm screen on Wiley.

IV. Reagents:

- A. McDougall's Buffer (see In Vitro Dry Matter Disappearance procedure)
- B. Saturated Benzoic Acid (C₇H₆O₂), 0.2% (w/v).
 - 1. Add 4 g benzoic acid to 2 L of dH₂O.
 - 2. Place on stir plate, stir and heat until dissolved.
- C. 1.2 M Acetate Buffer, pH 5, in 0.2% benzoic acid.
 - 1. Dissolve 70.4 g anhydrous sodium acetate (C₂H₃NaO₂) in 800 ml 0.2% benzoic acid solution.
 - 2. Add 20.5 ml glacial acetic acid (C₂H₄O₂)
 - 3. Adjust pH, if necessary.
 - 4. q.s. to 1 L with 0.2% benzoic acid solution.
- D. α-Amylase
 - 1. Sigma Catalog No. A-3403 Type XII α-amylase from *Bacillus globigii*
 - 2. Depending on lot #, α-amylase contains approximately 25, 000 units/mL
- E. Amyloglucosidase
 - 1. Sigma Catalog No. A-3042 Amyloglucosidase from *Aspergillus niger*
 - 2. Depending on lot #, amyloglucosidase contains approximately 6,000 units/mL.
- F. D-(+)-Glucose Solution (10%)
 - 1. Sigma Catalog No. G-8644
 - 2. Solution is 100g D-(+)-Glucose /L
- G. Glucose Trinder
 - 1. Sigma Catalog No. 315-100

V. Standards:

- A. Pipette into 100 mL volumetric flasks the reagent indicated in column 2 below:

1	2	3
Standard No.	Glucose Standard (mL)	Glucose (mg)
1	0	0
2	0.1	10
3	0.2	20
4	0.3	30
5	0.4	40
6	0.5	50

B. q.s. to 100 mL with ddH₂O.

VI. Procedure:

A. Preliminary

1. Before the run, collect approximately 50 ml of rumen fluid and determine pH.
2. If pH is not approximately 6.0, this may indicate potential problems and you may need to wait a day before you begin your run.
3. Freeze a portion of the sample for glucose and VFA analysis.

B. In Vitro

1. Weigh approximately 0.5 g of sample in triplicate into 100 mL in vitro tubes. Include cornstarch and cobs standard as controls.
2. Place McDougall's buffer in 39°C water bath and add 1 g urea/L. Reduce by bubbling CO₂ through buffer.
3. Inoculum Preparation
 - a. Follow procedure outlined in In Vitro Dry Matter Disappearance procedure
 - b. Mix rumen fluid and buffer in a ratio of 1:2.
4. Dispense 30 ml inoculum into in vitro tube trying to disperse sample.
5. Vortex or swirl by hand, be sure to break up all clumps.
6. Gas each tube with CO₂, stopper and place in 39°C water bath.
7. Gently swirl tubes several times during incubation, taking care not to leave DM on the walls of the tube.
8. Kill tubes at 4, 8, 12, 16, and 24 hr with 15 ml of ice-cold 1.2 M acetate buffer in saturated benzoic acid if you want to determine rate of starch disappearance.
9. Kill tubes at 8 hr with 15 ml of ice-cold 1.2 M acetate buffer in saturated benzoic acid if you want to determine starch remaining.
10. Place tubes in cooler and process within 48 hr of kill.

C. Conversion of Starch to Glucose (after tubes are killed)

1. Add 660 µL α-amylase (340 U/µL)
2. Boil @ 105°C for 30 minutes.
3. Cool to 60°C, police sides.
4. Add 50 µL amyloglucosidase (200 U/mL) and mix.
5. Incubate overnight at 60°C, swirl after two hours.
6. Transfer into 100 ml volumetric flasks.
7. Rinse tubes three times with 0.2% benzoic acid.

8. Rinse funnel and q.s. with 0.2% benzoic acid.
9. Mix and transfer aliquot into 12 x 75 mm centrifuge tubes.
10. Centrifuge at 3,000 x g for 10 minutes.
11. **Place tubes in 60°C water bath unless pipetting.** Glucose tends to form crystals when cooled.
12. Determine glucose in supernatant using Glucose Trinder
 - a. Pipette 40 µL standard into wells of a microtiter plate.
 - b. Pipette 40 µL dH₂O into wells of a microtiter plate to serve as a blank.
 - c. Pipette 20 µL of sample plus 20µL dH₂O into wells (dilution may be changed).
 - d. Pipette 250 µL Glucose Trinder to all wells.
 - e. Incubate in plate reader (SpectraMax 250) for 12.5 minutes at 37°C.
 - f. Read absorbance at 492nm.
13. Samples can be stored at 4°C until analysis.

VII. Calculations:

$$\frac{(\text{Sample glucose, mg/dL} * \text{dilution (2)} - \text{blank}) (.9)}{(\text{Sample wt., g})(1000)(\text{DM})} \times 100 = \begin{matrix} \% \text{ starch remaining} \\ \% \text{ of original starch} \end{matrix}$$

$$100 - \% \text{ starch remaining as \% of original starch} = \% \text{ starch disappearance}$$