RUMINAL FLUID VFA PROCEDURE

Updated September 2013

I. Reference:
   a. Erwin et al. 1961. J. Dairy Sci. 44:1768 (for luminal fluid preparation).

II. Personal Protective Equipment:
   a. Lab coat
   b. Safety glasses/goggles
   c. Latex gloves

III. Reagents:
   a. 25% (w/v) meta-phosphoric acid
      i. Determine amount of 25% MPA needed for entire VFA procedure. Will need at least 400mL for the smallest run.
      ii. Weigh 100g m-phosphoric acid
      iii. Add 200mL ddH_2O to a 400mL volumetric flask with a stir bar, quantitatively transfer m-phosphoric acid to the flask and dissolve.
      iv. q.s. to 400mL with ddH_2O
   b. 25 % (w/v) meta-phosphoric acid (H_3PO_4), 25mM 2-EB (internal standard)
      i. Add about 50mL of 25% m-phosphoric acid to a 100mL volumetric flask.
      ii. Weigh ca. 0.2904g 2-ethlbutyrate ($C_6H_{12}O_2$) and quantitatively add to flask. (**Note:** This is a critical step for the samples to be run on the GC. You must get all the 2-EB into the 25% m-phosphoric acid).
      iii. Note the exact weight of the 2-EB added to calculated molarity.
      iv. q.s. to 100mL with 25% meta-phosphoric acid (MW of 2-EB is 116.16).
      v. Place in bottle and shake thoroughly. Store at 4^{o}C.

IV. Sample Preparation:
   a. Freeze and thaw sample (helps to precipitate protein).
   b. Centrifuge at 5,000 x g for 10 minutes.
   c. Pipette 2.0mL supernatant into pre-labeled tube in duplicate.
   d. Add 0.5mL ice cold 25% m-phosphoric acid/2-EB solution to the sample. Vortex tubes after addition of 2-EB solution.
   e. Allow to stand in refrigeration for 30 minutes. After refrigeration centrifuge at 10,000 x g for 15 minutes.
   f. Fill 3mL tuberculin syringe with supernatnant without disturbing the pellet.
   g. Filter through filter-tip syringe into GC vial. Fill vial at least ¾ full. (**Note:** use a 25mm GDX Disposable filter, Polethersulfone Filter Media with Polypropylene Housing and a 0.45µm Pore Size).

V. Standard:
    a. Stock Standard:
        i. Add the following approximate amounts to a 200mL volumetric flask. Note the exact weights for calculation of actual concentrations.

VFA	Approx. g/200mL	Approx. Stock Conc. mM	MW	Approx. Work Conc. mM
Ac-	0.7500	62.50	60.0	50.0
Pr-	0.2775	18.75	74.0	15.0
Ibut-	0.1101	6.25	88.1	5.00
But-	0.2202	12.5	88.1	10.0
Ival-	0.1021	5.00	102.1	4.00
Val-	0.1021	5.00	102.1	4.00

        ii. q.s. to 200mL with 25% m-phosphoric acid (w/o 2EB).

    b. Working Standard:
        i. Pipette 2mL stock std to 8 tubes and add 0.5mL of ice cold 25% m-phosphoric acid and 2-EB solution.
        ii. Vortex tubes and incubate in the refrigerator for 30 minutes.
        iii. Mix standard tubes and transfer to eight GC vials.
        iv. It is important that the standards and the samples have the exact same amount of ISTD (2-EB) in them. Use the same preparation of 25% m-phosphoric acid, 25mM 2-EB for all standards and samples.

VI. Column Specifications and GC Conditions:
    a. Column
        i. Packing
            1. Supelco 11965
                a. 10% SP-1200
                b. 1% H_3PO_4
                c. 80/100 Chromosorb W AW
            2. Supelco 12144
                a. 15% SP-1220
                b. 1% H_3PO_4
                c. 100/120 Chromosorb W AW
        ii. Measurements

1. Length: 8 feet
2. I.D.: 2mm
3. O.D.: ¼ inch

iii. Special Instructions

1. Leave 3 inch space on injector end
2. Leave 2 inch space on detector end

b. GC Conditions

i. Temperature

1. Oven: 145^{o}C
2. Injector: 185^{o}C
3. Detector (FID): 200^{o}C

ii. Flow Rates

1. Nitrogen (N_2): 20mL/minute
2. Hydrogen (H_2): 20mL/minute
3. Compressed Air: 200mL/minute

iii. Slope Sens: 0.10

iv. Attenuation: 2^{8}

c. Supplies

i. Syringe

1. Hamilton 80358 w/ Hamilton 80458 needle
2. Hamilton 90393

ii. Septum

1. Thermogreen LB-2 Septa 11.0mm (Supelco 20654)

iii. Inlet Liner

1. PureCol Inlet Liner 20mm (Supelco 20536)

iv. Filters

1. Nitrogen
    a. Agilent OT3-2
    b. OMI-2 (Supelco 23906)
2. Hydrogen
    a. Agilent OT3-2
    b. OMI-2 (Supelco 23906)
3. Air
    a. Supelco 20618 w/ Supelco 20298 replacement mesh