DAPA Determination Procedure

September 2013

I. Reference:

Webster, P.M., W.H. Hoover, and T.K. Miller. 1989Determinantion of 2, 6 diaminopimelic acid in biological materials using high performance liquid chromatography. Anim. Feed. Sci. Technol., 30:11-20.

II. Personal Protective Equipment:

1. Lab coat
2. Safety glasses/goggles
3. Designated chemical gloves

III. Reagents:

1. Mobile Phase A:
   a) ≈ 1600 mL of buffer solution- use this to q.s. to 2 liters
      - Buffer Solution:
        - 1600 mL ddH_2O
        - 3.2812 g sodium acetate
        - 5.67 g anhydrous sodium phosphate
        - Adjust pH to 6.8 w/ 85% H_2PO_4

   b) 370 mL of methanol
   c) 30 mL Tetrahydrofuran (THF-**peroxide former**)

2. Mobile Phase B:
   a) 1300 mL methanol
   b) 700 mL H_2O

3. Methanesulfonic acid (4N solution)
   a) 259 mL methane sulfonic acid
   b) 2 g 3-(2-aminoethyl) indole (0.2% w/v)
   c) 100 mL of β-aminoadipic acid stock solution
   d) Q.s. mixture with ddH_2O
      - Store at 4°C and flush w/ nitrogen after each use

4. DAPA Stock Solution
   a) 1 mg DAPA/5mL ddH_2O

5. β-aminoadipic Acid Stock Solution
   a) 1 mg β-aminoadipic acid/5mL ddH_2O

IV. Working Standard Prep:

a) Combine 1 mL DAPA stock solution w/ 1 mL β-aminoadipic stock solution
b) 10 mL of ddH_2O
c) 1 mL of working standard solution added to 1 mL of OPA derivatizing solution in small test tube

d) Vortex contents
e) Place on HPLC w/ prepped samples

V. Sample Prep:

b) Weigh 0.10-0.20 g of sample into screw cap glass tubes
c) Add 3 ml 4.0 N methanesulfonic acid containing catalyst and internal standard
d) Securely cap the tubes (tighten caps and slightly release pressure) and place on hotplate for 22 hours (high setting on hot plate)
e) Allow samples to cool after 22 hours
f) Neutralized the solution with 10 N sodium hydroxide-use pH probe and adjust to pH of 7.0-7.5
    - Note if you need to bring the pH down to 7.0 instead of up use 1.0 N sulfuric acid
g) Sample must then be vortexed to ensure the sample is uniform
h) 0.2000-0.2500 g of HCl-washed activated charcoal was added to each tube
i) Contents were mixed and transferred to smaller centrifuge tubes
j) Centrifuge tubes at 1000 x g for 20 min
k) Supernatant was passed through a disposable filter assembly (pore size 0.45 µm)
l) A second clean-up procedure utilizing the Sep-Pak C-18 cartridge (Waters Inc.) was performed on the sample as follows:
    i) Sep-Pak cartridge was activated with 20 mL methanol, washed with 20 mL of 0.1% TFA in water, followed by 10 mL of 0.1% TFA in a 80:20 mixture of water: methanol
    ii) 1 mL of sample was mixed with 2 mL of 0.1% TFA in 70:30 water: methanol (sample should be free of particulate matter) and the mixture passed through the Sep-Pak cartridge
    iii) The first milliliter was discarded and the next 2 mL, which contained all the amino acids, were collected.
m) 1 mL of OPA derivatizing solution was added to 1.0 mL of sample in a small test tube
n) Contents were mixed thoroughly and placed on the HPLC