Ruminal Ammonia Concentrations

Principle:

Ammonia reacts with alkaline hypochlorite and phenol in the presence of a catalyst, sodium nitroprusside, to form indophenol. The concentration of ammonia is directly proportional to the absorbance of indophenol, which is measured spectrophotometrically at 570 nm.

Materials:

Centrifuge
25 ml centrifuge tubes with caps
16 x 125 mm borosilicate glass tubes
Vortex mixer
Pipettes, 0-1,000 ul, 1-5 ml capacity
Spectrophotometer

Reagents:

18 MOhm water

Phenol nitroprusside solution (Sigma Cat # P6994-120ML) Store at 4 °C. Note expiration date.

Alkaline hypochlorite solution (Sigma Cat # A1727) Store at 4 °C. Note expiration date.

Ammonium Ion Standard solution, concentration of 100 mM NH_3 (Fluka Cat # 09683-100ML, FW 53.49, CAS # 12125-02-9)

> Dissolve 0.06607 g $(NH_4)_2SO_4$ (dried) (Mallinckrodt Cat # 7725, FW 132.13, CAS # 7783-20-2) in 90 ml 0.1 N HCl (EMD Cat #HX0603-3, FW 36.46, CAS # 7647-01-0). Bring to volume in 100 ml volumetric flask. Store at 4 °C. You may also use a commercial NH_4 standard.

0.1 N Hydrochloric acid, (8.04 ml HCl (EMD Cat #HX0603-3, FW 36.46, CAS # 7647-01-0) / liter water)

Working standards (Store at 4 °C. Solutions are stable 1 month.)

Standard	ml Stock NH_4	ml Diluent (0.1 N HCl)	Conc. (mg/dl) NH_3	Conc. (mM) NH_3
1	0.05	4.95	1.70	1
2	0.10	4.90	3.40	2
3	0.20	4.80	6.80	4
4	0.40	4.60	13.60	8
5	0.60	4.40	20.40	12
6	0.80	4.20	27.20	16
7	1.00	4.00	34.00	20
8	1.20	3.80	40.80	24
9	1.60	3.40	54.40	32

A standard curve using standards 1-5 is usually sufficient for most samples.

Procedure:

1. Centrifuge rumen fluid for 10 minutes at 13,800 x g at 4 °C. Save supernatant. There is no need for centrifugation with rumen fluid prepared for VFA analysis.
2. Rumen fluid and working standards should be diluted by a factor of 10. For example, 0.100 ml sample or standard + 0.900 ml dd water.
3. Pipette 100 ul diluted sample or standard into 16 x 125 mm borosilicate tubes, in duplicate.
4. Prepare blanks (100 ul dd water) in duplicate.
5. To each tube, add the following reagents in the order listed:
    a. 1.0 ml phenol nitroprusside solution, vortex.
    b. 1.0 ml alkaline hypochlorite solution, vortex.
    c. 5.0 ml dd water, vortex.
6. Allow samples to react with reagents for 30 minutes at room temperature. Begin timing after step 5b.
7. Read absorbency at 570 nm on spectrophotometer. Color is stable for 1-3 hours.

Calculations:

Read absorbencies of standards and samples, and use a linear regression of the standards to calculate sample concentrations.

References

Sigma Technical Bulletin #640. The colorimetric determination of urea nitrogen. Sigma Diagnostics, St. Louis, MO 63178.

Chaney, A. L., Marback, E. P., Modified reagents for determination of urea and ammonia. Clin. Chem., 8:130, 1962.

Horn, D. B., Squire, C. R., An improved method for the estimation of ammonia in blood plasma. Clin. Chem. Acta, 17:99, 1967.

Weichselbaum, T. E., Hagerty, J. C., Mark, H. B. Jr. A reaction rate method for ammonia and blood urea nitrogen utilizing a pentacyanonitrosyloferrate catalyzed Berthelot reaction. Anal. Chem., 41:848, 1969.