

PROTEASE-PEPSIN INSOLUBLE NITROGEN

Updated September 2013

I. Reference:

Pichard and Van Soest. 1977. Protein Solubility of Ruminant Feeds. Proc. Cornell Nutr. Conf. P. 91

Poos-Floyd, Klopfenstein and Britton. 1985. Evaluation of Laboratory Techniques for Predicting Ruminant Protein Degradation. J. Dairy Sci. 68:829.

II. Personal Protective Equipment:

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

III. Reagents:

A. Sodium Azide (N_3Na) – 1% (W/V).

B. In Vitro Rumen Buffer.

1. Dissolve 4 g Ammonium Bicarbonate (CH_5NO_3) and 35 g Sodium Bicarbonate ($CHNaO_3$) in ~ 800 ml distilled H_2O .

2. q.s. to 1 liter.

C. Macromineral Solution.

1. Dissolve the following in ~ 800 ml distilled H_2O .

a. 5.7 g Sodium Phosphate (Na_2HPO_4) (dibasic)

b. 6.2 g Potassium Phosphate (K_2HPO_4) (dibasic)

c. 0.6 g Magnesium Sulfate ($MgSO_4 \cdot 7H_2O$)

2. q.s. to 1 liter

D. Triton X-100 – 1% (V/V).

E. Protease Enzyme Solution (Sigma # P-5147, 5.2 units/mg solid).

1. Add 2.5 g protease to ~ 800 ml distilled H_2O .

2. Stir gently to dissolve.

3. q.s. to 1 liter.

F. Pepsin – HCl solution. (Sigma # P 7000, Activity: 1:10,00).

1. Add 33 ml conc HCl to ~ 800 ml distilled H_2O .

2. Add 10 g pepsin and stir gently to dissolve.

3. q.s. to 1 liter.

IV. Procedure:

A. Using 100 ml in vitro tubes weigh out sample containing 15 mg N in triplicate.

B. Pre-wet samples at 39°C for 2 hours with 10 ml pre-warmed 1:1 rumen buffer: macromineral solution. Add 1 ml 15% N_3Na and 0.5 ml 1% Triton X-100 to each tube.

C. After pre-wetting, add 10 ml of a pre-warmed protease enzyme solution. Incubate for 8 hours.

D. After 8 hours incubation, slowly add 10 ml pepsin-HCl solution. You will get foaming.

E. Incubate additional 24 hours at 39°C.

F. Filter through Whatman #541 filter paper. Dry and run nitrogen analysis on them.