**METHODS FOR BINDING RARE EARTHS TO SPECIFIC FEED PARTICLES**
**Updated September 2013**

I. References:

Ellis, W.C. and D.E. Beever. 1984. "Methods for binding rare earths to specific feed particles. In: P.M. Kennedy (Ed.)." Techniques in Particle Size Analysis of Feed and Digesta in Ruminants. pp. 154-165. Canadian Society of Animal Science, occasional publication no. 1, Edmonton, Alberta, Canada.

II. Theory:

A. Based on theoretical expectations and results (Table 1), it appears possible to bind rare earths with sufficient affinity on feedstuff particles to resist displacement by the highest concentration of $H^+$ found within the ruminant's gastrointestinal tract.
   1. Further work is required to extend these approaches to other feeds.
   2. Improved criteria are needed for measuring and expressing binding together with measurement of factors affecting disassociation.
   3. The effect of actively growing microbes and abomasal/duodenal secretions need to be considered as additional possibilities of acting as competitive or exchange ligands.

B. Binding procedures recommended at this time to minimize proton-induced disassociation are summarized in Section IV.

C. The use of rare earth-acetate (alternative 1) has the advantage of achieving relatively high levels of rare earth binding (mg/g DM) to the higher affinity binding sites.
   1. This level is less than would occur if inorganic salts were used.
   2. Subsequent washing with 0.1 M acetic acid ($C_2H_4O_2$) has the added advantage of further removal of rare earths from the weaker spectrum of binding sites.
   3. This results from the combined effects of acetate acting as a ligand and the elevated concentrations of $H^+$.
   4. This alternative has been used for pulse dose techniques where rare earths were bound to esophageal masticate.
   5. Due to the limited size of DM dose feasible by oral dosing, high concentrations of rare earth per unit dry matter are required to provide good analytical detection in the feces over 3_5 turnover times.

D. The use of EDTA allows binding at pH 7 since this ligand's high Ka prevents formation of rare earth-hydroxide.
   1. This alternative is suggested when protection of acid liable entities of the feedstuff is a high priority to the research objective.
   2. Based on the present results, this alternative is also suggested when experimental objectives require strict indelibility throughout the gastrointestinal tract for specific residues.

**Table 1. Transfer (T) vs. competitive (C) vs. removal (R) soluble ligands**

| Ligand Initially Bound | | Ligand Ac. | Asp. | Cit. | NTA | EDTA |
|---|---|---|---|---|---|---|
| | | ----------mg Yb/g CWCi[a] ---------- | | | | |
| | T | 50.2 | 49.7 | 36.2 | 35.7 | 21.8 |
| | C | 44.9 | 41.0 | 48.2 | 49.8 | 17.9 |
| | R | 37.8 | 32.8 | 27.3 | 14.2 | 16.1 |
| After pH 1.5 | | ------(Yb/g CWCr[b] Ubg CWCi) x 100------ | | | | |
| | T | 61.8 | 68.8 | 71.9 | 46.6 | 86.7 |
| | C | 67.7 | 50.1 | 76.7 | 78.8 | 86.6 |
| | R | 62.6 | 71.8 | 94.3 | 62.4 | 92.7 |

[a] Weight of CWCi initially subjected to Yb.

[b] Weight of CWCr after subjecting to pH 1.5.

III. Personal Protective Equipment:

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

IV. Reagent:

A. 1 M Phosphate Buffer pH 7

1. 0.1 M solution of monobasic sodium phosphate ($NaH_2PO_4$) (13.9 g/1000 ml DDW)
2. 0.1 M solution of dibasic sodium phosphate (26.825 g $Na_2HPO_4 \cdot 7H_2O$ or 35.85 g $Na_2HPO_4 \cdot 12H_2O$ in 1000 ml DDW)
3. Mix 39.0 ml solution A and 61.0 ml solution B, dilute to 200 ml

IV. Recommended Binding Procedures:

A. Preparation of cell wall constituents

1. Reflux with pH 7, 0.1 M phosphate buffer with 3% (w/v) sodium lauryl sulfate ($C_{12}H_{25}NaO_4S$) and thoroughly wash to remove residual phosphate which can form insoluble salts with rare earths

B. Binding

1. Alternative 1: Rare earth acetate = transfer ligand
   a. 50+ mg of rare earth as acetate/g DM in 5-10 ml of 0.01 M acetic acid (pH = 3.4)
   b. Soak 24-48 hr
   c. Soak for 2-6 hr in 10 ml of 0.1 M acetic acid/g DM and then rinse with water
2. Alternative 2: Rare earth EDTA = transfer ligand
   a. 5 ml/g DM of 0.06 M $YbAc_3$ in 0.1 M HAc_$NH_4OH$, pH 7 buffer plus 5 ml/g DM of 0.06 M $Na_3HEDTA$, pH 7
   b. Soak 24-48 hr
   c. Wash by soaking 2-6 hr. with 0.03 M $Na_3HEDTA$, pH 7 and rinse with

water

3. Compute dose of rare earth required based on anticipated digesta dilutions and analytical detection levels
4. Compute dose of rare earth bound DM required based on measured or conservative estimates of rare earth bound to DM