SIMULTANEOUS EXTRACTION OF Yb, Dy AND Co FROM FECES WITH DCTA, DTPA OR EDTA

A. R. Karimi[1], F. N. Owens[2] and G. W. Horn[3]

Story in Brief

Markers are frequently fed to animals to determine digestibility. Analyses of these markers has been difficult. A new extraction procedure was developed to simplify analysis. Three orally administered digesta markers, ytterbium, dysprosium and cobalt were extracted from dried feces with three chelating agents (EDTA, DTPA and DCTA), filtered and measured by atomic absorption spectrophotometry. Presence of a low level of unlabeled fecal ash increased spectral response but higher amounts suppressed the signal. Extraction was complete within 30 min. DTPA extraction yielded higher absorption, less interference, higher recovery of elements and more stable values than extraction with the other two chelating agents while EDTA gave higher recovery than DCTA. Extraction was maximized at the highest (.1M) concentration of chelating agents tested. For DPTA, extraction was most complete at a final pH near 6.3 versus 5.5 for DCTA. Hence, buffering of the extraction solution is needed for uniform recovery. Compared to the ashing procedure for isolation of these markers, extraction with chelates simplifies analysis of digesta samples without reducing reliability of measurement.

(Key Words: Digesta Markers, Yb, Dy, Co, EDTA, DCTA, DTPA.)

Introduction

Many substances have been added to animal diets as indigestible markers. Common ones include cobalt (Co), chromium (Cr), ytterbium (Yb) and dysprosium (Dy) (Ellis et al., 1982). Due to the number of samples obtained in research trials, analytical methods need to be rapid as well as sensitive and accurate. Extraction of marker from dry digesta avoids the need to ash the samples to be analyzed.

Ethylene-diamine-tetra-acetic acid (EDTA) is a useful agent to extract metal ions from dried or wet samples (Hart and Polan, 1984). Formation of a metal-EDTA complex is pH dependent making EDTA attractive for extraction of markers from a complex media like feces. Hart and Polan (1984) developed a rapid and relatively sensitive method for simultaneous extraction of Yb and Co with EDTA from digesta.

Spectrophotometric measurements of extracted fecal samples are often imprecise due to presence of other minerals or compounds in fecal samples. Presence of one rare earth can quench or enhance absorption of another rare earth and complicate interpretation of spectral data. Certain metal chelating agents such as trans-1,2-diaminocyclohexane-N,N,N, N-tetraacetic acid (DCTA) and diethylene-triaminepentaacetic acid (DTPA) due to their high affinity for lanthanid ions, are more selective and more powerful extracting agents than EDTA.

---

[1]Lab Manager [2]Regents Professor [3]Professor
The authors thank L.P. Varga, Dept of Chemistry for helpful discussion.

was designed to compare EDTA, DCTA and DTPA solutions for extracting Yb, Dy, and Co from dried fecal samples.

Materials and Methods

Apparatus

A Perkin-Elmer 4000 Atomic Absorption Spectrophotometer was used for all measurements. Sample pH was measured with a Beckman Zeromatic pH meter.

Reagents and Materials

Reagent grade chemicals and deionized distilled water were used throughout the experiments. Diethylenetriaminepentaacetic acid (DTPA) and trans-1,2-diaminocyclohexane-N,N,N,N-tetraacetic acid (DCTA) were purchased from Sigma Chemical Company, St. Louis, MO.

Sample Preparation

One Hereford steer was fed a wheat forage diet treated with Yb ($YbCl_3$), Dy ($DyCl_3$) and Co (CoEDTA) solution. Fecal samples were composited to form one large sample which was ground in a Wiley Mill through a 2 mm screen to use for all analyses. Another large fecal sample not containing mineral markers was obtained from the same steer as a blank and to test for interference.

Procedures

The extraction procedure developed by Hart and Polan (1984) for EDTA was employed. Solutions with KCl (.5 g per liter) were prepared with EDTA at .01 and .1 molar. The pH was adjusted to 6.5 prior to use.

Standard Solutions

Two-hundred mg of the fecal sample from the steer not fed markers was added to 100 ml of standard mineral solutions to correct for effects of the fecal matrix on atomic absorption of the samples. To determine the percent of each element recovered by extraction, known amounts of Yb and Dy were added to 200 mg of dried ground feces containing neither Yb nor Dy in 100 ml beakers. These samples were dried and extracted with chelating agents.

DTPA Extraction

Extraction solutions (20 ml) contained KCl (.5 g per liter) plus concentrations of .01, .02, .05 or .1 molar DTPA. Subsamples of each were then adjusted to pH 4, 5, 6, 6.5 and 7 by addition of ammonium hydroxide. Each of these 9 solutions was added to 200 mg of feces samples in a 50 ml screw cap tube. Solutions were shaken on a wrist action shaker at a rate of 200 strokes/min for 30 min. Tubes were analyzed following filtration through Whatman filter paper #4 or centrifugation at 5000 RPM for 15 min. Ytterbium and Dy concentrations were determined by atomic absorption at 398.8 and 421.2 nm, respectively, in a red nitrous oxide flame.

DCTA Extraction

Extraction solutions contained KCl (.5 g per liter) plus DCTA concentrations of .01, .025 or .1 molar. Again, subsamples were adjusted to pH 4, 5, 6 and 7 and extraction and measurement procedures were the same as for DTPA.

Results and Discussion

Addition of 200 mg of fecal ash to samples containing Yb and Dy increased the signal whereas addition of 400 and 800 mg of fecal ash decreased the signal as noted previously by Hart and Polan (1984). This decrease in the absorption signal is presumably due to interference by other minerals in the fecal matrix. Hence, all subsequent fecal samples being extracted were weighed precisely at 200 mg and were extracted with 20 ml of chelating agent solution. To counter fecal matrix problems, fecal dry matter was added to all standards as well.

Shaking Time

To determine the optimum time for extraction, shaking durations of 30, 60, 90 and 120 min were tested. Increasing the shaking time beyond 30 min did not increase extraction or recovery of these elements by DCTA and DTPA (Figures 1 and 2). This indicates equilibrium had been reached within this time period. Equilibration of Yb between intra-

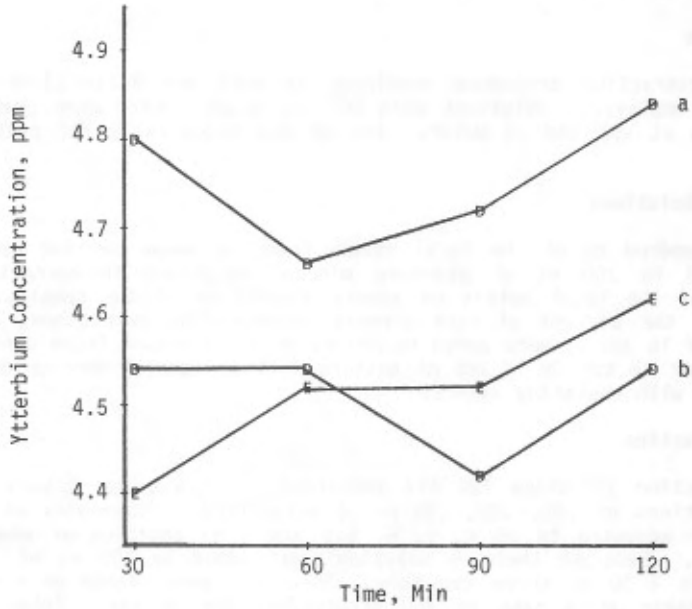

Figure 1. Effect of shaking time on Yb-extraction. a) .1M DTPA, b) .1M DCTA, c) .1M EDTA.

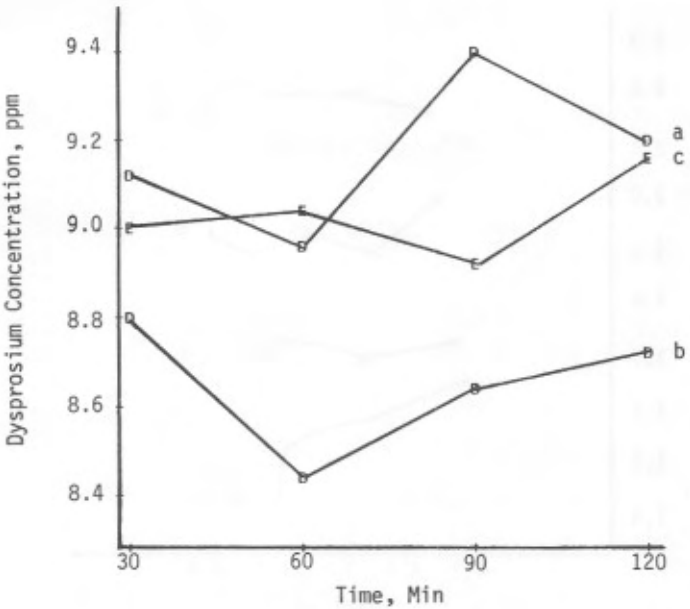

Figure 2. Effect of shaking time on Dy extraction.
a) .1M DTPA, b) .1M DCTA, c) .1M EDTA.

and extra-cellular fluids in feeds was measured previously by perturbation kinetics to be about 15 min by Teeter et al. (1981). For Yb-EDTA, times exceeding 30 min tended to increase Yb recovery.

Highest recovery among these chelating agents was attained by DPTA extraction (95% for Yb and 92% for Dy). Due to its higher formation (K_f) constants and higher affinity for lanthanids (Moeller, 1970), one might expect greater recovery by extraction with DCTA than with EDTA. However, EDTA resulted in higher ($P<.01$) recovery than DCTA for both Yb (92 vs 89%) and Dy (90 vs 87%). This is presumably due to the presence of organic and inorganic compounds in fecal samples which either reduce the capacity of DCTA to complex with Yb and Dy or interfere with measurement of extracted Yb and Dy.

After samples had been shaken, they were filtered or centrifuged prior to reading. Filtration or centrifugation did not reduce the amount of metal extracted by each chelating agent. Some method of sample cleanup was necessary before reading in order to prevent aspiration of floating particles into the nebulizer of the atomic absorption spectrophotometer which can clog the uptake tube. Compared to centrifugation, filtration requires more time and glassware and increases the possibility of contamination and interference. Hence, centrifugation following extraction was preferable.

DTPA Extraction

Effect of pH and concentration on extraction of Yb is shown in Figure 3. Higher concentrations of DTPA increased extraction of Yb

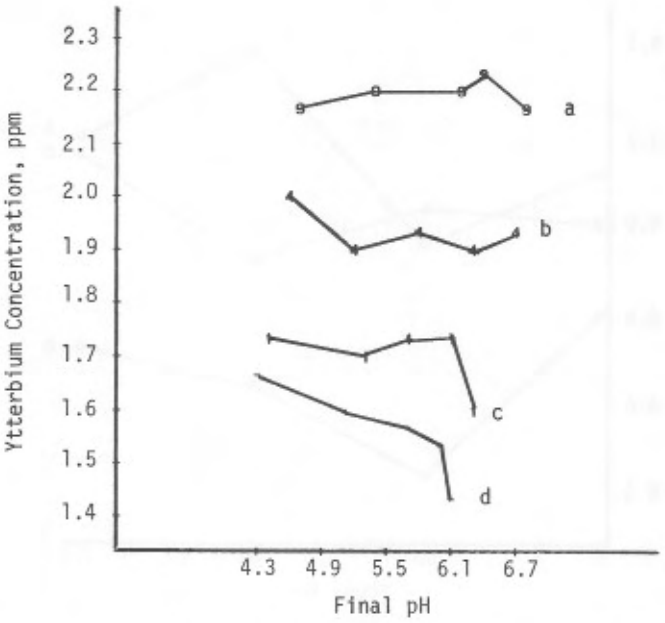

Figure 3. Effect of pH on Yb-DTPA extraction at various DTPA concentrations.
a) .01M, b) .05M, c) .02M, d) .01M.

(P<.01). Extraction plateaued (P<.01) at an initial pH of 6.5 or final pH of 6.4. Final pH of the Yb-DTPA solution dictates both the completeness of extraction and stability of the complex in the media.

For Dy extraction, similar effects of DTPA concentration (P<.01) and extraction pH (P<.04) were detected. The best combinations of pH and concentration for Yb and Dy extraction from fecal sample were pH 6.5 and .1M DTPA solution (Figure 4). Since fecal samples have a tendency to buffer the solution toward pH 5, it appears best to adjust the DTPA extraction solution to an initial pH of 6.5 so that the final pH is near 6.3.

Cobalt also was extracted more completely with DTPA at higher concentrations (P<.01) and at pH 6.5 (Figure 5) though a second extraction peak occurred at pH 5.

DCTA Extraction

Effects of pH and concentration of DCTA on extraction of Yb, Dy and Co from feces are shown in Figures 6, 7 and 8. Extraction of Yb and Dy again were maximum at .1M DCTA concentration (P<.01) for Yb and .15 for Dy). But the optimal pH was lower, with greater extraction at an initial pH of 5 which resulted in a final pH of 5.5. Extraction pH had more impact on Dy (P<.01) than on Yb (P=.27) recovery. Final rather than initial pH of the complexed mixture dictates recovery of the elements by chelation. DCTA produced a lower recovery of both Yb and Dy than DPTA and EDTA which makes it less attractive for extraction.

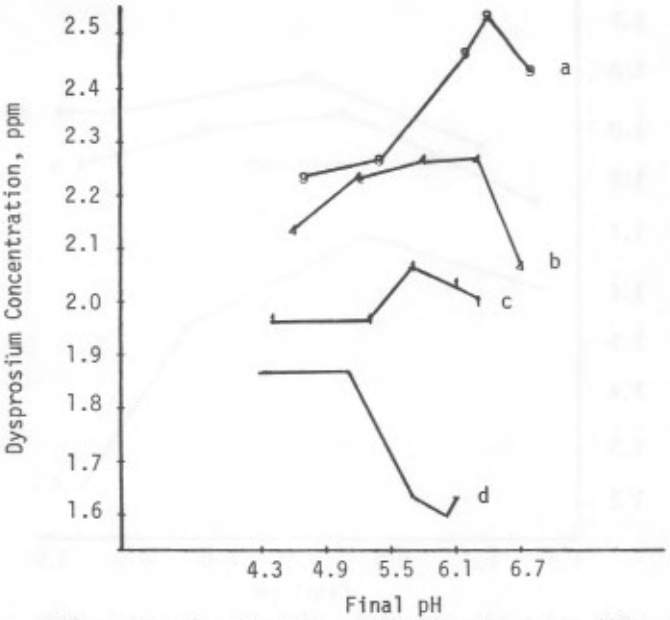

Figure 4. Effect of pH on Dy-DTPA extraction at various DTPA concentrations.
a) .1M, b) .05M, c) .02M, d) .01M.

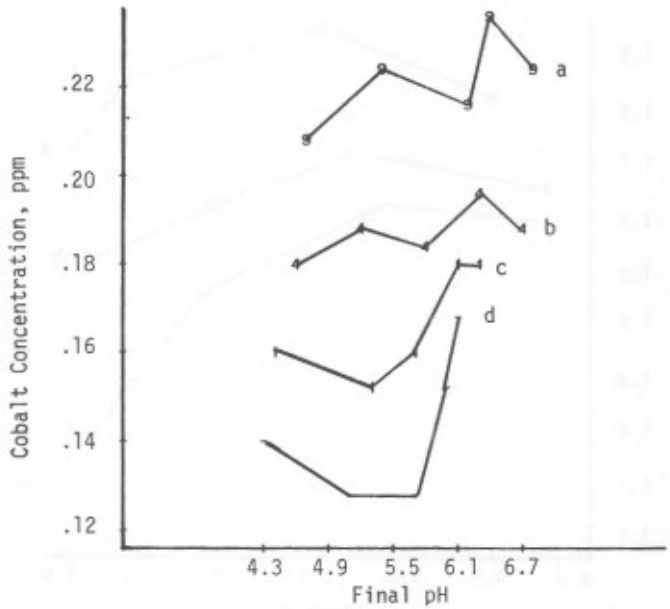

Figure 5. Effect of pH on Co-DTPA extraction at various DTPA concentrations.
a) .1M, b) .05M, c) .02M, d) .01M.

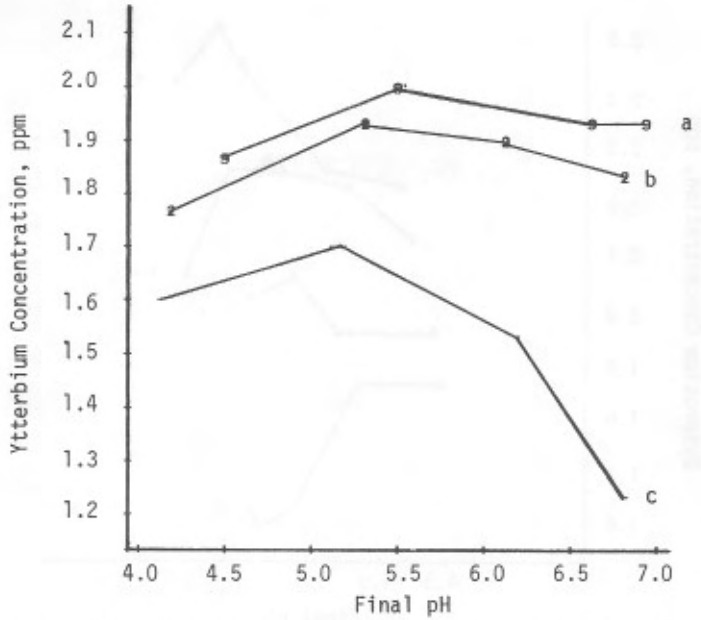

Figure 6. Effect of pH on Yb-DCTA extraction at various DCTA concentrations.
a) .1M, b) .025M c) .01M.

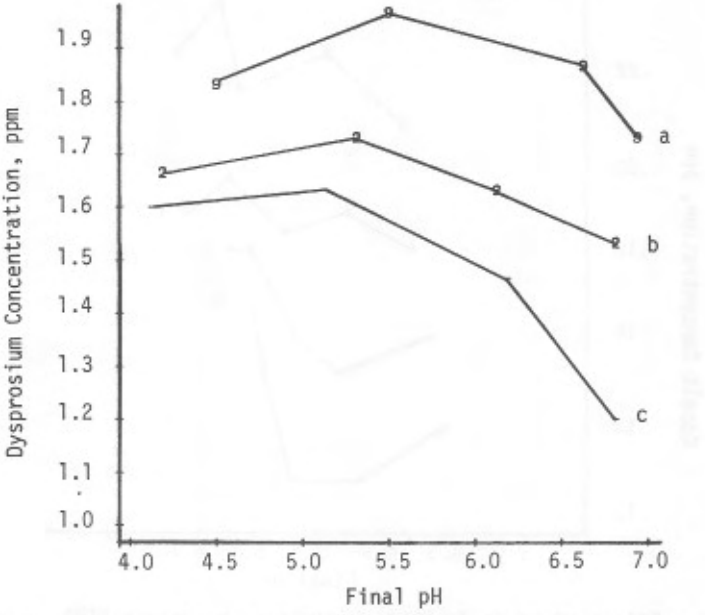

Figure 7. Effect of pH on Dy-DCTA extraction at various DCTA concentrations.
a) .1M, b) .025M c) .01M.

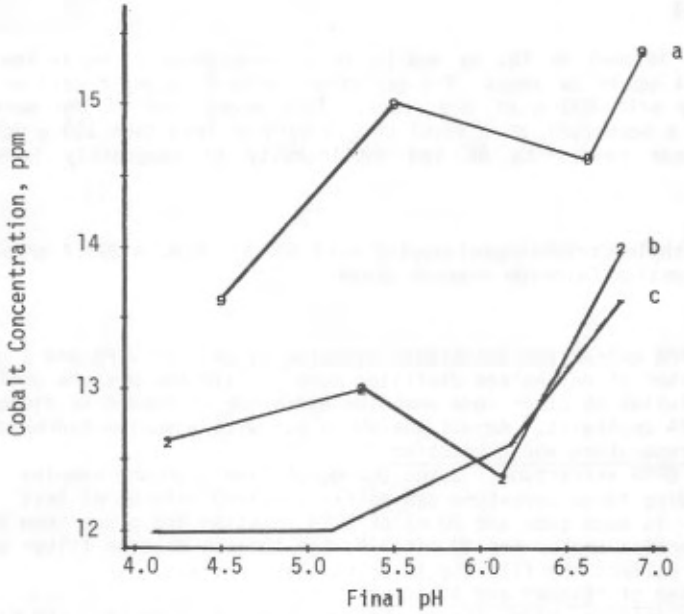

Figure 8. Effect of pH on Co-DCTA extraction at various DCTA concentrations.
a) .1M, b) .025M c) .01M.

Extraction of Co with DCTA was not consistently altered by DCTA concentration (P=.99) or pH of extractant (P<.04). Bizarre behavior of the Co-DCTA complex may be due to several factors such as DCTA competition with EDTA from the CoEDTA dosed for Co and interference by other elements present in the fecal sample.

EDTA Extraction

The .1 M EDTA concentration extracted more of the Yb, Dy and Co (P<.01) from the sample than .01M EDTA. Extraction was conducted at an initial pH of 6.5 based on findings of Hart and Polan (1984).

Adopted Method

Extractants and extracting conditions have not been tested with other mineral quantitation methods for markers such as fluorometry, neutron activation analysis and X-ray diffraction. Among these three chelates, currently, cost is least for DTPA. At .1M, DTPA extraction of DY, Yb and Co for 30 min proved repeatable and reasonably complete.

As a result of this experiment we have adopted the following procedure for extraction of Yb, Dy and Co in feces, duodenal and ileal samples:

Dose Level

Daily intakes of Yb, Dy and Co to produce about 2 ppm in the final extractant would be about .8 g per steer (with 8 lb dry feces) or .08 g per sheep with 800 g of dry feces. This means that if the marker is bound to a feedstuff at a level of 7.3 mg/g of feed then 110 g would be the minimum needed to be fed continuously to adequately label the feces.

Chemicals

1. Diethylenetriaminepentaacetic Acid (DTPA) M.W. = 393.4 g/mol
2. Potassium Chloride reagent grade

Reagents

.1 M DTPA extraction solution: Dissolve 39.34 g of DTPA and 1 g KCl in 1 liter of deioinized distilled water. Stir the mixture until the solution is clear (add ammonium hydroxide if needed to dissolve the DTPA crystals). Adjust the pH to 6.5 with ammonium hydroxide.

Sample Preparation and Extraction

Direct DTPA extraction: Weigh 200 mg of finely ground samples (including three zero-time ash matrix samples) into 50 ml test tubes. To each tube add 20 ml of DTPA solution and place them on wrist action shaker for 30 min. Filter through Whatman filter paper #4 and collect the filtrate in scintillation vials.

Preparation of "Blank" and Standards

Blank solution: Place one of the zero-time filtrate in a 100 ml vol. flask and make up to 100 ml with DTPA solution.

Standard Solutions: Place the two remaining zero-time filtrate in two ml vol flask. To the first flask add .5 ml of reference Yb standard (1000 ppm) and make up to 100 ml with DTPA solution to form a 5 ppm solution. Use this one for checking sensitivity of atomic absorption spectrophotometer. To the second flask add 1 ml of reference Yb standard and make up to 100 ml with DTPA solution. Use this 10 ppm standard to set the calibration on the spectrophotometer. Follow the same procedure for Dy and Co standard solutions.

Read the concentration of your samples against the above standards on atomic absorption spectrophotometer.

Literature Cited

Ellis, W. C., and J. E. Huston. 1968. ^{144}Ce-^{144}Pr as a particulate digesta flow marker in ruminants. J. Nutr. 95:67.

Greweling, T. 1961. Flame photometry of calcium and magnesium in ashed plant material; Resolution of anion interferences with ethylenediaminetetraacetate. Chemist Analyst 50:40.

Hart, S. P., and C. E. Polan. 1984. Simultaneous extraction and determination of ytterbium and cobalt ethylenediaminetetraacetate complex in feces. J. Dairy Sci. 67:888.

Moeller, T. 1970. Periodicity and the lanthanides and actinides. J. Chem. Educ. 47:417.

Teeter, R. G. et al. 1981. Ytterbium chloride as a particulate phase marker for ruminant animals. J. Anim. Sci. 53(Suppl. 5):436.