Methods of ytterbium analysis for predicting fecal output and flow rate constants in cattle

K.P. COFFEY, E.E. PICKETT, J.A. PATERSON, C.W. HUNT, AND S.J. MILLER

Abstract

Continuous or pulse doses of Yb-labeled feedstuffs with subsequent fecal sampling can be used to estimate digesta passage rates and fecal output in ruminants. However, the validity of such estimates is affected by mineral elements in fecal samples that interfere with atomic absorption analysis of Yb. A procedure was developed involving co-precipitation (CoP) of Yb with lanthanum (La) oxalate at pH 1.0 to separate Yb from interfering elements present in the fecal matrix. The procedure was tested for accuracy of Yb determination, repeatability, and for validity of predicting fecal output. Repetitive analysis of the same sample resulted in a coefficient of variation of 2.2% for the CoP technique. An experiment using 12 mature Angus cows offered 1 of 4 diets tested the accuracy of predicting fecal output using one- and two-compartment models. Cows were pulse-dosed with Yb-marked orchardgrass neutral detergent fiber, and fecal samples were collected from the rectum at 9, 12, 15, 18, 24, 32, 40, 48, 60, 72, 84, and 96 h after dosing. Ytterbium content of fecal samples was determined by neutron activation (NA) or atomic absorption spectrophotometry after slow oscillation of the fecal ash for 12 h in 3 M nitric and 3 M hydrochloric acid (acid leaching; AL) or CoP of Yb with La oxalate. For fecal Yb concentrations fit to the one-compartment model, the k_0 parameter (scaling factor related to initial marker in the age-dependent compartment) was greater ($P<.05$) for CoP than for AL or NA. Likewise, calculated first appearance of marker (Tau) and the age-dependent rate constant (k_1) were greater ($P<.05$) for CoP than for NA. For the two-compartment model, the initial marker concentration estimate (λ_0) was greater ($P<.05$) for CoP than for NA or AL, and Tau was less ($P<.10$) for NA than for CoP. Rate constant estimates (λ_1, λ_2) were not affected by method of analysis. For both models, fill and retention time estimates differed ($P<.05$) between CoP and NA. Fecal output estimated from both models was similar to actual fecal output for CoP, but the one-compartment model estimate of fecal output for AL and NA over-estimated ($P<.05$) actual fecal output. Likewise, the two-compartment model estimate of fecal output for NA was greater ($P<.05$) than actual fecal output. Co-precipitation of Yb with La oxalate appears to be a valid analytical procedure that may yield more accurate estimates of fecal output than other reported procedures.

Key Words: ytterbium analysis, external markers, digestibility, fecal sampling

Accurate determination of particulate passage rate, fecal output and voluntary intake requires accurate measurement of external digesta-marker concentrations. Presently, a number of procedures exist for the solubilization of ytterbium (Yb) from fecal matter before atomic absorption spectrophotometry. Ellis et al. (1982) proposed a procedure in which the Yb is "leached" from ash residue with a mixture of 3 N nitric acid (HNO_3) and 3 N hydrochloric acid (HCl). The use of ethylenediamine-tetraacetic acid (EDTA; Hart and Polan 1984) or diethylenetrinitrilo-pentaacetic acid (DTPA; Firkins et al. 1984) have also been proposed as leaching agents for Yb. Iron, Ca, Mg, Na, Al, K, Ba, and Sr have suppressive effects on the atomic absorption spectrophotometric determination of Yb (Mazzucotelli et al. 1982) that are curvilinearly dependent upon the interfering element to Yb ratio. Solutions of .1 M HCl or .1 M EDTA have been used to extract Ca, Mg, K, Cu, Mn, Zn, Fe, Al, P, B, and Sr from plant tissues (Baker and Greweling 1967). The problem of solubilizing interfering elements can be addressed by preparing standards from fecal samples taken before Yb dosage (Ellis et al. 1982, Firkins et al. 1984, Hart and Polan 1984). This approach, however, assumes solubilization of a constant amount of interfering elements from each fecal sample. The purpose of this study was to develop an atomic absorption spectrophotometric technique for Yb analysis which replaces a matrix of unknown element fluctuation between sampling times with one of consistent and known elemental composition. The new technique was compared to an acid leaching procedure (Ellis et al. 1982) and neutron activation (Gray and Vogt 1974) for determining particulate passage rate constants and predicting fecal output in cattle.

Materials and Methods

Trial 1. Analytical Technique

A procedure involving co-precipitation (CoP) of Yb and lanthanum (La) with oxalic acid was developed and tested in the following manner.

1. Fecal samples were collected, dried at 55° C for 48 hr, allowed to equilibrate to atmospheric moisture, then ground to pass through a 1-mm screen using a Wiley mill.
2. Approximately 1 g air-dry feces was weighed into dry 50-ml narrow mouth, heavy glass plasma containers or 50-ml narrow mouth Kimax erlenmeyer flasks (Kimble 26500).
3. Samples were dried at 100° C for 6 h to determine the sample dry matter then ashed at 500° C for approximately 10–12 h to ensure complete ashing of the sample in the narrow-mouth containers.
4. The ash residue was boiled in 5 ml concentrated HCl for 15 minutes with occasional swirling, then transferred to 50-ml volumetric flasks, diluted to volume with deionzied water and mixed thoroughly. The solution was allowed to stand for a minimum of 6 h to allow undissolved ash residue to settle.
5. A 25-ml aliquot was removed from the upper portion of each flask and placed in a 50-ml graduated screw-cap centrifuge tube (38 × 114 mm; Corning 25339).
6. One milliliter of La (20,000 ug/ml) was added to the solution and pH adjusted to 1.0 ± .05 using dilute (1:1; v/v) ammonium hydroxide with deionized water or concentrated HCl. The pH electrode was soaked in pH 1 buffer for 1–2 d prior to use and at all times between uses.
7. Two milliliters of a 5% (w/v) aqueous solution of oxalic acid were added to the tube resulting in formation of a cloudy white precipitate. The solution was stirred vigorously with a glass rod.
8. Tubes were placed in a 90° C water bath for 25 min, cooled for 25 min and centrifuged for 20 min at 3,000 rpm.

Authors are assistant professor, Southeast Kansas Branch Experiment Station, P.O. Box 316, Parsons, Kans. 67357; professor-emeritus, Department of Biochemistry, 322 Chemistry Hall, University of Missouri, Columbia 65211; associate professor, Department of Animal Science, University of Missouri, Columbia 65211; assistant professor, Department of Animal Science, University of Idaho, Moscow 83843; and research specialist, Department of Animal Science, University of Missouri, Columbia 65211.

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9. Supernatant fluid was decanted and .5 ml concentrated perchloric acid ($HClO_4$) was added and swirled to dissolve the precipitate. Tube walls were rinsed with approximately 4–5 ml of deionized water.

10. Three drops of 5% (w/v) aqueous potassium permanganate ($KMnO_4$) were added and the tube swirled using a vortex until the purple $KMnO_4$ color disappeared. Tubes were inspected to insure that all the precipitate was in solution. If not, the tubes were vortexed until the precipitate dissolved. The titration procedure was repeated with dropwise additions until the solution was no longer colorless, indicating the oxalic acid had been consumed. At this point, swirling by hand was sufficient.

$$2MnO_4^- + 5H_2C_2O_4 + 6H^+ \leftarrow 2Mn^{2+} + 10CO_2 + 8H_2O$$

11. One drop (.04 ml) of 30% hydrogen peroxide was added to reduce excess MnO_4^- to Mn^{2+} and the solution was swirled. Deionized water was added to dilute the final solution to 25 ml total volume. Because the Yb standard curve was nonlinear above 6 ug/ml, solutions estimated as having greater than 6 ug/ml Yb were diluted to 50 ml with deionized water and compared with standards which were similarly prepared.

12. Ytterbium concentrations of the final solutions were determined by atomic absorption spectrophotometry at a wavelength of 398.8 nm using a nitrous oxide/acetylene flame.

Validation of the CoP procedure was evaluated using fecal samples obtained from cattle offered a range of Yb-free diets (Table 1). Approximately 1 g dried (55° C for 72 hr) feces was ashed, boiled for 15 min in concentrated HCl, and diluted to a final volume of 50 ml. One hundred micrograms of Yb were added to a 25-ml aliquot of the Yb-free solution to provide a final Yb concentration of 4 ug/ml. Ytterbium concentrations were determined using the described CoP technique. Fecal composite samples were obtained by mixing equal quantities of each of the previously used fecal samples. Composite samples were ashed and 100 ug Yb added to the 25-ml aliquot prior to CoP to estimate a coefficient of variation (Snedecor and Cochran 1980) for the procedure. All standards were prepared using the CoP technique without the addition of fecal ash to the original solutions.

Table 1. Absorbance readings of various fecal matrices to which 4 ug/ml Yb were added before extraction of Yb by a La oxalate co-precipitation procedure.

Animal diets (% of each ingredient)	Absorbance units	% of standard
Corn:Corn silage:Soybean meal (70:25:5)	.166	94.9
Corn:Timothy hay (25:75)	.173	98.9
Silage:Soybean meal (95:5)	.180	102.8
Timothy hay	.173	98.9
Corn gluten feed:Timothy hay (30:70)	.177	101.1
Fescue pasture	.179	102.3
Alfalfa haylage	.183	104.7
Alfalfa haylage:Corn (40:60)	.172	98.2
Mean	.175	100.2
Standard error	.0019	1.10
4-ppm Yb standard	.175	100

Trial 2.

Twelve mature Angus cows that were in their last third of gestation were used in a total collection study to evaluate the accuracy of fecal output prediction from Yb data when dietary composition varied. Chopped (10-cm screen) orchardgrass (*Dactylis glomerata* L.) hay was fed ad libitum and was either offered as the only dietary source or was supplemented once daily with isocaloric and isonitrogenous supplements consisting of either 1,360 g chopped alfalfa (*Medicago sativa* L.) hay, 419 g soybean meal plus 502 g cracked corn, or 60 g urea plus 1,315 g liquid cane molasses. Cows were grouped by age and body weight and randomly assigned to 1 of 4 dietary treatments. A dietary adaptation period of 16 d was followed by 5 d of total feces and urine collection. Feces were collected daily, weighed, mixed, subsampled and dried at 55° C to determine fecal output. At 1800 hr of the first day of total collection, cows were pulse-dosed with 200 g (air dry basis) of Yb-marked orchardgrass hay which had previously been extracted with neutral detergent fiber solution (Goering and Van Soest 1970) without EDTA. Hay was marked according to the immersion technique of Teeter et al. (1984). Fecal samples were collected from the rectum at 9, 12, 15, 18, 24, 32, 40, 48, 60, 72, 84, and 96 hr after dosing and dried at 55° C. Total fecal output was corrected for the amount of dry feces taken via rectal samples. Ytterbium content was determined by neutron activation (NA; Gray and Vogt 1974), or by atomic absorption spectrophotometry after; (1) acid leaching (AL; Ellis et al. 1982) with standards made from similar fecal matrices, or (2) CoP with standards processed similarly to samples but without addition of fecal ash. Ytterbium content of the ground (1mm) marked forage was determined by the 3 previously described techniques using smaller sample sizes. Marked forage analyzed by the acid leaching technique was compared with standards made from matrices of orchardgrass hay NDF diluted similarly to the marked forage sample.

Statistical Analyses

Concentrations of Yb in fecal samples determined from each procedure were fitted to one- and two-compartment models proposed by Pond et al. (1982) and further described by Judkins et al. (1987). These models produced rate constants that were used to estimate particulate passage rates, gastrointestinal tract fill, and fecal output. With the exception of fecal output, orthogonal contrasts (Snedecor and Cochran 1980) were used to compare CoP with NA and AL. Orthogonal contrasts were also used to compare fecal output estimated by total collection with that predicted by each model and analytical technique.

Results and Discussion

Analytical Methods

Absorbance readings from a known quantity of Yb added to various fecal matrices and compared with a pure chemical standard prepared according to the CoP technique are shown in Table 1. These data indicate that Yb may be quantitatively recovered from a broad range of fecal matrixes by CoP of Yb with La oxalate and that this procedure may be used to overcome matrix interference. When a known quantity of Yb was added to the same fecal samples following ashing and acid solubilzation but analyzed before the CoP step of the procedure, Yb absorbance readings ranged between 55 and 89% of that of a pure chemical standard (Coffey and Pickett, unpublished data). The CoP procedure is not protected from matrix interference, having in the final solution elements which may either suppress or enchance the absorbance signal of Yb (Mazzucotelli et al. 1982). However, by quantitative addition of reagents and titration with $KMnO_4$, interfering elements should be in similar quantities in each sample. Also, comparisons of pure chemical standards prepared by the CoP technique with pure chemical standards which were not prepared by the CoP technique indicated a 98% recovery of Yb by the CoP technique. Therefore, the matrix remaining after co-precipitation of Yb with La oxalate does not appear to cause absorbance interferences, but preparing samples and standards similarly should standardize the matrix and eliminate any concerns. Precipitation of other elements is prevented by adjusting the pH to $1.0 \pm .05$ preceding oxalic acid addition. Calcium, the most common precipitated element was not precipitated in measurable quantities at a pH below 1.4.

Results of analyses on samples having a similar matrix (Table 2) suggest that acceptable precision and accuracy may be attained by the CoP procedure when samples are compared with similarly prepared standards. The differences in absorbance reading between the standards used in Tables 1 and 2 resulted from daily variation of the atomic absorption spectrophotometer or minor optimization differences because the samples were compared with the same prepared standard. Standard errors of absorbance readings across sample types (Table 1) and within a constant sample type (Table 2) were similar, indicating the variation between samples in Table 1

Table 2. Precision of Absorbance readings from the same fecal matrix to which 4 ug/ml Yb was added before extraction of Yb by a La oxalate co-precipitation procedure.

Sample	Absorbance units	% of standard
1	.178	103.5
2	.170	98.9
3	.168	97.6
4	.176	102.5
5	.172	100.0
6	.175	101.8
Mean	.173	100.7
Standard Error	.0016	.92
Coefficient of variation	2.21%	2.23%
4-ppm Yb standard	.172	100

was more likely due to variation in technique rather than variation in the ability to overcome matrix interferences.

Fecal Output and Passage Rate

Mean values for each parameter derived from both the one- and two-compartment models (Pond et al. 1982) were evaluated by dietary treatment. Although numerical differences were present among dietary treatments, no significant ($P>.10$) diet effects were observed for any of the variables measured. Therefore, data were pooled across diets within each analytical technique. Parameters k_0 (scaling factor related to initial marker in the age dependent compartment), k_1 (age dependent rate constant) and tau (calculated time of first appearance of marker in the feces) derived from the one-compartment model differed ($P<.05$) between CoP and NA. Likewise, k_0 and tau differed ($P<.05$ and $P<.10$, respectively)

Table 3. Parameter estimates from one- and two-compartment analysis of cow fecal Yb excretion data with Yb analysis by 3 analytical techniques.

Item	Technique: Acid leaching	Co-precipitation	Neutron activation	SE[a]
	one-compartment model			
k_0[bc]	29933	33642	26150	1694.2
k_1[c]	.048	.048	.042	.0022
Tau[cd]	18.5	20.1	17.8	.81
	two-compartment model			
λ_0[bc]	887	1156	742	112.9
λ_1	.156	.131	.126	.1062
λ_2	.029	.033	.027	.0043
Tau[e]	16.5	16.1	14.4	.85

[a]Standard error (N=12).
[b]Co-precipitation vs Acid leaching ($P<.05$).
[c]Co-precipitation vs Neutron activation ($P<.05$).
[d]Co-precipitation vs Acid leaching ($P<.10$).
[e]Co-precipitation vs Neutron activation ($P<.10$).
k_0 = concentration of marker in the feces if instantaneously mixed.
k_1 = age dependent rate parameter.
Tau = time delay.
λ_0 = concentration of marker if instanteously mixed in the rumen.
λ_1 = time-dependent turnover rate.
λ_2 = time-independent turnover rate.
Tau = time lag.

between CoP and AL (Table 3). Ellis et al. (1982) reported 98.8% solubilization of Yb from feces by the AL technique. Therefore differences in parameter estimates probably resulted from matrix fluctuations. Ellis et al. (1982) attempted to reduce matrix problems by preparing standards from a zero-hour sample matrix, an approach that assumes a constant matrix at each sampling time. Gray and Vogt (1974) minimized matrix interference by allowing a decaying period to permit deterioration of shorter-lived isotopes. It is possible, however, that these allowances are not completely effective.

The parameter estimate k_0 is used to calculate undigested dry matter fill and fecal output. Therefore, inaccurate values for k_0 result in erroneous values for fill and fecal output. Particulate flow rates are estimated using k_1, from the one-compartment model and therefore may be similar between AL and CoP, depending upon the degree of matrix fluctuation from the zero-hour sample. Tau represents the estimated time, postdosing, at which the marker first appears at detectable levels in the feces and is used to calculate retention time of the marked feedstuff in the intestinal tract.

Lambda$_0$ values derived from the two-compartment model and estimated from Yb concentrations determined by CoP were greater ($P<.05$) than those estimated by AL and NA techniques. Tau values estimated by CoP were similar ($P>.10$) to those estimated by AL but tended to be greater ($P<.10$) than those estimated by NA. Values for λ_1 and λ_2 estimated by CoP were similar ($P>.10$) to those estimated by AL and NA.

Fecal output predictions using parameter estimates derived from both the one- and two-compartment models are shown in Table 4. Orthogonal contrasts were used to compare values estimated by each technique with actual fecal output (avg. 3,781

Table 4. Fecal output, flow rates, fill and retention time in cows as estimated by one- and two-compartment models and 3 techniques for Yb analysis.

Item	Technique: Acid leaching	Co-precipitation	Neutron activation	SE[a]
Actual fecal output, g/day		3781		
	one-compartment model			
Fecal output, g/day	4306[b]	4051	4816[b]	243.4
Flow rate, h^{-1}[c]	.028	.029	.025	.0013
Fill, g[c]	6317	5883	8111	361.4
Retention time, h[c]	61.3	61.6	66.0	1.73
	two-compartment model			
Fecal output g/day	4092	3871	4552[b]	254.6
Fill, g[c]	6023	5171	7453	503.7
Retention time, h[c]	66.2	65.1	71.9	2.66

[a]Standard error (N=12).
[b]Values followed by superscript differ ($P<.05$) from total collection (avg. 3781 g/day).
[c]Co-precipitation vs Neutron activation ($P<.05$).

g/day). When one-compartment values are considered, fecal output values estimated by CoP were similar statistically ($P = .27$) to total collection but overestimated by 7%. Fecal outputs estimated by AL overestimated ($P<.05$) actual fecal output by 13.9% while NA over-estimated ($P<.05$) fecal output by 27.4%.

Two-compartment model values appeared to more closely estimate actual fecal output. Fecal output values estimated by CoP and AL were similar ($P = .73$ and .23, respectively) to total collection, over-estimating actual fecal output by 2.4 and 8.2% respectively. Neutron activation values were 20.4% greater ($P<.05$) than total collection estimates of fecal output. Hunt et al. (1984) and Mader et al. (1984) have reported acceptable agreements between actual fecal output and fecal output estimated using Yb concentrations from a pulse dose. However, Mader et al. (1984) corrected for matrix fluctuations by use of the standard additions technique (Beukelman and Lord 1960). Other workers have not been able to repeat these results (Paterson, J.A., R.R. Worley and W. Martin, unpublished data) with grazing animals or have otherwise obtained

questionable results from Yb generated data (Turner, K.E., J.A. Paterson, C.S. Saul, unpublished data; W.G. Bergen, personal communication). These discrepancies possibly result from a greater degree of fluctuation in the fecal mineral content than was present in the study by Hunt et al. (1984).

One-compartment model estimates of passage rate, as well as one- and two-compartment model estimates of undigested dry matter fill and retention time estimated by CoP differed ($P<.05$) from those estimated by NA. All values for flow, fill and retention time were similar ($P>.10$) between CoP and AL.

Co-precipitation of Yb with La oxalate appears to be a viable technique for determination of Yb concentration in fecal samples. The method provides a means of standardizing the sample matrix as well as reducing the number of and quantity of interfering elements. Others have proposed to minimize matrix interferences by using dilute solutions of solubilizing agent and preparing standards from matrices of similar composition. The AL procedure specifies dilute acids and slow solubilization of Yb to decrease extraction of undesirable salts (Ellis et al. 1982). However, Baker and Greweling (1967) used .1 M HCl to extract many of the interfering elements from plant material. Hart and Polan (1984) attempted to minimize the fecal matrix by extracting with dilute (.05 M) EDTA and by using a small sample size (200 mg). Furthermore, atomic emission has been used to attain greater sensitivity (Kniseley et al. 1969) than atomic absorption. However, the increased sensitivity would only be expected from a flame emission spectrophotometer with specifications similar to that indicated by Kniseley et al. (1969). It is suspected that interfering elements are solubilzed by dilute EDTA (.05M) and that the solubilization of these elements may fluctuate, resulting in matrix differences.

The effect of the presence of foreign elements on the atomic absorption spectrophotometric characteristics of Yb are vastly different from those effects on other elements in the Lanthanum series (Mazzucotelli et al. 1982). These authors concluded that only a few of the differing effects could be explained by usual spectral considerations such as spectral buffering or other general optical causes. Although a number of elements interfere with the atomic absorption signal of Yb (Mazzucotelli et al. 1982), it is presently unknown how the elements interact when combined in a solution such as a fecal matrix. In one example, single element additions of 5,000 ug/ml La or potassium (K) enhanced the atomic absorption signal of a 4-ug/ml Yb solution (Coffey and Pickett, unpublished data). These results are expected in light of the data by Mazzucotelli et al. (1982). However, when a mixture of 5,000 ug/ml La plus 5,000 ug/ml K was added to a 4-ug/ml Yb solution, the atomic absorption signal was highly suppressed, indicating undesirable and unpredictable effects of mixed solutions on the absorption reading of Yb. Therefore, to correct for matrix interference, Yb shall be carefully precipitated from the fecal matrix unless the technique of standard addition is used.

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