Methods of administering ytterbium for estimation of fecal output

PATRICK G. HATFIELD, DONALD C. CLANTON, DAVID W. SANSON, AND KENT M. ESKRIDGE

Abstract

Three experiments were conducted with grazing and penned animals to evaluate the accuracy and precision of different methods of administering ytterbium (Yb) as a marker to estimate fecal output. A paired *t* test was used to evaluate differences between marker estimates of fecal output and fecal output measured by total collection. Marker administration methods within an experiment were compared in a one-way analysis of variance using the absolute percentage deviation of the marker estimates from total collection. In Experiment 1, wethers were confined in metabolism crates and orally administered either a pulse dose or a once daily dose. Both methods overestimated fecal output (7.3% for the once daily and 11.2% for the pulse dose method); however, the once daily dose was more precise (SE = .78%) than the pulse dose (SE = 2.17%). In Experiment 2, a crossover design with steers grazing dormant bromegrass pasture was employed to compare estimates of fecal output by pulse dosing and once daily dosing of Yb via rumen cannula. Although both methods underestimated fecal output (4.0% for the once daily and 11.5% for the pulse dose), once daily dosing was more precise (SE = 2.36%) than pulse dosing (SE = 3.64%). In Experiment 3, an intraruminal constant release Yb bolus was compared with daily feeding of Yb-labeled supplement using grazing and pen fed steers. The constant release bolus overestimated fecal output 13.8% on pasture and 41.3% in the pen study. Standard errors for the constant release bolus were 4.02% (pasture) and 4.76% (pen). Although use of Yb in a labeled supplement did not provide an accurate estimate of fecal output (80.1 and 106.0% of total collection on pasture and in pen, respectively), it had a lower SE than the constant release bolus method during both the pasture and pen studies. Once daily dosing was more precise than a pulse dose for estimating fecal output in Experiments 1 and 2. The constant release bolus used in Experiment 3 does not appear to be suitable for estimating fecal output of grazing cattle.

Key Words: ytterbium, indicators, fecal output, grazing animals.

Forage intake of grazing ruminants has often been determined by dividing total fecal output by an estimate of forage indigestibility. This technique is expensive, labor intensive, and may alter normal grazing behavior (Corbett 1960, Cordova et al. 1978). However, Galyean et al. (1986) suggested that total collections may still be the best method of estimating fecal output. Pulse dosing a rare earth element is another method of estimating fecal excretion (Ellis et al. 1982). Daily dosing of inert markers is also commonly used to estimate fecal output (Galyean et al. 1986). Galyean et al. (1986) reviewed a number of studies in which fecal output was estimated for grazing and pen fed animals. Results of these studies indicated a greater degree of variability when markers were used with grazing animals compared with pen fed animals.

At the time this article was written the authors were with the West Central Research and Extension Center, University of Nebraska, North Platte 69101. Senior author's current address is U.S. Sheep Experiment Station, HC 62, Box 2010, Dubois, Idaho 83423; Eskridge is with the Biometrics Center, University of Nebraska, Lincoln 68583-0712.

Journal paper 8808, Univ. of Nebraska Agr. Res. Div. contributing to Regional Res. Proj. W-151.

Manuscript accepted 13 November 1989.

The use of rare earth markers in grazing studies raises an important question: how does the method of marker administration and the associated analytical approaches affect fecal output estimates? To answer this question, various methods of administering ytterbium (Yb) were investigated in 3 separate experiments. The 2 hypotheses for the 3 experiments were: (1) differences would exist between total fecal output and marker estimates of fecal output, and (2) accuracy of marker estimated fecal output would differ within an experiment depending on method of administration.

Methods

Experiment 1

Ten crossbred wethers (avg wt 39.5 kg) were used in a completely random design to test methods of estimating fecal output. The wethers were fitted with fecal collection bags and confined in metabolism crates. Five wethers were assigned to either a pulse dose (P) or a daily dose (C) of Yb-labeled forage.

During the first 8 days of the trial, sheep were individually fed coarse, chopped bromegrass hay (Table 1) at 0730 hour in amounts sufficient to allow ad libitum consumption. Hay was chopped in a tub grinder to approximately 2 cm lengths. Orts were collected and weighed each day. During the remainder of the experiment, bromegrass hay was limited to 85% of ad libitum intake. Hay was fed in approximately 2 equal size meals at 0730 and 1930 hour. A portion of the hay fed during the pre-trial period was labeled with ytterbium chloride ($YbCl_3 \cdot 6\ H_2O$) using a modified procedure based on that described by Teeter et al. (1984) and McCollum and Galyean (1985). Before labeling, forage was boiled in a sodium laurel sulfate solution to remove cell solubles, thus only the neutral detergent fiber fraction of the hay was labeled, which ensured a greater concentration of Yb per gram of labeled feed. Fiber was labeled by soaking 50 g of air dry fiber in 1 liter of distilled water

Table 1. Chemical composition of hay, pasture forage, and supplement used in Experiments 1, 2, and 3.

	CP[a]	NDF[b]	IVDMD[c]	OM[d]
Experiment 1				
Hay[e]	7.6	71.2	50.5	89.8
Experiment 2				
Pasture forage[f]	25.9	63.5	59.4	81.4
Hay[e]	9.8	65.0	55.1	87.8
Supplement[e]	23.9	39.4	84.9	91.9
Experiment 3				
Pasture forage[f]	22.7	66.4	63.4	84.0
Hay[e]	7.2	72.5	60.1	89.0
Unlabeled supplement[e]	23.9	39.4	84.9	91.9
Labeled supplement[e]	22.1	38.1	84.0	92.4

[a]CP = crude protein
[b]NDF = neutral detergent fiber
[c]IVDMD = in vitro dry matter digestibility.
[d]OM = organic matter
[e]Dry matter basis.
[f]Samples were collected using esophageally fistulated steers. Values are expressed on an organic matter basis.

Table 2. Marker estimates of fecal output compared with total fecal collections (Experiments 1 and 2).

Experiment	Location	Method[a]	Fecal output g dry matter/day: Total collection	Fecal output g dry matter/day: Marker estimate	Mean difference g dry matter/day	SE[b]	P-value[c]
1	Metabolism Crates	C	670	719	48.8	3.8	.00
1	Metabolism Crates	P	657	733	76.1	19.4	.02
2	Pasture	CR	3844	3773	71.5	103.0	.54
2	Pasture	PR	3871	3785	85.4	283.2	.78
3	Pasture	CRB	3512	3941	429.2	266.6	.21
3	Pasture	YLS	4004	3241	762.9	108.5	.01
3	Pen	CRB	3606	5043	1436.8	424.5	.04
3	Pen	YLS	4018	4255	237.7	148.2	.21

[a]C = daily oral dosing.
P = oral pulse dose.
CR = daily dosing via rumen cannula.
PR = pulse dose via rumen cannula.
CRB = intraruminal Yb constant release bolus.
YLS = Yb labeled supplement.
[b]Standard error associated with mean difference between total collection and marker estimated fecal output.
[c]P-value for hypothesis of no difference.

containing 2.5 g $YbCl_3 \cdot 6\ H_2O$. After soaking for 48 hours, the fiber was rinsed 6 times in distilled water to remove unbound Yb and dried in a 55° C oven for 48 hours.

Wethers on the C treatment were dosed orally with a gelatin capsule containing 5 g of Yb labeled forage every morning at 0800 hour beginning on day 1 and continuing until the end of the study. On day 7, the wethers receiving the P treatment received a 10 g pulse dose of Yb labeled fiber at 0800 hour. Rectal grab sampling of feces began on day 7 for both groups. Wethers receiving the C treatment were sampled daily at 0700 hour for 7 days. Pulse dose treatment wethers were sampled 0, 4, 8, 12, 20, 24, 28, 32, 36, 42, 48, 54, 60, 72, 84, 96, 108, and 120 hours after dosing. Samples were identified by animal, day and time, and frozen for later analysis.

Fecal bags for all animals were emptied at the same time fecal samples were taken from P wethers. Total collections were composited by animal within day then frozen for later determination of total fecal dry matter. Weight of fecal grab samples was included in the determination of total fecal output.

Labeled forage for both C and P contained 18.4 mg of Yb per gram of forage dry matter. Marker concentrations in the feces for C wethers were determined by averaging the Yb concentration of the 7 daily fecal samples taken during the trial.

Experiment 2

Four ruminally cannulated steers (avg wt 536 kg) were used in a cross-over design to compare daily administration (CR) of Yb-labeled forage via a rumen cannula and a pulse dose (PR) of Yb-labeled forage via a rumen cannula. Steers grazed irrigated bromegrass pasture (Table 1) for 7 days before the beginning of two 14-day periods. On day 1 of period 1, steers were placed on pasture and CR steers began receiving a daily dose of 15 g labeled hay (chopped 2 cm) containing 20.9 mg Yb/g forage dry matter. On day 6, all steers were fitted with bags for total fecal collection. On day 7, PR treatment steers were pulse dosed with 248 g of labeled forage containing 15.8 mg Yb/g forage dry matter. Forage for PR labeling was collected from esophageally fistulated steers grazing the same pasture. By labeling masticate for the PR treatment and chopped bromegrass hay for the CR treatment, source of labeled forage and treatment were intentionally confounded. Ytterbium was bound to the chopped hay to associate the Yb with the solid phase of the digesta. This process should have enhanced marker recovery, whereas masticate was labeled in the PR treatment to ensure similar rate of passage with the ingesta. Labeling procedure was the same as in Experiment 1 except that forage was not boiled in sodium laurel sulfate. Rectal grab sampling of feces began on day 7 and continued at 8-hour intervals (0700, 1500 and 2300 hour) until day 14 for both CR and PR steers.

During the 10 days between periods 1 and 2, all steers were allowed to graze undisturbed and allowed to pass the marker residue from the digestive tract. In the second 14-day period, treatments were crossed over steers. Steers in period 2 were treated in the same manner as they were treated in period 1. However, binding efficiency of Yb for PR forage during period 2 was higher, resulting in 25.8 mg Yb/g forage dry matter. The same Yb-labeled hay used in period 1 for the CR treatment steers was used in period 2 for the CR treatment. In addition, hay was fed for the last 3 days of period 2 because of snow cover limiting forage availability. The method of collecting fecal samples was the same as in period 1.

Experiment 3

Eight crossbred steers (avg wt 438 kg) were used in a split plot design to compare an intraruminal constant release Yb bolus (CRB)[1] and a Yb-labeled supplement (YLS) as methods of estimating fecal output. Steers grazed dormant bromegrass pasture and then were confined individually and fed chopped bromegrass hay.

Before the study, 6 additional boluses were placed in 2 ruminally cannulated steers (3/steer) grazing the same pasture as the previously mentioned steers to estimate Yb release. Boluses were removed, washed, and weighed at 7-day intervals for a 112-day period. Mean 24-hour Yb release for the 112 days was 148 mg. This value was used as the dose amount; however, there was considerable variation in release rate among boluses.

The CRB and YLS steers received 400 g • $head^{-1}$ • d^{-1} of supplement at 0700 hour. The supplement was 25% corn, 25% soybean meal, and 50% alfalfa. Two batches of supplement were mixed and pelleted. The first contained Yb-labeled alfalfa. The alfalfa was labeled in the same manner described in Experiment 1 and resulted in the supplement containing 403 mg Yb/g of supplement dry matter. The second supplement did not contain Yb and was fed to the CRB steers (400 g • $head^{-1}$ • d^{-1}). On day 1, 4 steers were allocated randomly to the CRB treatment and orally administered 1 bolus per steer. Feces were not sampled for 21 days to allow Yb concentration in the digestive tract to stabilize. On day 21, the 4 steers assigned to the YLS treatment began receiving 400 g • $head^{-1}$ • d^{-1} of the Yb-labeled supplement at 0700 hour. On day 27, all

[1]Constant release boluses were developed and provided by Elanco Corp., Indianapolis, Indiana.

steers were fitted with fecal collection bags to determine total fecal output. Fecal samples were collected via rectal palpation at 8-hour intervals (0700, 1500, and 2300 hour) starting on day 28 and continuing for 7 days. Fecal bags were weighed, feces subsampled, and bags changed at the same time grab samples were collected.

At the end of the pasture phase of the study, all animals were placed in individual feeding pens and fed chopped Sandhills meadow hay (Table 1). Hay intake was limited to 90% of the daily intake determined in a 10-day ad libitum feeding period. On day 38, YLS steers began receiving the Yb-labeled supplement. All steers were fitted with fecal collection bags. Total and grab sample fecal collections began on day 44 and continued for 7 days in the same manner as described in the pasture phase of the study.

Laboratory Procedure

Hay and fecal samples were dried at 55° C for 48 hours. Dried samples were ground through a 2-mm screen in a Wiley mill, then through a 1-mm screen in a Udy mill. Hay samples were analyzed for dry matter, ash and Kjeldahl-N (AOAC 1980), neutral detergent fiber (Goering and Van Soest 1970), and in vitro dry matter digestibility (Tilley and Terry 1963).

Ytterbium-labeled forage and fecal samples were prepared for analysis by the DTPA extraction method (Karimi et al. 1986) for Experiments 1 and 2. In Experiment 3, Yb was extracted by solubilizing sample ash with 25% hydrochloric acid (Krysl et al. 1985). After filtration and dilution, the Yb content of the solutions were analyzed by atomic absorption spectroscopy with a nitrous oxide/acetylene flame. Ytterbium standards for fecal and forage analysis (0 to 5 ppm) were made from 0 hour fecal collections that contained no Yb and unlabeled forage, respectively. All samples and standards contained potassium at 2,000 ug/ml as an ionization buffer.

Calculations and Statistical Analysis

Fecal Yb excretion curves were analyzed by nonlinear regression procedures (Marquardt method) of SAS (1985) using a one-compartment model (Krysl et al. 1988): $Y = k_0 (t - \tau) \bullet (k_1)^2 \bullet e^{-k_1(t-\tau)}$; where Y = expected concentration in the feces sampled at time t; k_0 = scaling factor such that when t = 0, $k_0 \times k_1 = C_0$; C_0 = initial concentration of marker within the age-dependent compartment; k_1 = age-dependent rate parameter for a γ_2 distribution of passage rates which increase with age, t, in the age-dependent passage compartment, and τ = time from dose until first appearance of marker in feces. Parameters estimated by fitting the above model to fecal marker concentration were then used to calculate fecal output (g/h), which is defined as marker dose divided by k_0.

In all experiments, the animal was considered the experimental unit. Methods within experiments were compared using the percentage deviation (absolute value) of the estimate from the fecal output determined by total fecal collection: (Marker estimate/Total collection) × 100. When calculated in this manner, no distinction was made between over and underestimations, only that the estimate varied from total collection.

Percentage deviation was then used as the dependent variable in an analysis of variance. Experiment 1 was a one-way analysis of variance. The model used to analyze Experiment 2 included effects for animal, period, and administration method (Federer 1967). Experiment 3 was analyzed as a completely random split-plot design. The model for Experiment 3 included method × location interaction, location, method, animal within method, and animal within method × location. Animal within method was used to test the main effect of method, and animal within method × location was used to test the main effect of location and interaction.

Accuracy of marker estimates within experiment was compared with total collection using a paired *t* test (McClave and Dietrich 1982). The *P*-value for each comparison provides insight into the accuracy of the method (i.e., difference between marker estimates and total collection). Small *P*-values indicate inaccuracy, but not necessarily lack of precision (repeatability of the estimates). Standard errors of the estimates indicate precision of methods.

Results

Experiment 1

The mean difference between total collection and Yb estimates of fecal output for C and P methods was not equal to 0 ($P<0.05$; Table 2). However, the estimates provided by the 2 methods were not different ($P>0.13$; Table 3). The methods overestimated fecal output by 7.3 and 11.2% for C and P, respectively, when compared with total collection (Table 4). The lower SE associated with C indicates C is a more precise estimate than P (Table 4).

Table 3. Comparison of methods within experiment for estimating fecal output expressed as percentage deviation (absolute value) from total fecal collections (Experiments 1, 2, and 3).

Experiment	Location	Method[a]	SE[b]	P-value[c]
1	Metabolism crates	C	1.67	.13
1	Metabolism crates	P		
2	Pasture	CP	1.57	.08
2	Pasture	PR		
3	Pasture	CRB	6.67	.61
3	Pasture	YLS		
3	Pen	CRB	9.48	.04
3	Pen	YLS		

[a]C = daily oral dosing.
P = oral pulse dose.
CR = daily dosing via rumen cannula.
PR = pulse dose via rumen cannula.
CRB = intraruminal Yb constant release bolus.
YLS = Yb labeled supplement.
[b]Standard error associated with mean difference between marker methods
[c]P-value for hypothesis of no difference between marker methods.

Prigge et al. (1981) and Krysl et al. (1985) found no difference between estimates of total fecal output using rare earth marker techniques and total fecal collections. However, estimates of fecal output published by Prigge et al. (1981) indicated a range of variation similar to the variation noted in this study. Krysl et al. (1985) pulse dosed sheep in metabolism crates with Yb-labeled forage via ruminal cannulas and estimated fecal output within 3% of output measured by total collection. Krysl et al. (1985) found that the difference between marker estimates and measured fecal output was not significantly different from 0.

Table 4. Summary of marker estimates of fecal output as a percentage of total fecal collections (Experiments 1, 2, and 3).

Experiment	Location	Method[a]	Marker Estimate	SE[b]
1	Metabolism crates	C	107.3	.78
1	Metabolism crates	P	111.2	2.17
2	Pasture	CR	97.7	2.36
2	Pasture	PR	98.9	3.64
3	Pasture	CRB	113.8	4.02
3	Pasture	YLS	80.1	2.47
3	Pen	CRB	141.3	4.76
3	Pen	YLS	106.00	2.39

[a]C = daily oral dosing.
P = oral pulse dose.
CR = daily dosing via rumen cannula.
PR = pulse dose via rumen cannula.
CRB = intraruminal Yb constant release bolus.
YLS = Yb labeled supplement.
[b]Standard error associated with mean difference between animals on the same treatment.

Experiment 2

The mean difference between total collection and estimated fecal output for CR and PR methods was not different ($P>0.53$) from 0 (Table 2). However, the estimates were different ($P<0.08$) from one another (Table 3). An ideal marker method for estimating fecal output of grazing animals should give reliable results under a variety of conditions common to a grazing situation. The snow storm that occurred at the end of period 2 could have affected marker estimates of fecal output. Although the study was not designed to evaluate how snow cover affects the reliability of marker estimates of fecal output, it is interesting that the standard error associated with the PR treatment is almost 3 times greater than the standard error for the CR treatment (Table 4). Possibly, the changes in weather and diet (pasture forage to hay) affected the rate at which digesta flowed through the tract, which may have more adversely affected PR estimates of fecal output than CR estimates. Christopherson and Kennedy (1983) stated that a cold environment will reduce retention time or markers of the particulate phase of digesta in the reticulo-rumen. Judkins et al. (1984), who used a pulse dose of Yb-labeled forage, and Musimba et al. (1987), who used a once daily dose of Yb labeled forage, found similar variability in estimates of fecal output by grazing animals.

Experiment 3

The interaction of treatment and location was significant ($P<0.05$) in Experiment 3; thus, Tables 3 and 4 show the effects of treatment within location. During the pasture portion of Experiment 3, the SE for the CRB estimate was greater than the SE for the YLS estimate of fecal output (Table 4). The accuracy of the 2 methods was similar ($P>0.60$; Table 3).

When the YLS method was used on individually fed animals in confinement, the difference between marker estimates and total collection was not different from 0 ($P>0.20$; Table 2). The CRB estimate was different ($P<0.05$) from total collection (Table 2). The 2 administration methods were different from one another ($P<0.05$) in their accuracy of estimating fecal output (Table 3).

The YLS method performed better in the pen study than the pasture study. The accuracy and precision of the CRB method was poorer in the pen study than on pasture (Table 4). This may indicate inconsistency of Yb release from the bolus rather than any effect location and feeding behavior had on the method. Preliminary investigation of Yb release from boluses placed in the rumens of 2 ruminally cannulated steers indicated a high degree of variability in Yb release from the boluses. This variation was apparent not only between boluses but also in Yb release from a bolus across time. Ytterbium release from the bolus was dependent on decay of the co-polymer which held the Yb in the bolus. Extreme variation in surface area of the co-polymer-Yb complex which was exposed to the ruminal environment was observed. Changes in surface area and the rate at which the co-polymer decayed may have affected Yb release. If Yb release was not consistent then the quantity of Yb in the feces would vary not only with changes in fecal output but also with changes in Yb release from the bolus.

Discussion

Many of the recommendations by Raleigh et al. (1980) for chromic oxide used in range nutrition are also applicable to rare earth elements. An item which appears particularly important is the suggestion that intake results based on markers should only be used in a comparative fashion and should not be viewed as providing quantitatively correct values.

By knowing how the marker functions and varies under the conditions of the study, precision becomes more important than accuracy because total collection may be used to adjust estimates (Raleigh et al. 1980). On this basis, it would appear that a once daily dose may be a more reliable method of estimating fecal output than a single pulse dose. Galyean et al. (1986) concluded on the basis of their review that dosing twice daily may produce more uniform excretion patterns than dosing once daily. However, Pond et al. (1987) suggested that additional dosing may alter the animal's grazing behavior.

In Experiment 2, rectal grab samples of feces were taken every 8 hours for both the CR and PR steers. Although this collection scheme was probably adequate for the CR treatment (Raleigh et al. 1980) more frequent fecal collections (near the peak of fecal Yb excression) may be more desirable when employing the pulse dose method (Galyean et al. 1986). Taking rectal grab samples at 8-hour intervals on the PR treatment steers may have been inadequate and adversely affected K_0 in the equation for calculating fecal output in the one-compartment model. However, more frequent collections are difficult under typical grazing conditions and may alter animal grazing behavior.

In all of the methods tested in this study, accuracy of the calculated dose was a concern. Inconsistencies in co-polymer decay resulting in irregular Yb release from the intraruminal Yb bolus would definitely cause uncertainty in the dose value used in the fecal output equation. Based on preliminary observations of the boluses in ruminally cannulated steers, irregular co-polymer decay probably caused poor estimates of dose, and hence fecal output, in the CRB treatment.

In general, a major problem with rare-earth labeled feeds is accuracy of dose. Chromic oxide is a purified chemical and thus lends itself to accurate doses. There is always concern, however, with the degree to which an analyzed sample of rare earth labeled feed represents the labeled feed actually dosed. In this study, selected samples of labeled feed were analyzed using 2 different methods of extraction at 2 different university laboratories. Results were identical, giving us confidence in our analytical procedures.

An intraruminal constant release device would be ideal for administering a fecal output marker. However, the results of this study indicate more work is required to improve the consistency of marker release from such a device.

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