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Controlled release chromic oxide and alkaline peroxide lignin marker methods

P. A. MOMONT, R. J. PRUITT, R. J. EMERICK, AND R. H. PRITCHARD

First, second and fourth authors are former graduate assistant, associate professor, and associate professor, Department of Animal and Range Sciences, South Dakota State University, Brookings 57007-0392; third author is professor, Station Biochemistry Section, Department of Chemistry, South Dakota State University, Brookings 57007-0392. P. A. Momont is currently assistant professor, Department of Animal and Veterinary Science, University of Idaho, Caldwell Research and Extension Center, 83605.

Abstract

Two digestion trials, using 20 ram lambs (Experiment 1) and 8 cows (Experiment 2) provided ad libitum access to mature prairie grass hay, were conducted to evaluate controlled release intraruminal chromic oxide boluses and alkaline hydrogen peroxide lignin as markers for estimating forage intake by the fecal output/indigestibility ratio. A soybean meal and 3 urea based supplements were fed to lambs in Experiment 1. For both experiments, daily fecal output was weighed and sampled for 6 days (Experiment 1) and 5 days (Experiment 2) beginning 7 days after oral administration of Cr_2O_3 controlled release boluses. Rectal fecal grab samples were also collected at 1000 daily and at 4-hour intervals on day 4 of collections for Experiment 2. For both experiments Cr_2O_3 excretion rates based on total collections were used to evaluate Cr_2O_3 controlled release boluses and alkaline hydrogen peroxide lignin predictive value in place of manufacturer's stated release rate. In experiment 1, fecal Cr_2O_3 output was 224 mg/day ± 3.9 compared to the manufacturer's stated release rate of 201 mg Cr_2O_3/day. Fecal alkaline hydrogen peroxide lignin recovery was 97.8% ± 1.9. Samples composited over the 6-day collection period predicted fecal output, apparent dry matter digestibility, and dry matter intake similar (P = .44, .15 and .55; respectively) to actual values. Supplemental treatment and dry matter intake had no effect ($P \geq .38$) on daily fecal Cr_2O_3 output or alkaline hydrogen peroxide lignin recovery. In Experiment 2, fecal Cr_2O_3 was 1,662 mg/day ± 63 compared to the manufacturer's stated release rate of 1,505 mg Cr_2O_3/day. Fecal alkaline hydrogen peroxide lignin recovery was 95.9% ± 7. Using 5-day composited samples, predicted fecal output, dry matter digestibility, and dry matter intake were similar (P = .49, .21 and .49; respectively) to actual values. Increasing the number of daily grab samples increased R^2 values between actual and predicted fecal output and dry matter digestibility. Fecal grab samples and total fecal collection samples provided a similar relationship (R^2=.71) between actual and predicted dry matter intake when each were composited over 5 days. Time of day did not affect fecal Cr_2O_3 or alkaline hydrogen peroxide lignin concentrations. These results suggest that grab samples collected once daily on 5 consecutive days can be used to predict fecal output when Cr_2O_3 controlled release boluses are used. Although recoveries of fecal alkaline hydrogen peroxide lignin were near 100% in these experiments, digestibility estimates using this internal marker were variable and adversely influenced predictions of dry matter intake.

Key Words: markers, fecal output, digestibility, dry matter intake, ruminants

Aspects of marker procedures used to estimate dry matter intake by the fecal output/indigestibility ratio have been reviewed extensively (Wallace and Van Dyne 1970; Ktob and Luckey 1972; Kartchner and Campbell 1979; Raleigh et al. 1980; Fahey and Jung 1983; Cochran et al. 1987). Ideally, an internal marker for estimating apparent dry matter digestibility would be 100% recoverable in the feces. Fecal lignin recoveries are variable with both positive (Fahey et al. 1979) and negative (Krysl et al. 1988) values reported. Cochran et al. (1988) suggested that residue from sequential alkaline hydrogen peroxide and acid detergent lignin analysis, called alkaline hydrogen peroxide lignin, was more indicative of the in vivo indigestible lignin fraction than acid detergent lignin alone.

Numerous procedural adaptations of the Cr_2O_3 external marker system have been made. Although some modifications (e.g., Cr_2O_3 impregnated paper) have led to improvements in fecal recovery and reduction of diurnal variation in confinement trials (Nelson and Green 1969), the benefits of these procedures have not always been observed in grazing trials (Kiesling et al. 1969). Single or twice daily dosing of Cr_2O_3 has resulted in diurnal excretion of Cr_2O_3, sedimentation of a fraction of the Cr_2O_3 in the reticulo-rumen and(or) disruption of animal grazing patterns (Ktob and Luckey 1972; Raleigh et al. 1980). The recent development of a Cr_2O_3 controlled release intraruminal device may improve marker performance with grazing animals. The objectives of these trials were to evaluate alkaline hydrogen peroxide lignin and Cr_2O_3 controlled release boluses as internal and external markers for estimating fecal output, dry matter digestibility, and dry matter intake of ruminants consuming mature prairie hay.

Materials and Methods

In Experiment 1, a digestibility trial was conducted with

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Table 1. Composition of supplements fed to lambs in Experiment 1.

Ingredient	Supplements: Soybean meal	Urea + methionine	Urea + sulfur	Urea
	----------(%)----------			
Soybean meal	91.4	19.8	19.9	21.4
Corn	3.1	57.4	57.7	59.1
DL-methionine	-	3.3	-	-
Urea	-	8.3	8.3	8.3
Sodium sulfate	-	-	3.0	-
Sodium bentonite	1.7	5.6	5.6	5.6
Liquid molasses	3.4	3.3	3.3	3.4
Potassium chloride	-	1.1	1.1	1.1
Dicalcium phosphate	-	1.1	1.1	1..1

[a]Percentage of dry matter.

20 Hampshire ram lambs (mean wt 53 kg ± 3.3) allowed ad libitum access to mature prairie grass hay and fed 1 of 4 protein supplements that provided 45 g of crude protein·head^{-1}·day^{-1}. Composition and daily nutrient intake, based on laboratory analyses, of supplements fed to lambs in Experiment 1 are listed in Tables 1 and 2, respectively. Analytical and species composition of prairie hay consumed by animals in both Experiments 1 and 2, are listed in Table 3. Lambs were housed in metabolism pens (.5 x 1.0 m) and provided free access to water. The experiment consisted of a 21-day adaptation period followed by two 3-day collection periods for weighing and sampling feed, orts, and feces. Seven days before the beginning of the collection period, lambs were orally administered intraruminal boluses (Captec Chrome, Nufarm Industries, Aukland, New Zealand) designed to release 201 mg of chromic oxide/day (test completion date 17 June 1988; batch number 61125-1).

Experiment 2 consisted of a digestibility trial with 8 mature cows (mean wt 631 kg ± 34) that had ad libitum access to mature prairie hay (Table 3) with no supplement. Cows were housed in individual pens (4.9 x 4.9 m) and provided free access to water. The trial began with a 14-day adaptation period and was followed by a 5-day collection period for weighing and sampling feed, orts, and feces. Seven days before the first fecal collections, cows were orally administered Cr_2O_3 controlled release boluses designed to release 1,505 mg of chromic oxide/day (test completion date 12 December1988; batch number 81122-8). Total fecal collections were accomplished by scraping concrete floors of each pen a minimum of 4 times a day. Daily fecal output was weighed, mixed, and an approximate 400-g sample of each cow's total daily fecal output was taken and frozen (-30° C) for later laboratory analyses. Rectal fecal grab samples (approximately 200 g) were taken at 1,000 each day to compare marker recoveries from grab samples and total daily fecal output samples. On day 4, fecal grab samples were taken at 4-hour intervals to evaluate diurnal variation in marker recoveries. Weights from fecal grab samples were recorded and included as part of the total daily fecal output of each cow.

Table 2. Daily nutrient intake from supplements fed to lambs.

Ingredient	Supplements: Soybean meal	Urea + methionine	Urea + sulfur	Urea
Dry matter, %	87.79	88.61	89.68	87.88
Dry matter intake, g	97.45	108.10	114.79	109.85
Crude protein, g	44.83	45.01	45.32	46.16
Nonprotein nitrogen, g	.11	4.06	4.29	4.28
Methionine, g	.68	4.37	.32	.38
Sulfur, g	.46	.82	.99	.22
Calcium, g	.42	.48	.48	.49
Phosphorous, g	.58	.55	.59	.63
Potassium, g	2.57	1.65	1.66	1.74
Metabolizable energy, Mcal[a]	.26	.26	.26	.26

[a]Calculated values.

Table 3. Chemical and species composition of prairie hay consumed by lambs and cows in experiments 1 and 2.

Item	
	----------(%)----------
Dry matter	84.1
Crude protein	5.8
Neutral detergent fiber	70.2
Acid detergent fiber	39.0
Alkaline peroxide lignin	1.57
Calcium	.40
Phosphorus	.11
Sulfur	.10
Western wheatgrass (*Agropyron smithii*)[b]	65.0 ± 10.0
Japanese brome (*Bromus japonicus*)[b]	35.0 ± 11.0
Unidentified forage[b]	2.0 ± 3.0

[a]Percentage of dry matter.
[b]Mean and SD from 15 samples.

For both experiments, prairie hay was fed twice daily at a level approximately 10% above ad libitum intake. Hay was fed long-stem, having been previously shredded through a screenless bale grinder and stored in barrels. Individual orts were weighed and refed each day. Final orts were weighed and sampled at the end of the trial. Feed, final orts, and fecal samples were oven dried at 60° C until a constant weight was achieved, ground through a 1-mm screen with a Wiley mill, and stored for later analysis. To determine species composition of the hay, samples from 15 bales were subsampled, sorted by species, oven dried at 100° C, and weighed.

Fecal dry matter and ash content (AOAC 1980) were determined on duplicate samples (.2 g). Fecal Cr_2O_3 concentrations were determined by sulfuric/phosphoric acid digestion and bromate oxidation of ashed samples (Costigan and Ellis 1987). After hydration of samples Cr_2O_3 concentration was measured using a Perkin-Elmer 503 atomic absorption spectrophotometer. Chromic oxide-free fecal samples, Cr_2O_3 recoveries, and Cr_2O_3 standards accompanied each analysis. An aluminum block capable of holding 64 test tubes (15 ml) was used for the digestion and oxidation steps. Recovery of added Cr_2O_3 from ashed Cr_2O_3-free fecal samples was 103% ± 1.9 for all analyses.

Forage, ort, and fecal alkaline hydrogen peroxide lignin concentrations were determined on duplicate 10-g samples by sequential 48-hour alkaline hydrogen peroxide (pH = 11.5, 1% H_2O_2) digestion (Cochran et al. 1988) followed by acid detergent lignin procedures (Robertson and Van Soest 1981). Alkaline hydrogen peroxide lignin concentrations in grain supplements have been shown to be negligible in high roughage diets (Cochran et al. 1988) and were not included in laboratory analyses or mathematical computations. Modifications of the alkaline hydrogen peroxide procedure as suggested by Sunvold et al. (1989) included the use of 100-ml polyethylene tubes and a horizontal shaker (48 hours). To facilitate filtration in Experiment 2, the shaker was turned off after 44 hours, allowing particulate matter to settle. Excessive bumping during the acid detergent fiber procedure was prevented by not tearing the filter paper containing the alkaline hydrogen peroxide residue. Blank tubes and standard forages accompanied each analysis.

Equations used in the process of estimating dry matter intake are listed in Table 4. For each experiment, a mean Cr_2O_3 excretion rate across all animals was determined based on daily total fecal collec-

Table 4. Equations used with marker procedures in process of dry matter intake estimations.

Cr_2O_3 excretion rate, mg/day = (mg of Cr_2O_3/gm of fecal dry matter) x (gm of fecal dry matter output/day)

Percentage alkaline hydrogen peroxide lignin recovery =

$$\frac{\text{(percentage of fecal alkaline hydrogen peroxide lignin) x (gm of fecal dry matter/day)}}{\text{(percentage of dietary alkaline hydrogen peroxide lignin)}^a \text{ x (gm of dry matter intake/day)}}$$

Predicted fecal dry matter output gm/day = $\frac{\text{(mean } Cr_2O_3 \text{ excretion rate, mg/day)}^b}{\text{(mg of } Cr_2O_3\text{/gm of fecal dry matter)}}$

Predicted dry matter indigestibility = $\frac{\text{(percentage of dietary alkaline hydrogen peroxide lignin)}}{\text{(percentage of fecal alkaline hydrogen peroxide lignin)}}$

Predicted dry matter intake = $\frac{\text{(predicted fecal dry matter output, gm/day)}}{\text{(predicted dry matter indigestibility)}}$

[a] Mean dietary alkaline hydrogen peroxide lignin = 1.57%.
[b] Mean excretion rate with 20 lambs during 6-day collection period = 224 mg Cr/day. Mean excretion rate with 8 cows during 5-day collection period = 1,662 mg Cr/day.

tions and fecal Cr_2O_3 concentrations. These mean Cr_2O_3 excretion rates were used in calculations to estimate fecal output in place of manufacturer's values. For each animal, estimated daily dry matter digestibility (based on feed and fecal alkaline hydrogen peroxide lignin concentrations) were compared to actual dry matter digestibility (average dry matter digestibility during the entire collection period, based on dry matter intake and fecal output). Calculated dry matter intake for each individual were compared to the average actual dry matter intake during the entire collection period.

Using the GLM procedure of SAS (1985) in a split-plot in time design (Damon and Harvey 1987) the effects of supplemental treatment (Experiment 1) on Cr_2O_3 controlled release boluses and alkaline hydrogen peroxide lignin marker recovery and predictive value were analyzed. Main effects in the whole plot were treatment and lamb within treatment. The lamb within treatment error term was used to test treatment effects. Residual error was used to test for the split-plot effects (period and period x treatment, with period being one 3-day collection). Dry matter intake was included as a covariate to determine the effect of intake level on Cr_2O_3 controlled release boluses excretion rate of Cr_2O_3, alkaline hydrogen peroxide lignin recovery and predictive value of these markers. Predicted minus actual values for fecal output, dry matter digestibility, and dry matter intake were generated as dependent variables. Coefficients of determination and variation (R^2 and C.V., respectively) were determined by regressing predicted over actual fecal output, dry matter digestibility, and dry matter intake values. Predicted and actual values were also compared using MEANS procedure with the paired t-test option (SAS 1985).

In Experiment 2, alkaline hydrogen peroxide lignin and Cr_2O_3 fecal concentrations were regressed over sampling time of day, with quartic through linear responses being tested, using GLM procedures of SAS (1985). Sampling time was also used as a discrete independent variable to generate least squares means. Mean separation was accomplished using the L.S.D. procedure (SAS 1985). Coefficients of determination (R^2) and variation (C.V.) values were determined by regressing predicted fecal output, dry matter digestibility, and dry matter intake over the actual values. These regressions were conducted by progressively compositing data from collection days 1 to 5 for fecal grab samples and from total fecal collection samples in order to evaluate the number of daily samples required and to determine if grab samples were representative of the total daily fecal output. Predicted and actual values were also compared using the MEANS procedure with the paired t-test option (SAS 1985).

Results

In experiment 1, mean dry matter intake of prairie hay for lambs during the collection periods was 2.0% of body weight ± .05. Based on total fecal collections and sample Cr_2O_3 concentrations, mean Cr_2O_3 excretion rate for lambs was 224 ± 4.4 mg of Cr_2O_3/day (n = 20, C.V. = 11) compared with the manufacturer's reported value of 201 mg of Cr_2O_3/day. Minimum and maximum excretion rates were 187 and 257 mg of Cr_2O_3/day, respectively. Mean alkaline hydrogen peroxide lignin fecal recovery was 97.8% ± 2.5 (n = 20, C.V. = 11.6) with a range from 82.4 to 118%.

Supplementation of lambs fed mature prairie hay did not affect daily Cr_2O_3 excretion rate (P = .38) or fecal alkaline hydrogen peroxide lignin recovery (P = .47, Table 5). Daily Cr_2O_3 excretion rate and fecal alkaline hydrogen peroxide lignin recovery values were not affected by dry matter intake (P = .41 and .52, respectively). Predicted minus actual fecal output, dry matter digestibility, and dry

Table 5. Effect of supplemental treatment on marker performance and predictive value.

	Treatment						
Item	Soybean meal	Urea + mehtionine	Urea + sulfur	Urea	SE[a]	p[b]	p[c]
Total dry matter intake, kg/day	1.02	1.06	1.09	1.04	0.8	.66	-
Alkline hydrogen peroxide lignin recovery, %	96.4	102.1	92.4	98.1	4.3	.47	.52
Cr_2O_3 excretion rate, mg/day	225	218	216	236	8.8	.38	.41
Predicted minus actual:							
fecal output, g/day	-11.9	15.6	30.7	-25.6	23.6	.36	.40
dry matter digestibility, %	-2.6	.9	-4.4	-1.3	2.3	.62	.77
dry matter intake, g/day	-11.1	69.4	-20.7	-69.4	77.6	.82	.20

[a] Least squares mean standard error (n=5).
[b] P = Probability of a significant supplemental treatment effect
[c] P = Probability of a significant dry matter intake effect.

Table 6. Relationships between actual and predicted fecal output, apparent dry matter digestibility and dry matter intake.

Item	Actual	Predicted	Difference[a]	p[b]
Experiment 1				
fecal output, g	572	580	8±10	.44
dry matter digestibility, %	45.6	43.9	-1.5±1.0	.15
dry matter intake, g	1,053	1,033	-20±33	.55
Fecal grab samples (Experiment 2)				
fecal output, g	4,531	4,653	122±174	.49
dry matter digestibility, %	56.0	53.6	-2.4±1.8	.21
dry matter intake, g	10,304	10,016	-287±410	.49
Total daily fecal collection samples (Experiment 2)				
fecal output, g	4,531	4,519	-12±149	.94
dry matter digestibility, %	56.0	55.5	-.5±2.2	.85
dry matter intake, g	10,308	10,155	-149±309	.63

[a]Predicted minus actual and SEM.
[b]P = Probability that mean differences in different from zero.

matter intake values were similar ($P > .20$) across supplemental treatments and dry matter intake. Utilizing paired comparisons, predicted minus actual fecal output, dry matter digestibility, and dry matter intake values were not different ($P = .44$, .15 and .55, respectively) from zero (Table 6).

Regression of predicted over actual fecal output, dry matter digestibility, or dry matter intake, however, resulted in varied R^2 values. Estimated fecal output values were closely related to actual fecal output ($R^2 = .83$, and C.V. = 8.4%), using total fecal collection samples composited over the entire 6-day period. The relationship detected between predicted and actual dry matter digestibility was low ($R^2 = .01$, C.V. = 18.25) and when dry matter digestibility was used to calculate dry matter intake, the relationship between predicted and actual dry matter intake was adversely affected ($R^2 = .54$, C.V. = 17.0).

In experiment 2, mean dry matter intake of prairie hay for cows during the collection periods was 1.6% of body weight ± .05. Mean Cr_2O_3 excretion rate from cows was 1,662 mg of Cr_2O_3/day 63 (n = 8, C.V. = 10.6) with a range from 1487 to 2,020 mg of Cr_2O_3/day. Manufacturer's reported release rate for this allotment of boluses was 1505 mg Cr_2O_3/day. Mean alkaline hydrogen peroxide lignin fecal recovery from cows was 95.9% ± 3.2 (n = 8, C.V. = 10.1), ranging from 81 to 106%.

There was no diurnal variation of fecal Cr_2O_3 or alkaline hydrogen peroxide lignin excretion. Based on polynomial regression analysis of variance, sampling time of day did not affect ($P>0.69$, quartic through linear responses tested) fecal Cr_2O_3 or alkaline hydrogen peroxide lignin concentrations. Mean fecal Cr_2O_3 concentration for the 8 cows on day 4 was 42.2 µg/g, and fecal Cr_2O_3 concentration at each 4-hour interval was not affected by sampling time of day (P=.97, Fig. 1). Mean fecal alkaline hydrogen peroxide lignin concentration was 4.9% ± .24, and fecal alkaline hydrogen peroxide lignin did not differ (P=.94, Fig. 1) over sampling time. Utilizing paired comparisons, predicted minus actual fecal output, dry matter digestibility, and dry matter intake values were not different from zero (Table 6) for fecal grab samples (P = .49, .21 and .49, respectively) or samples from total fecal collections (P = .94, .85 and .63, respectively) in experiment 2.

The ability to predict fecal output, dry matter digestibility, and dry matter intake using Cr_2O_3 controlled release boluses and alkaline hydrogen peroxide lignin as markers was improved by increasing the number of daily samples collected and composited (Fig. 2 and 3). As expected, the amount of increase in R^2 and decrease in C.V. values

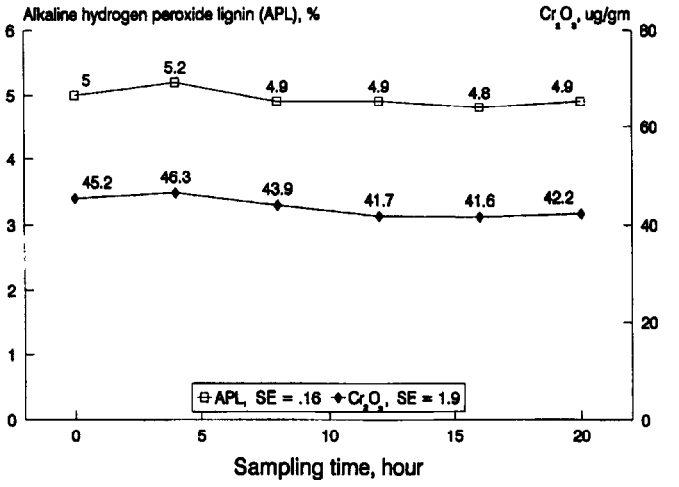

Fig. 1. Fecal alkaline hydrogen peroxide lignin (APL, % dry matter basis) and Cr_2O_3 (ugm Cr_2O_3/gm fecal dry matter) concentrations on d 4 of total collections for cows (n = 8) fed mature prairie hay (Experiment 2).

between predicted and actual fecal output, dry matter digestibility, and dry matter intake diminished with additional sampling days. Predicted fecal output was closely related to actual fecal output. Using 5-day composited samples, high R^2 values for both fecal grab and total daily fecal output samples were determined (.82 and .85, C.V. = 10.9 and 8.5 for Experiments 1 and 2, respectively). Lower R^2 values between actual and predicted dry matter digestibility (.70 and .65., C.V. = 4.9 and 6.1 for 5-day fecal grab and total daily fecal output samples) resulted in reduced accuracy of dry matter intake estimations (R^2 = .71 and .71, C.V. = 12.6 and 10.1 for grab and total daily fecal output samples).

Discussion

Mean Cr_2O_3 excretion rates were 11.4% higher in Experiment 1

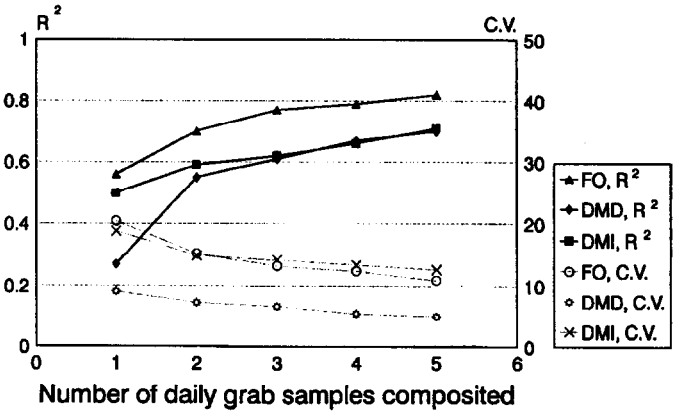

Fig. 2. Coefficients of determination and variation (R^2 and C.V., respectively) for relationships between marker based estimates from fecal grab samples and actual values derived from total collections for fecal output (FO), apparent dry matter digestibility (DMD), and dry matter intake (DMI) for cow trial (Experiment 2). The x-axis represents the number of daily fecal grab samples that were composited for marker analysis.

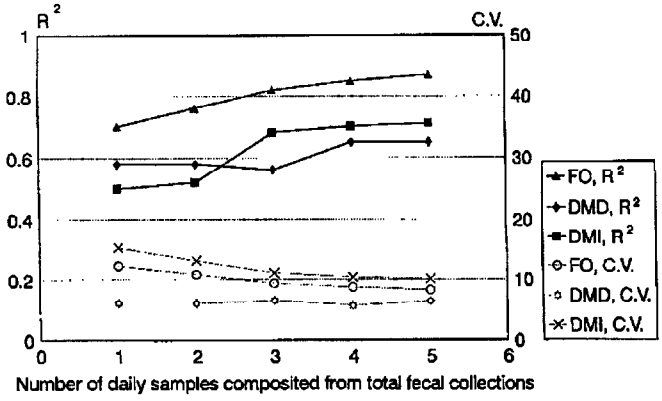

Fig. 3. Coefficients of determination and variation (R^2 and C.V., respectively) for relationships between marker based estimates from total daily fecal collection samples and actual values derived from total collections for fecal output (FO), apparent dry matter digestibility (DMD), and dry matter intake (DMI) for cow trial (Experiment 2). The x-axis represents the number of total daily fecal collection samples that were composited for marker analysis.

and 11.8% higher in Experiment 2 than manufacturer's stated release rates. R. H. Laby (unpublished data) suggests that Cr_2O_3 release from the bolus is directly related to H_2O kinetics in the rumen. If ruminal fluid dynamics differ with forage type, then different Cr_2O_3 release rates from the Cr_2O_3 controlled release boluses would be expected. Parker et al. (1989) determined that Cr release rates from Cr_2O_3 controlled release boluses were higher for less digestible feedstuffs. They detected higher Cr release rates (68.1 mg of Cr/day) with lambs consuming meadow hay (dry matter digestibility = 58%) and lower values (54.8 mg of Cr/day) for lambs fed fresh cut clover (dry matter digestibility = 77.5%). Lambs fed fresh cut ryegrass (dry matter digestibility = 63.8) and a mixture of ryegrass and clover had intermediate Cr release rates from Cr_2O_3 controlled release boluses. Using manufacturer's stated Cr_2O_3 release rates, Hatfield et al. (1991) over predicted fecal output by 18 to 45 percentage units with lambs in confinement consuming alfalfa pellets, and in 2 subsequent trials with lambs grazing growing range vegetation fecal output was underestimated by 4 to 13 percentage units. If the alfalfa pellets were more digestible than the range forage consumed by lambs, then variations in Cr_2O_3 excretion rate due to diet digestibility may explain these differences.

Using release rates suggested by the manufacturer in our studies would have resulted in underestimation of fecal output. In situations where only relative comparisons of fecal output are needed, validation of the Cr_2O_3 excretion from cattle administered Cr_2O_3 controlled release boluses may not be critical. Data from this trial suggests that when fecal output is being estimated using Cr_2O_3 controlled release boluses and determining the amount of forage consumed is a high priority, it is advisable to validate the rate of Cr_2O_3 excretion using total fecal collections on a small number of animals consuming forage similar to that consumed by a larger group. Although this will increase the labor required, it may be necessary to accurately predict forage intake when more than relative differences are needed. This is substantiated by other studies (Parker et al., 1989; Hatfield et al. 1991) where digestibility of the diet affected Cr_2O_3 release rates.

Fecal Cr_2O_3 concentration was not affected by sampling time or type of fecal sample used for cows administered Cr_2O_3 controlled release boluses. Ruminal Cr_2O_3 dissolution has been determined to be linear over time for boluses designed for sheep (Ellis and Rodden 1987; Barlow et al. 1988; Parker et al. 1990) and cattle (Ellis et al. 1982). Parker et al. (1990) determined that daily sampling time had little effect on hourly fecal Cr_2O_3 concentrations for sheep consuming a variety of forages in confinement or pasture environments. Fecal Cr_2O_3 concentrations and estimations of fecal output were comparable for grab samples and total daily fecal output samples. Nelson and Green (1969) also reported that Cr_2O_3 recovery was not affected by fecal sampling technique. A constant release of Cr throughout the day would improve fecal output estimations made from a single fecal grab sample and would allow greater flexibility in sampling time when conducting grazing studies.

Daily excretion rates of Cr_2O_3 for sheep fed mature prairie hay were not affected by level of forage intake or the supplemental protein treatments used in Experiment 1. Other studies, with sheep administered Cr_2O_3 controlled release boluses, have generated contradictory results concerning the effects of dry matter intake and supplementation on Cr_2O_3 release rates. Parker et al. (1990) suggested that daily feeding pattern and level of intake did not affect Cr_2O_3 release rate. However, Hatfield et al. (1991) determined that barley supplementation and level of forage dry matter intake influenced prediction of fecal output for lambs in confinement consuming alfalfa pellets. With several groups of cattle administered Cr_2O_3 controlled release boluses while grazing 1 of 3 pasture systems (high, medium, and low intake), Barlow et al. (1988) found no differences in Cr_2O_3 release rate between pasture groups. It seems that daily Cr_2O_3 excretion rates were similar across the levels of forage dry matter intake and supplementation schemes used in this study.

Within the allotment of Cr_2O_3 controlled release boluses used for each experiment, variation in fecal Cr_2O_3 excretion rates (C.V. = 11 and 10.6% for Experiments 1 and 2, respectively) were higher than previously reported values that ranged from 5 to 10% (Ellis et al. 1981, 1982; Ellis and Rodden 1987; Barlow et al. 1988; Parker et al. 1990). These lower C.V. values for daily Cr_2O_3 release rate were obtained when ruminal Cr_2O_3 dissolution from the surface of the Cr_2O_3 controlled release boluses was measured using ruminally fistulated animals. Movement of Cr_2O_3 out of the rumen and through the lower gastrointestinal tract would increase variation associated with determining daily Cr_2O_3 release rates based on fecal Cr_2O_3 concentration and total fecal collection.

Problems with Cr_2O_3 controlled release bolus regurgitation and unexplained failure of the bolus to release Cr_2O_3 in the rumen have been reported (Ellis et al. 1981; Parker et al. 1990). All animals administered Cr_2O_3 controlled release boluses in our experiments had reasonable fecal Cr_2O_3 levels. High failure rates primarily due to Cr_2O_3 controlled release bolus regurgitation (Momont, et al., 1993) were found in subsequent studies involving mature cows grazing dormant winter range.

Data from our study indicate that mean fecal alkaline hydrogen peroxide lignin recovery was nearly 100% in both experiments and predicted digestibility estimates were not different than dry matter digestibility values based on total collections. However, relationships between predicted and actual digestibility coefficients were variable as indicated by R^2 values. Previous studies indicated that consistent marker recoveries from alkaline peroxide treatment before acid detergent lignin analysis could be obtained for mature forage sources, which would provide accurate digestibility estimations. Using steers fed dormant bluestem-range grass, Cochran et al. (1988) reported a mean fecal alkaline hydrogen peroxide lignin recovery of 97.6% and determined that predicted and actual organic matter digestibility were not different (46.4% and 48.3%, respectively). In a follow-up study (Sunvold and Cochran, 1991), steers limit-fed prairie hay had a mean fecal alkaline hydrogen peroxide lignin recovery of 92.5%. While predicted and actual digestibility coefficients were not different (54.4% and 48.2%, respectively), alkaline hydrogen peroxide lignin did not appear as effective at predicting digestibility as in the earlier

study. Judkins et al. (1990) determined a fecal alkaline hydrogen peroxide lignin recovery of 100.4% for sheep provided ad libitum access to fescue hay, but predicted and actual digestibility coefficients were significantly different. Considerable animal to animal variation and intrinsic problems with the mathematics used to calculate digestibility were identified as problems associated with their use of lignin markers.

Fecal alkaline hydrogen peroxide lignin recovery was not affected by protein supplementation and level of dry matter intake for sheep fed mature prairie hay in our study. Cochran et al. (1988) determined that fecal alkaline hydrogen peroxide lignin recovery for steers fed several levels of sorghum supplement while consuming dormant range grass was not affected by level of supplementation. However, steers consuming immature range grass without supplements had lower fecal alkaline hydrogen peroxide lignin recovery values and organic matter digestibility estimates than supplemented steers.

In our study sampling time and type of daily fecal sample had little effect on fecal alkaline hydrogen peroxide lignin concentrations or prediction of dry matter digestibility and dry matter intake. McCann and Theurer (1967) reported that comparable estimations of dry matter digestibility using lignin as a marker were obtained from fecal grab samples versus samples from total fecal collections. Although predicted dry matter digestibility were similar to actual values based on total collections, relationships between these values were variable for our 2 experiments. The low R^2 between predicted and actual dry matter digestibility for sheep fed mature prairie hay may be partially attributable to the narrow range and variation in actual dry matter digestibility values (dry matter digestibility range from 41.2 to 50.4%, mean dry matter digestibility = 45.6 ± 2.7%).

Conclusions

Grab samples collected once daily on 5 consecutive days can be composited and used to reliably predict fecal output when Cr_2O_3 controlled release boluses are used. After verification of manufacturer's stated Cr_2O_3 release rate with a small number of animals, this technique has the potential to reduce animal handling time and disruption of animal grazing patterns compared with twice daily administration of Cr_2O_3 gelatin capsules. Although mean recovery of alkaline hydrogen peroxide lignin in the feces was nearly 100%, accuracy of digestibility estimates using alkaline hydrogen peroxide lignin as an internal marker were variable and adversely influenced predictions of dry matter intake.

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