

EFFECT OF LEVEL OF INTAKE AND SUPPLEMENTAL BARLEY ON MARKER ESTIMATES OF FECAL OUTPUT USING AN INTRARUMINAL CONTINUOUS-RELEASE CHROMIC OXIDE BOLUS¹

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ABSTRACT

Sixty wethers (average BW = 45 kg) fitted with fecal collection bags were used in four experiments to evaluate the ability of an intraruminal continuous-release chromic oxide bolus to predict fecal DM output. In Exp. 1, 18 wethers housed in metabolism crates were fed barley at either 0, 100, or 200g/d and allowed ad libitum consumption of alfalfa pellets. In Exp. 2, 18 wethers were housed in metabolism crates and fed alfalfa pellets at either 70, 85, or 125% of pre-study ad libitum consumption. In Exp. 3, 12 wethers grazed a sagebrush-bunchgrass range and were individually fed barley at 0 or 200g/d. In Exp. 4, 12 wethers grazed either an ungrazed (383 kg/ha herbaceous biomass) or a heavily grazed (175 kg/ha herbaceous biomass) sagebrush-bunchgrass range. Experiments 1 and 2 were balanced 3 × 3 Latin squares, and Exp. 3 and 4 were crossover designs. Chromium content was determined in rectal grab samples. Treatment effects were compared using marker-estimated fecal output divided by total fecal collection, multiplied by 100, as the dependent variable. Accuracy of the estimate was verified by comparing marker-estimated fecal output with total fecal collection using a paired *t*-test. In Exp. 1 and 2, treatments were different ($P < .05$). No differences ($P > .50$) were detected in Exp. 3 and 4. In Exp. 1, 2, and 3 accuracy was different ($P < .02$) among wethers within study and treatment. Only in Study 4 were minor or no differences ($P > .09$) in accuracy found. Accuracy (Exp. 1, 2, and 3) and precision (Exp. 1 and 2) of marker-estimated fecal output seemed to be affected by level of DMI and supplemental barley. These data indicate that total fecal collections on a subset of animals used in a study may be necessary to adjust marker estimates of fecal output.

Key Words: Chromium, Feces Collection, Rumen Boluses

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Introduction

Many methods of estimating fecal output using inert markers have been investigated. These include pulse-dosing rare earth markers

(Galyean et al., 1986), daily dosing of inert markers (Raleigh et al., 1980; Galyean et al., 1986), and intraruminal continuous-release bolus (Parker et al., 1989; Estell et al., 1990; Hatfield et al., 1990). Use of an intraruminal continuous-release chromic oxide bolus may provide an improved method of estimating fecal output in grazing ruminants (Harrison et al., 1981, 1982; Laby et al., 1984; Ellis and Rodden, 1987; Parker et al., 1989).

The application of an intraruminal continuous-release bolus in grazing studies raises two important questions. First, are marker estimates of fecal output affected by variations in

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level of DMI and supplementation, which are conditions common in grazing research? Second, how precise are marker estimates of fecal output compared with total fecal collections? Four experiments were designed to test the following two hypotheses. First, differences exist between total fecal output and marker estimates of fecal output. Second, precision of marker-estimated fecal output is affected by level of DMI and supplementation.

Experimental Procedure

Sixty Polypay wethers (average BW = 45 kg) were used in four separate experiments to investigate the reliability of fecal output estimates using an intraruminal continuous-release chromic oxide bolus⁵. Chromic oxide release rate from boluses used in Exp. 1 and 2 was reported by the manufacturer to be 201 mg of chromic oxide/d (138 mg of chromium/d). The rate of chromic oxide release in Exp. 3 and 4 was reported by the manufacturer to be 186 mg of chromic oxide/d (127 mg of chromium/d).

Experiments 1 and 2 were conducted with wethers housed in metabolism crates (61 × 123 cm) and fed alfalfa pellets as the basal diet. Experiments 3 and 4 were conducted in May while wethers grazed native range. Dominant vegetation on the native range was bluebunch wheatgrass (*Agropyron spicatum* [Pursh] Scribn. and Smith) and three-tip sagebrush (*Artemisia tripartita* Rydb.). The range site was a shallow loam that receives 200 to 300 mm of precipitation annually.

Wethers were adapted to wearing fecal bags and harnesses for 7 d before starting each experiment. In all experiments, fecal collection bags were emptied twice daily at 0730 and 1630. Feces were weighed and a 5% aliquot was retained from each sample and refrigerated for later determination of DM. Rectal grab samples of feces were taken at the same time that fecal collection bags were emptied. Weight of rectal grab samples was included in the calculation of DM fecal output, which was calculated for each animal in each period.

Rectal samples were composited across time and day within a period for each animal.

Chromium concentration was determined in the rectal grab samples but not in the total fecal collections. If total recovery of chromium was assumed, variation in marker release from the bolus could be investigated. However, this was not the intent of this study. In addition, analyzing for recovery of chromium in the feces would not be practical when the quantity of chromium released from the bolus may not be consistent or at the rate reported by the manufacturer.

Experiment 1

Experiment 1 examined effects of supplemental barley on precision and accuracy of marker estimates of fecal output. Eighteen wethers (six per treatment) were fed whole barley at the rate of 0, 100, or 200g/d and allowed to consume alfalfa pellets ad libitum (Table 1). Experimental periods consisted of a 10-d pre-study adaptation and three 6-d collection periods. Each animal was dosed orally with one intraruminal continuous-release chromic oxide bolus on d 3 of adaptation. This allowed 7 d for the bolus to achieve an equilibrium release rate before the first collection period (Parker et al., 1989). During pre-study adaptation, wethers were fed whole barley at the rate of 100g/d and allowed to consume alfalfa pellets ad libitum. There were no adaptation periods between collection periods, thus allowing the treatments to produce residual effects, if present, in the period immediately after the one in which the treatment was applied.

During collection periods wethers were fed daily at 0700. Orts were collected before feeding, weighed, subsampled, and composited by animal within period. After collection of Orts, alfalfa pellets were fed in a quantity sufficient to ensure at least a 10% refusal the following morning. Whole barley was top-dressed on the alfalfa pellets.

Experiment 2

Experiment 2 examined effects of level of DMI on precision and accuracy of marker estimates of fecal output. Eighteen wethers (six per treatment) were fed either 70 or 85% of pre-study ad libitum consumption or an ad

⁵Captec chrome manufactured by Captec Pty. Ltd., Australia, distributed internationally by Nufarm Limited, Otahuna, Auckland 6, New Zealand.

TABLE 1. CRUDE PROTEIN, NDF, ADF, AND ADL OF ALFALFA, BARLEY, AND MASTICATE SAMPLES FROM EXP. 1, 2, 3, AND 4

Exp.	Same description	% DM			
		CP	NDF	ADF	ADL
Exp. 1 & 2	Alfalfa	13.6	54.3	40.3	9.2
Exp. 2 & 3	Barley	10.5	9.3	5.1	2.3
Exp. 3	Masticate	12.0	52.6	31.7	9.4
Exp. 4	Masticate (grazed)	12.5	52.0	30.6	9.4
Exp. 4	Masticate (ungrazed)	11.8	49.2	30.9	9.3

libitum amount of alfalfa pellets (Tables 1 and 2). Experimental periods, adaptation, and dosing wethers with chromic oxide boluses were the same as described in Exp. 1. During adaptation, wethers were allowed to consume alfalfa pellets ad libitum. The quantity of alfalfa pellets consumed by each animal during this period was used to determine its average ad libitum DMI. Levels of feed DMI for the 70 and 85% of ad libitum DMI treatments were calculated based on DMI during the last 7 d of adaptation. There were no adaptation periods between collection periods. Feeding and orts were handled in the same manner as in Exp. 1. After orts had been collected from the ad libitum treatment group, alfalfa pellets were fed as prescribed by treatment.

Experiment 3

Experiment 3 evaluated the effect of supplemental barley on precision and accuracy of marker estimates of fecal output in grazing wethers. Twelve wethers (six per treatment) were individually fed barley at the rate of 0 or 200g/d at 0700. Wethers grazed a 4-ha sagebrush-bunchgrass pasture (Table 1) during

active forage growth for a 7-d adaptation period before two 6-d collection periods.

On d 1 of adaptation, wethers were orally dosed with one chromic oxide bolus per animal and fitted with fecal collection bags. After the first collection period, wethers were crossed-over treatments and allowed 5 d of adaptation before the second collection period. Wethers were confined in a large holding pen overnight and given water, but not fed, to facilitate collections.

Forage availability was determined before the adaptation period by clipping 15 randomly located .5-m² circular plots. All herbaceous biomass in each plot was clipped to ground level. Samples were identified as grass or forb, placed in paper bags, dried at 50°C for 48 h, and weighed (Table 3).

Two esophageally fistulated wethers (Harris et al., 1967) grazed with wethers given the chromic oxide boluses and were used on the last 2 d of adaptation for collection of diet samples. Esophageally fistulated wethers were fasted overnight before a 30-min grazing period at 0600. Masticate samples were composited across both days and wethers (Table 1).

TABLE 2. ALFALFA PELLET CONSUMPTION AND APPARENT IN VIVO DM DIGESTIBILITY IN EXP. 1 AND 2

Item	DMI, g/d	SE ^a	IVDMD	SE
Exp. 1				
0 g Barley	1,828	64.1	59.4	2.11
100 g Barley	1,781	53.4	61.5	1.40
200 g Barley	1,667	64.6	64.6	2.30
Exp. 2				
Adaptation	1,581	62.3	—	—
70% of Ad libitum	1,092	49.6	60.3	2.32
85% of Ad libitum	1,326	60.2	60.5	1.65
Ad libitum	1,867	50.9	60.0	1.73

^aStandard error associated with three periods and six wethers in each period.

TABLE 3. HERBACEOUS BIOMASS (kg DM/ha) FOR EXP. 3 AND 4

Item	ha	Grass	SE ^a	Forb	SE	Total	SE
Exp. 3	4	127	25.0	66	18.1	193	31.5
Exp. 4							
Grazed pasture	2	149	35.6	25	9.6	174	38.0
Ungrazed pasture	2	334	44.2	49	7.2	383	47.0

^aStandard error associated with 15 plots clipped in each pasture.

Experiment 4

Experiment 4 examined the effects of forage availability on the precision and accuracy of marker estimates of fecal output. Twelve wethers (six per treatment) grazed either a heavily grazed pasture (2 ha with 175 kg herbaceous biomass/ha) or an ungrazed pasture (2 ha with 383 kg herbaceous biomass/ha; Tables 1 and 3). Wethers and sample collections were handled as in Exp. 3. After the first collection period, wethers were crossed-over pastures (treatments) and allowed a 5 d adaptation period before beginning a second collection period.

The heavily grazed pasture had been grazed by 12 heifers and 24 wethers for 1 wk before beginning the adaptation period. After the pasture had been grazed, forage availability was determined by the method described in Exp. 3 (Table 3). For each pasture, masticate samples were collected using two esophageally fistulated wethers for 2 d. These samples were handled in the same manner described for Exp. 3 (Table 1).

Laboratory Procedures. Alfalfa, barley, masticate, and fecal samples were dried at 55°C for 48 h. Dried samples were ground to pass a 1-mm screen in a Cyclone mill. Masticate and feed samples were analyzed for DM, ash, Kjeldahl N (AOAC, 1980), nonsequential NDF, ADF, and ADL (Goering and Van Soest, 1970).

Feces from rectal grab samples were prepared for chromium analysis using the method described by Williams et al. (1962). A .5-g fecal sample was ashed in a silica basin for 90 min at 600°C. Samples were digested in 3 ml of phosphoric acid-manganese sulfate with a 4.5% (wt/vol) potassium bromate solution added until effervescence ceased or a light purple color appeared. Samples were brought up to volume in a 100-ml volumetric flask with deionized water and mixed thoroughly. Chromium concentration was determined by

atomic absorption spectrometry using an air/acetylene flame.

Statistical Analysis. In Exp. 1 and 2, precision of marker-estimated fecal output was tested using a balanced 3 × 3 Latin square design with three wethers/square, two squares/replicate, and three periods (Cochran and Cox, 1957; Freund and Littell, 1981). The model included replicates, square within replicate, animal within square within replicate, period within square within replicate, treatment, and residual effects. Treatments within experiments were compared using the term (marker-estimated fecal output/total fecal collection) × 100.

In Exp. 3 and 4, precision of marker-estimated fecal output was tested using a crossover design (Federer, 1967). The model included effects for animal, treatment, and period. Treatments within experiments were compared using the term (marker-estimated fecal output/total fecal collection) × 100.

Accuracy of marker-estimated fecal output for all four experiments was compared with total fecal collection by a paired *t*-test (Freund and Littell, 1981; McClave and Dietrich, 1982). The *P*-value for each comparison provides an estimate of the accuracy of marker-estimated fecal output within a treatment (i.e., difference between marker estimates and total fecal collection). Small *P*-values indicate inaccuracy, but not necessarily lack of precision (i.e., repeatability of the estimate).

Results

Experiment 1

Intake and apparent *in vivo* DM digestion are shown in Table 2. Wethers fed 100 or 200 g of barley consistently consumed all the whole barley offered within 5 min of feeding. The mean difference (g DM fecal output/d) between total fecal collection and marker estimates of fecal output for wethers fed barley

TABLE 4. MARKER ESTIMATES OF FECAL OUTPUT COMPARED WITH TOTAL FECAL COLLECTIONS IN EXP. 1, 2, 3, AND 4

Experiment	Treatment	N	Fecal output, g DM/d		Mean difference, g DM/d	SE ^a	P ^b
			Total collection	Marker estimate			
1	0 g Barley	18	743	995	252	44.6	.01
1	100 g Barley	18	726	1,004	278	48.8	.01
1	200 g Barley	18	668	962	294	42.7	.01
2	70% Ad libitum	18	437	635	198	23.9	.01
2	85% Ad libitum	18	524	696	173	37.2	.01
2	Ad libitum	18	742	875	133	51.5	.02
3	0 g Barley	12	392	350	41	12.2	.01
3	200 g Barley	12	395	350	45	10.9	.01
4	Grazed	12	405	383	17	10.7	.14
4	Ungrazed	12	399	382	22	11.9	.09

^aStandard error associated with mean difference between total collection and marker estimated fecal output.

^bP-value for hypothesis of no difference between mean difference and 0.

at the rate of 0, 100, or 200g/d was not equal to 0 ($P = .01$; Table 4). Mean differences increased with an increase in the amount of barley fed, which also corresponded with a decrease in total fecal output.

Precision, expressed as marker-estimated fecal output divided by total fecal collections \times 100, also was different ($P = .05$; Table 5) among treatments. Both the 0 and 200 g barley treatments differed ($P = .06$ and $P = .02$, respectively) from the overall estimate of fecal output. The 100 g barley treatment did not differ ($P = .54$) from the overall mean.

The test for residual effects indicated a marginal ($P = .10$) carryover effect of the previous treatment on the precision of fecal output estimates in the following period. Specifically, the 0 and 200 g barley treatments influenced ($P = .07$ and $P = .05$, respectively) estimates of fecal output in the following period. Estimated fecal output using the chromic oxide bolus was 134, 138, and 144% of total fecal collections for 0, 100, and 200 g barley treatments, respectively (Table 4).

Experiment 2

Intake and apparent in vivo DM digestion are shown in Table 2. Intake by the wethers on the ad libitum treatment increased during the collection periods compared with DMI during adaptation. Hence, DMI for the 70 and 85% of

TABLE 5. COMPARISON OF TREATMENTS WITHIN EXPERIMENT FOR MARKER-ESTIMATED FECAL OUTPUT WHEN EXPRESSED AS A PERCENTAGE OF TOTAL FECAL COLLECTIONS IN EXP. 1, 2, 3, AND 4

Exp. and item	SE ^a	P ^b
1		
Treatment effect	2.15	.05
0 g Barley	3.41	.06
100 g Barley	3.41	.54
200 g Barley	3.41	.02
Residual effect	2.15	.10
0 g Barley	4.57	.07
100 g Barley	4.57	.90
200 g Barley	4.57	.05
2		
Treatment effect	3.10	.01
70% Ad libitum	4.91	.01
85% Ad libitum	4.91	.73
Ad libitum	4.91	.01
Residual effect	3.10	.42
70% Ad libitum	6.58	.54
85% Ad libitum	6.58	.48
Ad libitum	6.58	.20
3		
Treatment effect	2.30	.59
Treatment effect	2.48	.85

^aStandard error associated with treatment and residual effects are standard errors of the means. Standard errors associated with a particular treatment are standard errors of the estimated percentage units from the overall mean.

^bP-value (treatment and residual) for hypothesis of no difference between treatments. P-value for a particular treatment tests hypothesis of no difference between a treatment and treatment means.

ad libitum DMI treatments were actually 58 and 71% of ad libitum, respectively.

The mean difference between total collection and marker estimates of fecal output for the 70, 85% of ad libitum, and the ad libitum treatments was not equal to 0 ($P < .02$; Table 4). Mean differences (g DM fecal output/d) tended to increase with lower levels of DMI, which corresponded with a decrease in total fecal output. Precision of fecal estimates also was different between treatments ($P = .01$; Table 5). The test for residual effects indicated no ($P = .42$) effects of previous treatment on fecal output estimates. Estimated fecal output using the chromic oxide bolus was 145, 133, and 118% of total fecal collections for 70 and 85% of ad libitum and the ad libitum treatments, respectively (Table 4).

Experiment 3

Mean difference between total collection and marker estimates of fecal output for 0 and 200 g of barley/d treatments was not equal to 0 ($P = .01$; Table 4). Precision of marker estimates of fecal output was not different between treatments ($P = .59$; Table 5). Estimates of fecal output using the chromic oxide bolus were 88 and 87% of total fecal collections for 0 and 200 g barley treatments, respectively (Table 4).

Experiment 4

Although the intent of the heavily grazed treatment was to reduce DMI, fecal output was similar for both treatments (Table 4). Therefore, differences in forage availability most accurately describe the treatments applied in Exp. 4. The grazed pasture had 174 kg of herbaceous biomass/ha and the ungrazed pasture had 383 kg herbaceous biomass/ha.

The mean difference between total collection and marker estimates of fecal output for the grazed and ungrazed treatments was not different from 0 ($P = .14$ and $P = .09$, respectively; Table 4). Precision of estimated fecal output also was not affected by treatments ($P = .85$; Table 5). Estimated fecal output using the chromic oxide bolus was 94 and 96% of total fecal collections for grazed and ungrazed treatments, respectively (Table 4).

Discussion

Laby et al. (1984) concluded that the release rate of chromic oxide from the bolus was not affected by diet or by whether the animal was penned or grazing. In Exp. 1 and 2, both accuracy and precision of marker estimates of fecal output were affected by level of DMI and supplementation. The bolus also seemed to provide better estimates of fecal output with grazing wethers than with confined wethers. There are at least three possible explanations. First, chromic oxide release during Exp. 1 and 2 may have been less than the manufacturer-specified rate. Parker et al. (1989) may have overcome this problem by placing three boluses in ruminally fistulated wethers, thus minimizing bolus variation and increasing the concentration of chromic oxide in the feces. Second, DMI patterns of grazing animals may be more constant than those of confined animals that are given a once-daily allowance of feed. Third, variation in chromic oxide release among animals might result from differences in the mixing action in the rumen caused by differences in diet or levels of DMI. In addition, inconsistencies in flow of the chromic oxide from the rumen through the gastrointestinal tract might have occurred because the chromic oxide was not bound to plant fractions.

Parker et al. (1989) observed 100% recovery of chromium under both confined and grazing conditions. They did not, however, compare marker estimates of fecal output with total fecal collections. Additionally, they estimated chromium release to be 62 mg/d, but the release rate varied from 52 to 70 mg/d. It is questionable how marker recovery can be quantified when the marker release rate is so variable.

Precision of marker-estimated fecal output in Exp. 3 and 4 (Table 5) was high and compared favorably with the results of Ellis and Rodden (1987); however, their results are suspect. The average DM fecal output for ruminally cannulated wethers in their study was 166 g. Ellis and Rodden do not report either the type of pasture or a digestion coefficient. Assuming a 65% digestion coefficient, DMI would have been 252 g based on the average fecal output reported. This is 18% of the NRC (1985) projected DMI for a 40-kg replacement ewe.

Treatment affected marker estimates of fecal output in both Exp. 1 and 2, indicating that a subset of bagged animals within treatment groups may be required to adjust estimates of fecal output in research using the bolus. The residual effect in Exp. 1 indicates that animals should be treated as consistently as possible.

We conclude that differences exist between estimates of fecal output based on an intraruminal continuous-release chromic oxide bolus and total fecal collections. This was apparent in all the experiments conducted except Exp. 4. Precision of marker estimates of fecal output was low in confinement (Exp. 1 and 2) but acceptable in the grazing trials (Exp. 3 and 4). Total fecal collections on a subset of animals used in a study seem to be necessary to validate estimates of fecal output using chromic oxide boluses.

Implications

Many of the recommendations for dosing with chromic oxide in range nutrition are also applicable to use of the intraruminal continuous-release bolus. Factors that seem particularly important are the following: 1) results obtained under drylot conditions are not applicable to grazing trials, 2) chromic oxide should be used only for estimating fecal output on a comparative basis, and 3) comparisons should be limited to one trial. Comparisons should not be made between trials under different conditions.

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