Recovery of Fecal Chromium Used as a Digestibility Marker in Cattle

P. S. MIR, C. M. KALNIN, and S. A. GARVEY
Agriculture Canada Research Station
3015 Ord Road, Kamloops, BC, Canada V2B 8A9

ABSTRACT

Chromium content of fecal samples that were mixed with Cr-mordanted rumen contents at 5 or 10 g/200 g feces in Experiment 1 and at 5 or 10 g/300 and 200 g feces in Experiment 2 was determined by atomic absorption spectroscopy. Recovery of Cr was estimated after digesting fecal samples, which were frozen fresh and later thawed or dried in either a forced draft oven at 60°C or in a microwave oven in Experiment 1. Experiment 2 evaluated the efficacy of each of three methods of digestion in recovery of Cr from fecal samples. Recoveries of Cr from feces were calculated relative to the Cr content of the Cr-mordanted rumen contents, which were determined by atomic absorption spectroscopy of samples digested with concentrated nitric acid in the presence or absence of the wetting agent Tween 80. Recovery of Cr from fecal samples frozen fresh and later thawed was greater than that from either forced draft oven or from microwave oven-dried samples, especially when the concentrated nitric acid digestion was used. Neither the double ashing procedure nor the digestion with the weaker nitric acid did not improve Cr recoveries from the samples.

INTRODUCTION

Chromium as chromic oxide has been used as a marker for determination of digestibility of feeds. The atomic absorption spectrophotometric (AAS) method of Williams et al. (9) and the spectrophotometric method of Fenton and Fenton (3) are among the various procedures that have been developed to determine Cr content of feeds and feces. However, loss of Cr due to volatilization with high temperature ashing (4) or through formation of volatile chromyl chloride compounds (1) with perchloric acid digestion is a problem related to using these methods.

To avoid these problems, Robinson et al. (5) used a procedure in which feces containing Cr-mordanted feed were digested with concentrated nitric acid at low temperature to release the Cr from the organic material for determination by AAS. These same authors (2) published an alternate procedure that required longer contact time between the digesting acid and the sample to ensure complete digestion of the organic matrix of the sample and release of the Cr. Nevertheless, variance in recovery with wet digestion procedures has been a problem when biological materials were analyzed for Cr content (1). Blincoe et al. (1) published a procedure where samples are ashed twice at 500°C for 4 h each time with a concentrated nitric acid treatment.

However, all these procedures require that the samples be dried and ground prior to digestion. These processes, in addition to being time-consuming and labor intensive, also cause sample particles to float above the acid during digestion, which then become deposited on the sides of the beaker. This results in loss of contact between the sample particles and the acid, despite periodic shaking during digestion, and longer digestion times, which ultimately result in incomplete digestion of the sample and variable recovery of Cr. The efficacy of Cr recovery from samples digested without drying and grinding has not been evaluated and requires investigation.

Therefore, the present study was conducted with the objectives of determining recoveries of Cr from Cr-containing feces samples without previously drying and grinding the samples and thus of minimizing time required for Cr analysis, and to determine whether method of drying or method of digestion of these samples affected Cr recovery. Recovery of Cr from feces

Received August 19, 1988.
Accepted March 10, 1989.

that were frozen fresh and later thawed (FFT) was compared with that from forced draft oven (FDO) or microwave oven (MWO)-dried feces, whereas the three digestion methods evaluated were those of Robinson et al. (5), Blincoe et al. (1), and Fadel et al. (2).

MATERIALS AND METHODS

Experiment 1

Rumen contents from a steer fed alfalfa hay were obtained and Cr-mordanted rumen contents (CrMRC) were prepared by following the procedure described by Uden et al. (8). The samples were dried and then ground in a hammer mill to pass through a 1-mm screen. The CrMRC was mixed into feces samples collected from steers.

Fecal samples from four steers were obtained by rectal stimulation and either 5 or 10 g of the CrMRC were thoroughly mixed into 200 g of fresh feces. One-half of these samples were dried in a FDO at 60°C to constant weight in order to determine DM content and then ground in a hammer mill with a 1-mm screen and saved for Cr determination. Three 2-g samples from the other half of each feces sample were weighed into beakers and frozen. The remaining feces containing the CrMRC were dried in a MWO at maximum power (720 W) for 2-min durations until the material achieved constant weight, then ground as the FDO dried samples were.

Six samples of CrMRC, each approximately .05 g, were weighed into beakers, and three of these samples were treated with Tween 80 (Fisher Scientific, Nutley, NJ) to immerse the sample and held at room temperature overnight. The previously frozen fecal samples were thawed at 5°C. Approximately .5 g of both the FDO and MWO dried feces were weighed into beakers in triplicate. To all of the samples, 15 ml of concentrated nitric acid were added and placed in a water bath and digested according to the procedure of Robinson et al. (5).

Experiment 2

Feces were obtained by rectal stimulation from a steer fed alfalfa hay. Five and 10 g of CrMRC were mixed with a glass rod into approximately 300 and 200 g of the fresh feces, respectively. As in Experiment 1, 2 g of fresh feces containing the CrMRC were placed in each of six beakers for the feces samples containing the two concentrations of CrMRC and then frozen. The remaining feces samples were again divided into two portions and either dried in a FDO at 60°C or in a MWO as described previously.

The Cr content of the feces samples, frozen in the beakers, and that of the FDO and MWO dried samples was determined by AAS after treating the samples in duplicate by each of the methods described by Robinson et al. (5), Blincoe et al. (1), and Fadel et al. (2). Approximately .5 g of each of the dried samples were used for digestion.

The data from Experiment 1 were analyzed as a 2 (concentration of CrMRC inclusion) × 3 (conditions of the feces) factorial design using the general linear models procedure (6). The data from Experiment 2 were analyzed for variance as a 3 (conditions of the feces) × 3 (methods of treatment) factorial design, and differences among the factors were estimated using the least significant difference method (7).

RESULTS AND DISCUSSION

The Cr content of the dried CrMRC was 3.2 ± .1 and 4.0 ± .1% for untreated samples and samples treated with Tween 80, respectively. Wetting treatment resulted in higher ($P<.05$) values for Cr, suggesting that the treatment increased Cr recovery from this sample. The DM and the expected Cr contents of the feces samples used in Experiment 1 are shown in Table 1. The calculated Cr content of the samples varied with the value used for the Cr content of the CrMRC; thus, estimates using both (Tween-80, untreated and treated) values are listed. The determined Cr content of the feces samples and percentage of recovery of Cr relative to the content of the CrMRC with and without treatment with Tween 80 are shown in Table 2. The values for the MWO dried fecal samples include only those from two and three animals for the low and high concentrations of CrMRC inclusion, respectively, because the other three samples were charred during drying, and the values obtained for the charred samples were omitted.

In Experiment 1 the Cr content of the MWO dried and FFT samples was greater ($P<.05$)

TABLE 1. Dry matter and expected chromium values of the feces samples.

Steer	Concentration of CrMRC[1] inclusion	Dry matter content	Chromium content relative to CrMRC content	
			Untreated	Treated with Tween 80
	(g/200 g feces)	(%)	(g/100 g DM)	
1	5	11.1	.72	.88
	10	13.4	1.19	1.45
2	5	17.7	.45	.55
	10	19.3	.83	1.01
3	5	14.4	.56	.68
	10	16.9	.95	1.16
4	5	10.7	.75	.91
	10	12.8	1.25	1.52
Mean ± SEM	5	13.5 ± 1.6	.62 ± .07	.76 ± .08
Mean ± SEM	10	15.6 ± 1.5	1.06 ± .10	1.29 ± .12

[1]CrMRC = Chromium-mordanted rumen contents.

than that of the FDO dried feces when the lower of the two amounts of CrMRC was added to the feces. However, when the higher amount of CrMRC was included, only the Cr content of the FFT was greater ($P<.05$) than that of the dried feces.

The percentage of Cr recovered from the FFT samples was greater than that of the dried samples with recovery of Cr from the MWO dried samples being better ($P<.05$) than that of the FDO dried samples. However, recoveries in this experiment were less than 100% when they were calculated relative to expected values, based on Cr content for Tween 80 treated CrMRC (Table 2). These results suggest that further improvements were required. Therefore, Experiment 2 was conducted to evaluate the effect of the three methods of digesting the sample prior to AAS for Cr determination.

In Experiment 2, recoveries were calculated relative to Cr content of Tween 80-treated CrMRC, and the expected Cr values were .39

TABLE 2. Content and percentage recovery of chromium from fecal samples.

Sample	Concentration of chromium-mordanted rumen contents (CrMRC) added to 200 g of feces					
	5 g			10 g		
	Cr, % of DM	Recovery		Cr, % of DM	Recovery	
		1[1]	2[2]		1[1]	2[2]
	(%)			(%)		
Oven dried	.52[b]	83.0[c]	68.3[c]	.88[b]	82.6[b]	67.9[b]
Microwave dried	.68[a,3]	92.6[b]	79.9[b]	.97[ab,4]	86.6[b]	71.5[b]
Fresh	.65[a]	106.1[a]	87.0[a]	1.05[a]	99.9[a]	82.4[a]
frozen, then thawed						
SEM	.05	2.4	1.9	.04	2.2	1.8

[a,b,c]Means with different letters in a column differ ($P<.05$).

[1]Recoveries were calculated relative to Cr content of CrMRC without treatment with Tween 80.

[2]Recoveries were calculated relative to Cr content of CrMRC treated with Tween 80.

[3]Values for feces from animals 1 and 4. Values from feces from the other animals were omitted because they were charred while drying.

[4]Values for feces from animals 1, 3, and 4. Values from feces from the other animals were omitted because they were charred while drying.

TABLE 3. Content and recovery of chromium from dried or frozen fresh feces analyzed by three methods.

Concentration of Cr-mordanted rumen contents (CrMRC)	Condition of feces	Method of analysis	Cr, % of DM	Recovery[1]
				(%)
1[2]	Oven dried	1[3]	.38[c]	96.4[c]
1	Oven dried	2	.30[ab]	75.9[ab]
1	Oven dried	3	.32[abc]	81.0[ab]
1	Microwave dried	1	.27[a]	69.0[a]
1	Microwave dried	2	.29[ab]	73.3[a]
1	Microwave dried	3	.35[bc]	88.7[bc]
1	Fresh frozen, then thawed	1	.38[c]	96.4[c]
1	Fresh frozen, then thawed	2	.28[a]	72.0[a]
1	Fresh frozen, then thawed	3	.34[bc]	86.1[bc]
2	Oven dried	1	.74[c]	80.4[b]
1	Oven dried	2	.74[c]	79.9[b]
2	Oven dried	3	.72[bc]	77.7[b]
2	Microwave dried	1	.53[a]	57.6[a]
2	Microwave dried	2	.73[c]	79.3[b]
2	Microwave dried	3	.67[b]	72.3[b]
2	Fresh frozen, then thawed	1	1.00[d]	108.6[c]
2	Fresh frozen, then thawed	2	.70[bc]	76.1[b]
2	Fresh frozen, then thawed	3	.67[b]	72.5[b]
	SEM		.02	5.2

[a,b,c,d]Means with different letters within each concentration of CrMRC inclusion in a column differ ($P<.05$).

[1]Recoveries were calculated relative to chromium content of CrMRC treated with Tween 80.

[2]For concentration 1, 5 g of CrMRC were added to 300 g of feces while for concentration 2, 10 g of CrMRC were added to 200 g of feces.

[3]1, Robinson et al. (5); 2, Blincoe et al. (1); and 3, Fadel et al. (2).

and .92% for the two concentrations of CrMRC inclusion. In this experiment, the best recoveries were noted for the FFT samples containing either amount of CrMRC and the FDO dried sample containing the lower concentration of CrMRC, when both were digested by the procedure outlined by Robinson et al. (5) (Table 3). However, digestion of MWO-dried samples by this procedure resulted in the lowest recoveries. These results are different from those obtained in Experiment 1, where recoveries of Cr from MWO-dried samples were higher than or equal to those from FDO-dried samples. The improved recovery of Cr from FDO dried samples in this experiment may be related to the lower ratio of CrMRC to feces used in the second than in the first experiment. However, the recovery was only 80% for the FDO dried samples when the higher concentration of CrMRC was added to the feces samples.

Values obtained for ashed samples (1) were lower than those obtained by the other two methods for FFT samples at lower concentration of CrMRC inclusion (Table 3). However, recoveries of Cr were similar to those obtained by the other two methods for the dried samples with the exception of the FDO dried samples containing the lower concentration of CrMRC. This is contrary to the claims of Blincoe et al. (1) and may be related to the smaller sample sizes in our experiment. However, Kumpulainen et al. (4) reported that acid-insoluble silicates formed during ashing of the samples can adsorb Cr, rendering that fraction unavailable for estimation.

The use of the procedure described by Fadel et al. (2) produced values for Cr similar to those obtained by the procedure of Robinson et al. (5) for the FFT and the FDO dried samples, whereas values for the MWO dried samples were greater ($P<.05$) when the lower concentration of CrMRC was added to the feces. However, at the higher concentration of CrMRC inclu-

sion, there was no advantage to using this relatively lengthy procedure.

Among the procedures examined, the rapid method of Robinson et al. (5) appeared to be adequate, especially if FFT samples could be used. The improvement in recovery with the use of the FFT samples may be related to the smaller DM content (.36 g if sample size is 2 g and DM content is 18%) of the sample than the .5-g sample recommended by Robinson et al. (5).

In conclusion, the results from the two experiments suggest that the rapid method of Robinson et al. (5) could be utilized successfully if FFT samples are used. Dry matter determinations of samples would be required, but time and labor invested in grinding the samples could be avoided if Cr determinations are performed with the FFT samples and later corrected for DM content. The digestion procedure is simple and relatively rapid. As a result, turnaround time for analysis can be reduced considerably, especially because DM and Cr determinations can be conducted simultaneously.

REFERENCES

1 Blincoe, C., M. O. Theisen, and K. Stoddard-Gilbert. 1987. Sample oxidation procedures for the determination of chromium and nickel in biological material. Commun. Soil Sci. Plant Anal. 18:687.

2 Fadel, J. G., P. Uden, and P. H. Robinson. 1987. Effect of nitrogen and energy supplements on intake and digestion of oat straw by non-lactating dairy cows. J. Agric. Sci. Camb. 109:503.

3 Fenton, T. W., and M. Fenton. 1979. An improved procedure for the determination of chromic oxide in feed and feces. Can. J. Anim. Sci. 59:631.

4 Kumpulainen, J. T., W. R. Wolf, C. Veillon, and W. Mertz. 1979. Determination of chromium in selected United States diets. J. Agric. Food Chem. 27:490.

5 Robinson, P. H., S. Taminga, and A. M. van Vuuren. 1987. Influence of declining level of feed intake and varying the proportion of starch in the concentrate on milk production and whole tract digestibility in dairy cows. Livest. Prod. Sci. 17:19.

6 SAS User's Guide. 1982. SAS Inst., Inc. Cary, NC.

7 Steel, R.G.D., and J. H. Torrie. 1980. Principles and procedures of statistics. McGraw-Hill, New York, NY.

8 Uden, P., P. E. Colucci, and P. J. Van Soest. 1980. Investigation of chromium, cerium and cobalt as markers in digesta. Rate of passage studies. J. Sci. Food Agric. 31: 625.

9 Williams, C. M., D. J. David, and O. Iismaa. 1962. The determination of chromic oxide in faeces samples by atomic absorption spectrophotometry. J. Agric. Sci. 59: 381.