Queensland Journal of Agricultural and Animal Sciences Vol. 40 (2), 141-143 (1983)
Published by the Queensland Department of Primary Industries

Collection and preparation of chromic oxide marked faeces for estimation of intake by grazing cattle

R.L. Roofayel, J. Evans and A.E. Burge

Summary

A procedure is described for collecting and subsampling faeces containing chromic oxide (Cr_2O_3) as marker for estimation of intake by cattle. It was developed, evaluated and adopted at Coolum Research Station.

Cr_2O_3 in glycerine was administered (oral-drip) to test animals before collecting faecal samples. Individual samples were mixed, subsampled, dried and ground before analysis for chromium.

The subsampling procedure was evaluated using 37 sample collections, analysing in each case both the finely-ground subsample and the finely-ground bulk sample. Subsamples were accepted as effectively representing the collected sample.

The effect of different milling processes was evaluated using 12 duplicated subsamples and two mill types (shatterbox and beater-cross). A 2-way replicated analysis of variance (ANOV) test showed no difference between mills. Particle-size distributions are presented for the two mills. The shatterbox mill gave a much finer grind.

INTRODUCTION

Intake of grazing animals can be measured if faecal output and coefficients of digestibility are obtained simultaneously. The technique of total collection for estimating faecal output is both difficult and tedious. A recognised alternative is to administer an indigestible marker (Smith and Reid 1955) and measure its concentration in the faeces.

Chromic oxide (Cr_2O_3) is generally considered the most satisfactory marker (Reid 1962, Kotb and Luckey 1972, Langlands 1975), although other markers have been used (Kotb and Luckey 1972). Fluctuating diurnal excretion patters make it difficult to obtain a representative sample even though several methods of administration have been tried (Raymond and Minson 1955, Pigden and Brisson 1956, Corbett, Greenhalgh, McDonald, and Florence 1960).

Tudor (1980, pers comm.) developed a gravity flow system for oral infusion at a fairly constant rate of Cr_20_3 suspended in glycerine.

The oral-drip apparatus being attached to the head of the animal did not affect its grazing habits. This method was combined with a procedure developed, tested and adopted at Coolum Research Station for collecting and preparing marked faecal samples for chemical analysis. The heterogeneity of chromium-marked faeces suggested that care be taken not only in the method of sample collection, but also in the procedures of subsampling and grinding before analysis (Utley, Bradley, Boling 1971).

The adopted procedure was evaluated by testing whether chromium concentration in the subsample was representative of that in the collected sample. Very fine grinding (shatterbox) was used in this test.

In a later experiment the effect of different milling processes was evaluated. A shatterbox and beater-cross mill were used. Since most Departmental research stations are equipped with beater-cross mills it would be convenient if the method of milling had no effect. Particle-size distributions for both mill types were compared.

METHODS

Faecal samples were collected twice daily at 7.30 a.m. and 3.30 p.m. from animals pretreated with Cr_2O_3 using the oral-drip technique. If voluntary defaecation took place at sampling time, the entire dropping was collected in a plastic bucket either as the faeces dropped from the animal or from the pasture surface. Care was taken to avoid contamination from soil, stone and debris when recovering samples from the ground. Rectal samples were manually collected from animals still to be sampled.

Individual faecal samples were stirred into a stiff slurry adding, if necessary, a *small* quantity of water. Collection buckets were covered during heavy rain. A subsample of about 100 g slurry was spread in a thin layer on a labelled plastic bag and dried in a mechanical convection oven at 70°C for 72 h. After 48 h the samples were turned to aid drying.

The procedure was evaluated using a total of 37 faecal samples which were collected, mixed and subsampled as described. Both the subsample and the residue of the sample were dried at 70°C and then very finely ground in a Spex shatterbox mill for 3 min using a large plain-steel vial. The method of Roofayel and Lyons (1984) was used to determine chromium concentrations in the subsamples and sample residues. These concentrations were compared using a paired *t*-test.

In a later experiment, the effect of different milling processes was evaluated. Twelve faecal samples were collected and prepared as described, with two subsamples being taken from each sample. One subsample was ground in a Spex shatterbox mill and the other in a Christy and Norris (C & N) beater-cross mill fitted with a 1 mm sieve. Each ground subsample was analysed four times for chromium as before. Duplicate instrument readings were recorded for each analysis. A 2-way replicated ANOV procedure was used to test for differences between mills.

Particle-size distributions were measured on four ground samples selected at random for each mill for comparison of fineness of grinding.

RESULTS AND DISCUSSION

Subsampling procedure

Data and paired *t*-test calculations (Table 1) show that variances for subsamples and sample residues were similar. Although the paired *t*-statistic of 2.49 is significant at the 2% level ($t_{2\%}$=2.44) the absolute mean difference between sample and subsample is small relative to sample values—a relative difference of only 0.7% (40.4×100/5547.6). Thus, the subsampling procedure was accepted as satisfactory.

Milling procedure

Shatterbox milling produced a much higher proportion of finer material than hammer milling (Table 2).

Table 1. Evaluation of subsampling procedure: paired *t*-test

	Chromium (μg/g)	
	mean	s.d.
Subsamples	5648.35	±757.40
Sample residues	5547.62	±799.25

Means paired-difference=100.73μg/g Cr

s.e.=±40.405μg/g Cr

Paired *t* (df=36)=2.49

Means significantly different (P<0.05)

Table 2. Particle-size distributions in samples ground in two different mills

		Shatterbox				Hammer mill			
		Samples				Samples			
		A	B	C	D	A	B	C	D
500μ	(%)	0.5	0.1	0.1	0.1	0.5	0.6	0.7	0.7
500-200μ	(%)	2.8	0.4	1.7	1.2	31.5	34.3	31.1	31.2
250-66μ	(%)	38.6	30.1	44.7	38.7	59.5	58.5	59.9	59.4
66μ	(%)	37.0	43.1	37.1	47.9	4.5	2.9	4.4	5.3
Initial weight	(g)	8.49	5.39	10.28	11.55	20.21	17.37	21.47	24.50
Loss	(g)	1.79	1.42	1.69	1.40	0.81	0.64	0.84	0.86
Loss	(%)	21.1	26.3	16.4	12.1	4.0	3.7	3.9	3.5

However, no significant difference in chromium analysis values was obtained between samples from each mill (Table 3), the means for which were 6098μg/g (shatterbox) and 6031μg/g (hammer mill). Standard deviations for S×M, replications and observations were in the expected decreasing order.

The more accessible C & N hammer mill was adopted for routine use in sample preparation.

Table 3. Evaluation of mill effect: 2-way ANOV replicated with repeated observations

Source	d.f.	Mean square	*F* ratio	s.d.
Mills (M)	1	214668.8	0.63	
Samples (S)	11	6078940.0	179**	
S×M	11	340523.3		583.5
Replications	72	108329.9		329.1
Observations	96	2627.08		51.26

** Significant at 1% level.

References

Corbett, J.L., Greenhalgh, J.F., McDonald, I. and Florence, E. (1960), The excretion of chromium sesquioxide administered as a component of paper to sheep, *British Journal of Nutrition* **14**, 289.

Kotb, A.R. and Luckey, T.D. (1972), Markers in nutrition, *Nutrition Abstracts and Reviews* **42** (3), 813.

Langlands, J.P. (1975), Techniques for estimating nutrient intake and its utilization by the grazing ruminant, in *Proceedings 4th International Symposium on Ruminant Physiology,* 1974. ed. I.W. Mcdonald and A.C.I. Warner, University of New England Press.

Pigden, W.J. and Brisson, G.J. (1956), Effect of frequency of administration of chromic oxide on its faecal excretion pattern by grazing wethers, *Canadian Journal of Agricultural Science* **32** (2), 146.

Raymond, W.F. and Minson, D.J. (1955), The use of chromic oxide for estimating the faecal production of grazing animals, *Journal of the British Grassland Society* **10** (4), 282.

Reid, J.T. (1962), Indicator methods in herbage quality studies, in *Pasture and Range Research Techniques,* American Society of Agronomy. Comstock Publishing Associates, New York.

Roofayel, R.L. and Lyons, D.J. (1984), Determination of marker chromium in faeces using inductively coupled plasma emission spectrometry, *Analyst*, **109**, 523.

Smith, A.M. and Reid, J.T. (1955), Use of chromic oxide as an indicator of faecal output for the purpose of determining the intake of pasture herbage by grazing cattle, *Journal of Dairy Science* **38** (5), 515.

Utley, P.R., Bradley, N.W. and Boling, J.A. (1971), Methods of sample preparation for chemical determination of chromic oxide in bovine faeces, *Journal of Dairy Science* **54** (7), 1091.

(Received for publication 21 March 1982)

The authors are officers of the Department of Primary Industries. Mr Roofayel and Mr Burge (formerly) are Chemists, Div. I in Agricultural Chemistry Branch, Indooroopilly Q 4068. Mr Evans is a District Experimentalist, Div. I in Research Stations Branch and is the Manager, Mutdapilly Research Station, Mutdapilly Q.