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Chromic oxide contamination of pasture previously used in marker studies

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Abstract

Fecal output of range cows was determined during 2 periods of summer (period I) and late summer (period II) grazing using a constant release intraruminal Cr_2O_3 bolus. Chromic oxide contamination was determined by analyzing forage for Cr_2O_3 and by obtaining fecal samples from cows prior to bolusing. Control cows were also monitored along with the experimental cows during grazing periods. The overall herd least squares mean for fecal output during period I was lower than the expected value by 48%. Forage during period I contained an average of 55.7 µg Cr_2O_3 g^{-1} of forage or about 45% of the daily dose of the bolus. Forage during period II contained an average of 38.3 µg Cr_2O_3 g^{-1} of forage or about 29% of the daily dose of the bolus. Our results indicate that comparisons of fecal output least squares means by period of the year can be biased by Cr_2O_3 contamination of forage in pastures which have been previously used for marker studies.

Key Words: fecal output, range cattle

Organic matter intake of free ranging beef cows is often estimated by the ratio technique (Cordova et al. 1978). Fecal output on an organic matter basis has often been determined using an external marker such as chromium sesquioxide. Methods of administering Cr_2O_3 have included daily dosage of 10 g Cr_2O_3 in a gelatin capsule (Raleigh et al. 1980), Cr_2O_3 impregnated in paper in a gelatin capsule and daily dosing (Kiesling et al. 1969), Cr mordanted to fiber (Pond et al. 1987), and more recently by using a constant release bolus (Harrison et al. 1981).

Many researchers have tested the accuracy of the constant release bolus (Laby et al. 1984, Parker et al. 1989, Furnival et al. 1990a, Furnival et al. 1990b, Adams et al. 1991, Butinx et al. 1992, King et al. 1992), but few have measured background Cr_2O_3 in the forage. Due to the low daily dose of the constant release bolus (1.68 to 1.74 g • day^{-1} in our study), and the fact that many forage intake or fecal output studies use pastures that have been previously used in large dose Cr_2O_3 (10 g • day^{-1}) studies, Cr_2O_3 contamination of forage could be a potential problem.

The objective was to determine if background levels of Cr_2O_3 were present in pastures previously used for intake studies where Cr_2O_3 had been used to estimate fecal output, and if the background levels could bias estimates of fecal output based on the constant release bolus data.

Materials and Methods

Fifty-one 2- to 4-year-old producing range cows from the Northern Agricultural Research Center herd near Havre, Mont. were randomly selected from 3 biological types: Hereford, Tarentaise, and Tarentaise × Hereford (or Hereford × Tarentaise), with 15, 15, and 17 cows in each group respectively.

Daily fecal output was estimated during 2 periods. Collection periods were (1) lactating, summer grazing (period I) and, (2) lactating, late summer grazing (period II). For each cow during each period, fecal output was determined using a constant release intraruminal bolus[1] containing Cr_2O_3. Following a 10 day equilibration period, 3 fecal grab samples, 3 days apart, were obtained, frozen, and analyzed later for Cr_2O_3 concentration.

Cows that lost a bolus by regurgitation during a sample period were eliminated by either finding a regurgitated number coded bolus or by an unreasonable fecal Cr_2O_3 concentration reading. Unreasonable Cr_2O_3 concentrations were defined as having a spectrophotometric absorbance less than 0.018, which would be equivalent to 170.3 µg of recovered Cr_2O_3 per g of feces. This would be equivalent to a dry matter intake for the heaviest cow in the experiment (639 kg) of 3.5 % of body weight. Steady decreases of absorbance from sample to sample within period (indicating a lost bolus) and large fluctuations of absorbance between samples (beyond expected daily variation in fecal output) were also considered and these cows were deleted for that sample period also. There were 8 cows which were eliminated in both sample periods.

Fecal samples were analyzed for dry matter and organic matter according to AOAC (1980) procedures. Fecal samples were ground through a 1-mm screen and chromium content was determined using the acid digestion and spectrophotometric procedure

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[1] "Captec Chrome", Nufarm, Auckland, NZ

Table 1. Chemical composition, fiber, IVOMD, forage production and Cr_2O_3 content of forage.

Pd	Type	DM[a]	Ash	PROT[a]	NDF[a]	IVOMD	PROD[a]	Cr_2O_3[a]
		----------	----------	%	----------	----------		
I	Grass Clipping	43.63	15.66	10.32	61.15	60.09	770 ± 92[b]	58.2 ± 13.0[b]
	Forb Clipping	28.38	14.71	14.87	38.68	59.52	486 ± 80	53.9 ± 11.3
II	Grass Clipping	79.63	10.93	5.96	63.23	57.13	775 ± 115	43.3 ± 7.9
	Forb Clipping	75.13	11.60	7.82	45.87	57.58	397 ± 89	33.3 ± 6.4

[a] DM = dry matter; PROT = protein; NDF = neutral detergent fiber; IVOMD = in vitro organic matter digestibility; PROD = kg DM production / ha; Cr_2O_3 = mg/g forage.
[b] Least-squares means ± SE.

described by Fenton and Fenton (1979). Two grams of feces were digested in 50 ml erlenmeyer flasks and then diluted to 50 ml due to the reduced amount of Cr_2O_3 dispensed daily by the constant release bolus.

During the study periods, cattle grazed range forage in a pasture in the Bears Paw mountains characteristic of the foothill range of North Central Montana. The area is classified in the Forest-Grassland complex of the western glaciated plains. Pasture elevation averaged 1,300 m with slopes ranging from 0 to 40%. Annual precipitation averages 46 cm and was 45 cm during this study. Upland areas were dominated by rough fescue (*Festuca scabrella* Torr.), Idaho fescue (*Festuca idahoensis* Elmer), and bluebunch wheatgrass (*Agropyron spicatum* (Pursh) Scribn. & Smith) with an open ponderosa pine (*Pinus ponderosa* Laws.) overstory. Kentucky bluegrass (*Poa pratensis* L.) dominated the lowland vegetation.

During the study periods, hand-clipped forage samples were collected once from sites located at the bottom, lower, middle, and upper slopes of a north- and south-facing aspect of a typical slope within the pasture. Vegetation clipped in 5 random 0.5 m^2 hoops was collected from each site and forage was pooled within site and separated into grass and forbs. Forage samples were analyzed for dry matter, ground through a 1-mm screen and analyzed for Kjeldahl N and organic matter (AOAC 1980), acid detergent fiber (Goering and Van Soest, 1970) and neutral detergent fiber (Robertson and Van Soest, 1977). In vitro organic matter digestibility was determined using the Barnes modification of the Tilley and Terry in vitro technique (Harris 1970). Digestibility was determined on an organic matter basis by filtering samples through glass crucibles with room temperature water after the pepsin digestion step, drying overnight at 100° C, and ashing at 450° C overnight. Three droplets of termamyl 120L enzyme (Novo Nordisk Bioindustrials, Inc., Danbury, Conn.) were added to each sample during the filtering process of the in vitro organic matter digestibility procedure to aid filtering. Chromium sesquioxide content of the forage was determined using procedures described previously.

Chromic oxide contamination of the forage was evaluated by: (1) analysis of forage as outlined above, and (2) by taking prebolus fecal samples from control cows and experimental cows during the study periods. During period I, Cr_2O_3 background was observed in the prebolus fecal samples of the 3 control cows and 3 of the 51 experimental cows tested. Therefore, in period II, all cows were sampled prior to bolusing. The 3 control cows were also sampled with the experimental cows during the 3 postbolus samples for periods I and II.

The average of all 3 fecal samples collected for each cow for each period was analyzed by period using least squares analysis of variance techniques (SAS 1987). Fixed main effects during both periods were breed and cow age. Chromium concentrations for the prebolus sample of period II were analyzed using least squares techniques with breed and cow age as main effects.

Results and Discussion

The results of the forage analyses are presented by period (Table 1). Production of grasses and forbs combined exceeded 1,000 kg • ha^{-1}. Digestibility of the forage during both summer grazing periods was adequate, exceeding 50%. Protein was adequate during period I, exceeding the 9.6 % requirement for lactating cows (NRC 1984). Protein in period II was 3% lower than the specified daily protein content for lactating cows.

The overall cow herd least squares means for fecal output were 2,678 and 3,798 g for periods I and II, respectively. In past studies at this location with similar organic matter digestion coefficients (50 to 61%; Wagner et al. 1986, Doornbos et al. 1987), lactating cows during early summer grazing (similar to period I) were found to have intakes averaging 28 g organic matter intake/kg body weight. Using the average organic matter digestibility in period I of 60%, the average fecal output of 2,678 g, and the average period I cow herd weight of 504 kg, the observed mean intake was 13.3 g organic matter intake/kg body weight during period I. This is a 48% reduction compared with the 28 g organic matter intake/kg body weight observed by Wagner et al. (1986) and Doornbos et al. (1987).

Forbs and grasses analyzed in period I were 14.87 and 10.32% protein and 59.5 and 60.1% digestibility, respectively. The forage contained an average of 55.7 ± 5.2 μg of Cr_2O_3 g^{-1} of forage (0.79 g • day^{-1} • cow^{-1}; based on 28 g organic matter intake/kg body weight or 14,112 g organic matter intake) or 45% of the daily Cr_2O_3 release by the bolus. This level of Cr_2O_3 in the forage combined with the normal bolus release rate explains why the fecal output least squares mean during period I was lower than previously reported values (Wagner et al. 1986, Doornbos et al. 1987).

Past studies by Wagner et al. (1986) using this pasture in late summer (similar to period II) with a forage digestibility of 57% reported forage intakes ranging from 19 to 24 g organic matter intake/kg body weight. During period II, forage had 7.82 and 5.96% protein and 57.6 and 57.1% digestibility for forbs and grasses, respectively. Cow weights in period II averaged 555 kg and the average fecal output was 3,798 g, or 16 g organic matter intake/kg cow weight. This is 16 to 33% less than organic matter intake values reported by Wagner et al. (1986). Chromic oxide concentrations in clipped forage in period II contained 38.3 ± 2.7 μg of Cr_2O_3 g^{-1} of forage (0.51 g • d^{-1} • cow^{-1}; based upon 24 g organic matter intake/kg body weight or 13,320 g organic matter intake) or an overdosage of 29% of Cr_2O_3 daily.

Fecal recoveries of Cr_2O_3 from the control cows during period I averaged 311 μg × g^{-1} of feces. Normal Cr_2O_3 fecal recoveries for

Table 2. Fecal recovery of Cr_2O_3 (µg / g) from control cows during period II. 1991.

Cow ID No.	Prebolus sample 29 Aug.	Sample 1 10 Sep.	Sample 2 13 Sep.	Sample 3 16 Sep.
Control cows		(µg/g)		
7173	339.7	269.9	11.0	70.7
8037	240.0	548.8	329.7	50.8
5505	379.5	260.0	90.6	120.5

experimental cattle during nongrazing periods (winter feedlot) ranged from ≈ 220 µg to 680 µg of Cr_2O_3 g^{-1} of feces (Sprinkle 1992). Barlow et al. (1988) had background fecal Cr_2O_3 readings of 4, 6, and 9 µg g^{-1} of feces for high, medium, and low quality pastures, respectively. Based on this data, our control cows had from 35 to 78 times the background Cr_2O_3 values Barlow et al (1988) reported. The data reported by Barlow et al. (1988) fits more closely in the expected range (1 to 5 µg • g^{-1}) which has been reported in plant material (Pratt 1966, Wallace and Romney 1977).

Fecal samples from control cows and experimental cows during the prebolus sample of period II contained large amounts of background Cr_2O_3. The overall least square mean for the experimental cow herd was 248.7 µg Cr_2O_3 g^{-1} of feces. There were no significant differences (P>0.10) for breed groups (n = 12, 13, and 15 for Tarentaise, Hereford, and Tarentaise × Hereford, respectively) or cow age (n = 10, 15, and 15 for 2-, 3-, and 4-yr-olds, respectively) in recovered Cr_2O_3.

Chromic oxide fecal concentrations for the control cows during period II are presented in Table 2. Sample 1 was high in recovered Cr_2O_3, but by sample 2, Cr_2O_3 concentrations had dropped 60% for the control cows. Therefore, period II fecal output least square means were not depressed to the same levels as in period I. For all cows in the experiment, the least squares mean for sample 1 Cr_2O_3 weight in period II was 64% greater than for sample 2, indicating a reduction over time in the amount of background Cr_2O_3 ingested during late summer.

Because Cr_2O_3 concentrations in the forage base rapidly changed, as evidenced by the control cow fecal outputs (Table 2) during period II, it would not be appropriate to adjust observed Cr_2O_3 fecal recoveries by the prebolus sample as did Barlow et al. (1988).

Normal Cr content for plant leaves is usually less than 5 µg x g^{-1}, and most often less than 1 µg • g^{-1} on non-serpentine soils (Pratt 1966, Wallace and Romney 1977). By calculating the molecular weights of Cr in the Cr_2O_3 background observed in this study, we found the plant material contained an average of 19.1 and 13.1 µg Cr g^{-1} of forage in periods I and II, respectively.

Researchers have found Cr concentrations by plants to be mostly confined to the roots (Shewry and Peterson 1974, Lahounti and Peterson 1979). However, plants exposed to Cr VI have much greater translocation of Cr to the shoots than plants exposed to Cr III (Lahounti and Peterson 1979). Forage intake studies at the summer pasture in the past used Cr_2O_3 with Cr in the Cr III state. It is possible that oxidizing agents in the soil oxidized the Cr III to Cr VI and increased Cr translocation to the leaves of plants in this study (Lahounti and Peterson 1979, James and Bartlett 1984). Plants vary in their ability to transport Cr to the shoots of the plant (Lahounti and Peterson 1979).

Period I forage may have had greater Cr_2O_3 content than period II due to the increased rainfall during that period (19.04 cm vs. 1.5 cm). Rain may have indirectly affected Cr_2O_3 background due to more soil splash on the forage or by oxidizing ions being more available in an active soil profile. Also, cows may have consumed more root material when grazing due to the softer soil. Forage samples for period I did have greater ash content than period II (Table 1), indicating a greater amount of mineral content in the forage.

Implications

A possible source of bias between animals exists in Cr_2O_3 contamination from forage when using pastures previously used in Cr_2O_3 marker studies. Fecal output means may be higher than observed during grazing periods in which Cr_2O_3 contamination is a problem. Bias can be introduced when interpreting trends from one period to another. Differences in fecal Cr_2O_3 background are likely to be random among individual animals due to random grazing patterns.

Researchers should consider background levels of Cr_2O_3 in the forage available to grazing cattle when comparing fecal grab samples to total collection and when interpreting fecal output means from different seasons of the year.

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