Chromic Oxide as an Indicator of Total Fecal Output in White-tailed Deer

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Highlight: Two captive rumen-fistulated white-tailed deer (*Odocoileus virginianus*) were used to evaluate chromic oxide as an external indicator of total fecal output. Acknowledging the limitation imposed by having only two rumen-fistulated animals, accurate estimates of total daily fecal output were made by combining average values obtained from sampling at different times during the day. Factors which contributed to diurnal variation in excretion of chromic oxide should be studied further before the technique can be of practical value.

The ratio or external indicator techniques for determining total fecal output (TFO) has advantages in that it eliminates the necessity of making total fecal collections and it permits digestion trials to be conducted in places other than metabolism cages. Hence, estimating total fecal output in field situations is one potentially valuable application. However, research in external indicator techniques has been limited primarily to domestic ruminants, and the applicability for wild ruminants is unsubstantiated. Moreover, the field situation for domestic ruminants is quite different than that for a wild ruminant like the deer. Consequently, the practical application of such a technique to problems involving wild free ranging ruminants is questionable and would necessarily become the object of investigation once the technique itself is perfected. In any case, the technique would be of practical value for various aspects of wild ruminant nutrition research under captive or semiwild conditions.

The background for the present study comes almost exclusively from the literature of domestic animal science. In 1955, Raymond et al. elucidated the necessary criteria for an external indicator as a substance which should be quantitatively recovered in the feces (i.e., neither absorbed nor abnormally retained in the digestive tract), be nontoxic, inexpensive, readily analyzed by physical or chemical methods, and present in only a small amount in the original diet. In this context, chronic oxide has been widely accepted as an external indicator substance.

Two basic assumptions for the use of an external indicator are (1) the tracer material becomes evenly distributed with the ingesta, and (2) the tracer material passes through the digestive tract in a manner similar to that of nutrients (Kane et al. 1952). In the case where either of these assumptions does not hold true, the general accuracy of TFO estimates would be impaired and any single fecal sample would fail to provide an accurate TFO estimate.

In an effort to determine sampling error using the chromic oxide technique, Kane et al. (1952) took 15 fecal "grab" samples from three dairy cows on five different days. Comparison between TFO estimates based on these samples and a total fecal collection led to the conclusion that TFO values obtained from any single fecal sample were not sufficiently accurate to be used in digestibility studies. The apparent reason for the unreliability of single fecal samples was a diurnal variation in the excretion rate of chromic oxide in the dairy cow, i.e., a highly significant difference was noted in the chromic oxide content of a.m. versus p.m. fecal samples.

Subsequent experimentation corroborates these findings, and diurnal variation in the excretion of chromic oxide appears to be typical regardless of ruminant species (Pigden et al. 1957; Putnam et al. 1957; Faulds et al. 1971). Balch et al. (1957) cautioned that the suitability of any given scheme of chromic oxide administration and of feces sampling should be checked against a complete collection of feces under the conditions of each experiment.

Although the literature is definite relative to diurnal variation of chromic oxide excretion, there is no agreement as to what times during a 24-hour period or what combinations of grab samples would yield an accurate estimate of fecal output. For example, Harding et al. (1953), in a study using beef and dairy cattle, reported that feces taken at 6:00 a.m. and 4:00 p.m. and compounded on an equal wet-weight basis during seven or more days resulted in reliable estimates of dry matter intake. This is in contrast to the results of Kane et al. (1952), who reported that fecal grab samples taken from cattle between 1:00 p.m.–3:00 p.m. and 4:00 a.m.–6:00 a.m. gave the best results.

In a study using sheep, Raymond et al. (1955) found that there was no standard excretion pattern on which accurate grab sampling could be based. Raymond also provides a review which further documents between study variability relative to this technique.

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Various methods for administering chromic oxide are recorded in the literature (Pidgen et al. 1957; Laglanda et al. 1968; Phar et al. 1970). In general, the problem of unreliable estimates based on single fecal samples has persisted as some function of diurnal variability in chromic oxide excretion.

Faulds et al. (1971) evaluated the chromic oxide technique in sheep and deer. After a 5-day equilibration period these workers administered 3.0 grams of chromic oxide daily to two deer via gelatin capsules. Grab samples were taken twice daily at 8:00 a.m. and 4:00 p.m. for 5 days. The results of their work were checked against a total fecal collection for the 5-day trial period, and their data indicated that estimates of fecal output for deer are generally lower if based on the a.m. fecal grab samples rather than the p.m. grab samples. Estimates in both cases were based on averages of the five samples taken at those times. Both experimental deer showed the highest concentration of chromic oxide in the feces at noon, with a steady decrease to midnight, followed by an increase to 8:00 a.m. They concluded that one grab sample does not yield a reliable estimate of fecal output in white-tailed deer.

Hence, it appears that deer are also subject to diurnal fluctuations in chromic oxide excretion, and therefore the time of grab sample collection is quite important as it affects TFO estimates. Faulds et al. suggest that more accurate TFO estimates might be obtained for deer by taking grab samples at different and more varied times during a 24-hour period.

The principal objectives of the present study were (a) to determine the accuracy of chromic oxide as an external indicator of total fecal output in the white-tailed deer, and (b) to determine a schedule of fecal sampling which maximizes the accuracy of TFO estimates using this technique.

Methods

During a 2-week conventional digestion trial, two 17-month-old rumen-fistulated white-tailed deer (one doe and one castrated buck) were each given three grams of chromic oxide at 9:00 a.m. daily. The dose of chromic oxide was packaged in a thin cellulose sheet and administered via the rumen fistula. This technique required that the deer remain relatively still during administration so that no dry matter was lost through the fistula. This was accomplished, and for the duration of the trial no rumen fluid or ingesta was lost.

The first 5 days of the trial served as an equilibration period (Faulds et al. 1971; Van Dyne 1968; Crampton 1951) to insure that the marker material, chromic oxide, was evenly distributed throughout the digestive tract and a steady-state excretion pattern of the indicator was reached.

The deer were housed in 3.7 m × 6.4 m pens and were fed *ad libitum* a concentrate ration in pelleted form (Table 1). Daily feed consumption was recorded to the nearest gram for each deer. For 11 days following the equilibration period, fecal "grab" samples were taken from each animal at 9:00 a.m. and 5:00 p.m. daily, and for the last 3 days of the trial period (days 12, 13, and 14), fecal samples were collected every 4 hours. Fecal samples were collected according to this schedule for the purpose of determining the best time of day for estimating total fecal excretion, using chromic oxide as an external indicator. In addition to daily fecal sample collections, a total collection of the daily fecal output from each deer was made. All pellet groups were carefully collected from the floor of the pens at regular intervals throughout the day in an effort to make an accurate total collection. Each day the pen floors were thoroughly washed to further insure an accurate total fecal output collection.

Fecal collections were oven dried at 100°C for 24 hours and ground in a Wiley Mill using a size 40-mesh screen. Analysis of the chromic oxide content of daily fecal grab samples was made according to the calorimetric method of Hill and Anderson (1958).

Table 1. Composition of pelleted deer ration fed to rumen-fistulated white-tailed deer during a 14-day digestion trial.

Constituent	Composition
Feed ingredients (kg/metric ton[2])	
Corn	140
Soybean oil meal	150
Alfalfa meal	300
Wheat bran	57.5
Oats	240
Molasses	80
PO_4[2]	7.5
Mineral salts	15
DSVP[3]	10
Proximate analysis[4] (%)	
Crude protein	19.06
Ether extract	2.97
Crude fiber	15.27
Ash	8.29
Nitrogen-free extract	54.41

[1] 1 metric ton = 2,200 pounds.
[2] Phosphate supplement: defluorinated rock phosphate.
[3] Dairy supplement vitamin package (DSVP): S=1.4%; Mg=7.5%; Fe=2.1%; Mn=1.3%; Zn=1.3%; Cu=0.2%; I=0.02%; Co=0.02%; Vitamin A=1.1×10^6 USP/kg; Vitamin D=4.4×10^5 USP/kg; Vitamin E=220 IU/kg.
[4] Expressed as percent of ration dry matter (90.19%).

Duplicates of each fecal grab sample were analyzed for their chromic oxide content. The chromic oxide content of feces was calculated as follows:

$$\text{mg } Cr_2O_3\text{/g of fecal dry matter} = \frac{\text{optical density xK}}{\text{fecal sample weight (g)}}$$

The K factor in this equation represents a slope value which is obtained by plotting the change in concentration of standard solutions of chromic oxide against the corresponding change in optical density for these solutions. The K value for this study, 37.3, was determined using a range of .01 mg Cr_2O_3/ml to .14 mg Cr_2O_3/ml (.01 mg increments). These standard solutions were prepared using chromic oxide, concentrated nitric acid, and a digestion mixture as described by Hill and Anderson (1958).

The fecal output estimates for all individual grab samples were calculated as follows:

$$\text{Fecal output (grams dry matter)} = \frac{\text{mg } Cr_2O_3 \text{ administered}}{\text{mg } Cr_2O_3\text{/g of fecal dry matter}}$$

A comparison was made between the observed daily total fecal output and estimates obtained for specific times during the day using the chromic oxide technique.

Results and Discussion

Estimated and observed daily fecal outputs for the study are summarized in Table 2. The data gathered on days 1 through 11 at the 9:00 a.m. and 5:00 p.m. sampling times, indicate that the 5-day equilibration period was not sufficient for uniform distribution of chromic oxide in the digestive tract. After the 11th day of the trial period the estimates of fecal output became more accurate, suggesting that a relatively long equilibration period is necessary for white-tailed deer.

The most accurate estimates of total fecal output (TFO) per day and throughout the study period are shown for each deer in the last three rows of Table 2. The last 3 days of the trial (post equilibration) were used in calculating these estimates. This was achieved by combining the 9:00 a.m. 3-day grab sample mean with the 1:00 a.m. 3-day grab sample mean and the 9:00 a.m. 3-day grab sample mean with the 9:00 p.m. 3-day grab sample mean. These sample combinations for the female and male, respectively, gave results which were 99.7% and 96.0% of the actual TFO for the first combination, and 102% and 103% for the second combination. The most accurate estimates for a

Table 2. Summary of total fecal output (TFO) estimates using the chromic oxide technique. Values represent means for the last three days of a standard (14 day) digestion trial.

Fecal grab sample collection	Chromic oxide concentration (mg/g feces)		Estimated TFO (g)		9-day average actual TFO (g)		Estimated TFO / Actual TFO (%)	
(time)	Female	Male	Female	Male	Female	Male	Female	Male
9 am	7.8	5.3	386	577	311	451	124.0	128.0
1 pm	6.5	3.3	458	922	311	451	149.0	204.0
5 pm	5.6	2.4	571	959	311	451	181.0	213.0
9 pm	12.1	9.1	250	354	311	451	80.0	78.5
1 am	13.1	10.5	234	289	311	451	74.0	64.0
5 am	10.6	7.9	298	387	311	451	91.0	86.0
9 am + 1 am			310	433	311	451	99.7	96.0
9 am + 9 pm			318	465	311	451	102.0	103.0

single sampling time were obtained from the 5:00 a.m. samples.

Figure 1 shows the diurnal variation in chromic oxide excretion and was prepared from data gathered on the last 3 days of the trial. This curve, representing chromic oxide excretion as a function of time, decreases from 5:00 a.m. to 5:00 p.m., after which time it increases rapidly to its peak at 1:00 a.m.

Chromic oxide is subject to a diurnal excretion pattern in white-tailed deer, similar to that which has been found for domestic ruminants. This diurnal variation may be accompanied by a seasonal variation which is suspected when comparing the results of this study with those of Faulds et al. (1971). These workers conducted their study in the early spring as compared to the study here reported which was done in early fall, about 6 months later in the year. A comparison of diurnal excretion for chromic oxide in both studies shows the same general pattern but with an apparent phase shift, e.g., the excretion curve in this study lags behind that of Fauld's by about 7 hours.

The diurnal nature of chromic oxide excretion indicates that single grab samples should not be used to estimate total daily fecal output. Accurate estimates of total fecal output were obtained by combining the 3-day mean estimate for fecal grab samples taken at two different times during each day. These times were 9:00 a.m. plus 9:00 p.m. and 9:00 a.m. plus 1:00 a.m.

It appears that reliable (accurate) estimates of TFO can be experimentally obtained using the chromic oxide technique, if fecal samples are collected at the time(s) during each day which will provide the best estimate. However, the practical application of the technique will be minimal until more data are available on the diurnal and seasonal variation in the excretion pattern of chromic oxide so that these times can be determined. Hence, variability in the excretion pattern should be measured for all animals which are to be used for estimating TFO by the "grab" sample method.

Data on the average consumption of forage by individual deer is important given numerous management considerations but is very difficult to collect. A technique which permits accurate total fecal output estimates coupled with accurate forage digestibility estimates would permit forage intake estimates. Hence one of the most significant uses of fecal output estimates is as a factor in the calculation of forage intake, providing that an accurate method for estimating forage digestibility is available. Such a technique, an in vitro microdigestion procedure, has been shown to provide accurate estimates of in vivo digestibility for white-tailed deer (Ruggiero and Whelan 1976). In this context, it would appear that further investigation of diurnal and seasonal variation in chromic oxide excretion is warranted.

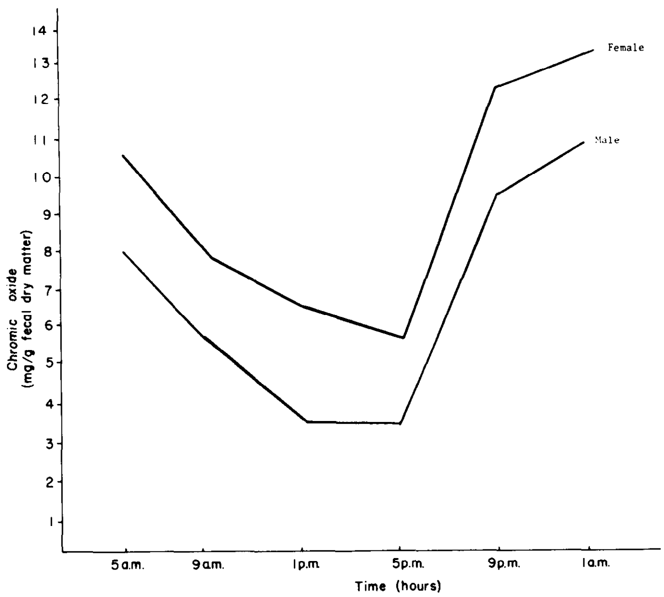

Fig. 1. *Diurnal variation in chromic oxide excretion by 17-month-old rumen-fistulated female and male white-tailed deer. (Values represent means for the last 3 days of a 14-day sampling period.)*

Literature Cited

Balch, C. C., J. T. Reid, and J. W. Stroud. 1957. Factors influencing the rate of excretion of administered chromium sesquioxide by steers. Brit. J. Nutr. 11:184.

Crampton, E. W., and L. E. Lloyd. 1951. Studies with sheep on the use of chromic oxide an index of digestibility of ruminant rations. J. Nutr. 45:319.

Faulds, C., D. T. Sanders, and J. E. Skeen. 1971. Evaluation of the chromic oxide technique in deer and sheep. Unpublished report. Dep. Fisheries and Wildlife Sci., Virginia Polytechnic Inst. and State Univ., Blackburg, Virginia.

Harding, W. A., and J. T. Reid. 1953. Use of indicators in the measurement of the dry matter intake of grazing animals. J. Nutr. 51:35.

Hill, F. W., and D. L. Anderson. 1958. Comparisons of metabolizable energy and productive energy determinations with growing chicks. J. Nutr. 64(4): 587-603.

Kane, E. A., W. C. Jacobson, and L. A. Moore. 1952. Diurnal variation in the excretion of chromic oxide and lignin. J. Nutr. 47:263.

Langlanda, J. P., J. L. Corbett, I. McDonald, and G. W. Reid. 1968. Estimation of the feces output of grazing animals from the concentration of chromium sesquioxide in a sample of feces. I. Comparison of estimates from samples taken at fixed times of day with feces outputs measured directly. Brit. J. Nutr. 17:211.

Phar, P. A., N. W. Bradley, C. O. Little, and L. V. Cundiff. 1970. Effects of confinement and level of feed intake on digestibility of nutrients and excretion of chromic oxide, crude protein, and gross energy in the bovine. J. Anim. Sci. 30:589.

Pigden, W. J., and G. L. Brisson. 1957. Note on a chromium oxide pellet to provide uniform release of this indicator in the rumen of cattle. Can. J. Anim. Sci. 37:185.

Putman, P. A., J. K. Loosli, R. G. Warner, and R. S. Searle. 1957. Diurnal excretion of chromic oxide and ash by dairy cows. J. Dairy Sci. 40:618 (Abstract No. 34).

Raymond, W. F., and D. J. Minson. 1955. The use of chromic oxide for estimating the fecal production of grazing animals. J. Brit. Grassland Soc. 10:282.

Ruggiero, L. F., and J. B. Whelan. 1976. In vitro and in vivo digestibility of feed consumed by captive white-tailed deer. J. Range Manage. 29:82-83.

Van Dyne, G. M. 1968. Measuring quantity and quality of the diet of large herbivores. p. 54-94. *In:* Guide to the Study of the Productivity of Large Herbivores. F. B. Golley and H. K. Beuchner, Eds., Handbook No. 7, Blackwell Sci. Publ. 308 p.