

Chromic Oxide in Range Nutrition Studies

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SUMMARY

A variety of studies were conducted by some 13 states and agencies to attempt to validate or develop parameters for using chromic oxide to determine intake and digestibility of the grazing ruminant animal.

Recovery trials were conducted under a variety of experimental conditions, ranging from confinement to grazing trials under range conditions. Included in these studies were various methods of and carriers for administration of the chromic oxide to the animal.

The diurnal variation of excretion of chromic oxide to determine time or number of administrations per day and established sampling time were evaluated. Methods of administration were further evaluated as to their effect on diurnal variation. Among methods used were the pure form of chromic oxide, paper impregnated chromic oxide, "solka floc" or cellulose-chromic oxide boluses, various modifications of a sustained release chromic oxide, and foliar application of chromic oxide.

The various "systems" of using chromic oxide to estimate fecal output and forage intake and digestibility were evaluated with different classes of animals and levels of production in with varying conditions in the western states. The use of chromic oxide as an external indicator in range nutrition will have limitations, but these data will help define those parameters. Guidelines or recommendations, for planning research and interpreting the results from chromic oxide studies are presented.

CHROMIC OXIDE IN RANGE NUTRITION STUDIES

INTRODUCTION

The accurate determination of intake and digestibility of range forage by grazing animals has been a problem plaguing investigators for many years. Many approaches have been utilized, however, none has proved entirely satisfactory. In keeping with the objective of the Western Regional Research Project W-34--Range Livestock Nutrition, "to improve techniques for measuring qualitative and quantitative forage intake of range animals and forage digestibility," several studies were initiated to evaluate chromic oxide for use as an external indicator in intake and digestibility studies. Some of this work was continued under the successor of W-34, Western Regional Research Project W-94.

A portion of this information has been published in a former regional publication (Harris *et al.*, 1967) as well as in other papers. These will be included here to bring all these data into a single publication.

CHROMIC OXIDE RECOVERY

The theory behind the use of an external marker to accurately estimate fecal output, and thus intake or digestibility, requires that the recovery of the indicator in the feces be near quantitative for a consistent fraction thereof with appropriate correction factors. Consequently, considerable effort has been expended to determine the extent to which chromic oxide can be recovered under a variety of experimental conditions.

While it is recognized that results from work done under closely controlled, confined conditions are not necessarily directly applicable to range conditions, confinement trials are a valuable tool to develop and test techniques that can be verified under range conditions.

Confinement Trials

New Mexico workers (Nelson and Green, 1969), in a drylot study, administered at 6 a.m. daily, 15.35 grams of chromic oxide on paper to each of six Holstein steers fed prairie hay and cottonseed meal. Total fecal collection was made with bags on days 1 through 11 and days 14 through 18. On days 12 and 13, fecal grab samples only were taken at two-hour intervals. Fecal grab samples were taken at 6:30 a.m. on days 14 through 18 to compare grab sample chromic oxide recovery with that obtained from total collection.

Recoveries for days 1 through 11 and 14 through 18 are shown in Table 1. Recoveries were low for the first two days but exceeded 100 percent on day 3 and continued near 100 percent through the 11th day, with an average recovery

of 103 percent for the nine-day period. For this period, neither the differences among steers nor those among days was significant ($P > .05$).

A relatively uniform recovery also was obtained for days 14 through 18. The mean recovery was 97 percent based on total collection and 93 percent for fecal grab samples. Differences among days, steers, and samples were not significant, but there was a day X steer interaction ($P < .05$).

In a second trial utilizing the same animals, ration, and equipment, and following a 13-day adjustment period, 78 grams of chromic oxide paper was administered with a balling gun at 6:15 a.m. on alternate days. Total feces was collected for nine days. On the 10th day, two-hour grab sampling was started at 7 a.m. and continued for 48 hours. Total collection was continued on days 12 and 13, and then a second period of grab sampling every two hours for 48 hours followed on days 14 and 15. During days 16 to 21, sampling of feces included single grab samples as well as total collection from each steer.

Recovery (Table 2) for the 24-hour period beginning 11 hours after initial administration of chromic oxide was very low (30 percent). By day 2, the recovery was 85 percent, indicating, again, relatively high recoveries within a few days of administration. For the next period following administration (day 4), recovery was 100 percent and remained high on days 6 and 8. The average recovery for alternate days beginning 11 hours after chromic oxide administration was 98 percent and that for the other days, 76 percent.

For days 16 through 21, more chromic oxide ($P < .01$) was recovered on days following administration of the indicator than on days of administration. Steer differences were not significant, but there was a steer X day of administration interaction ($P < .01$). Day of administration vs. day following administration was significant within each of the steers.

Nearly quantitative recoveries of chromic oxide were obtained in two digestion trials conducted at Lincoln, Nebraska (Streeter, 1966). Chromic oxide impregnated paper (15 grams paper/head/day) was administered twice daily in gelatin capsules to three steer calves in digestion crates. They were fed either bromegrass hay or fresh bromegrass clippings. A 10-day preliminary dosing period followed by a six-day sampling period resulted in recoveries of 99 percent for both trials.

At the Utah station (Border, 1962) chromic oxide was administered in a sustained-release pellet (equal parts of chromic oxide and gypsum) to three steers fed chopped meadow hay. Under conditions of this experiment it was suggested that an 8 to 10-day preliminary dosing period would be necessary to obtain a relatively uniform excretion pattern, although even then the day-to-day recoveries continued to be somewhat erratic (Figure 1).

In another study, 20 sheep were fed chromic oxide paper (32.85 percent chromic oxide) at five levels (4 sheep per treatment) in which 0, 1, 2, 4, or 8 grams of chromic oxide was administered daily. The sheep were fed alfalfa hay in individual stalls, and feces were collected in bags. All but one of the

sheep fed chromic oxide reached a constant output of indicator by day 6 (the other one by day 8). Recoveries for the last 14 days of the trial are given in Table 3. Treatments showed differences at the .069 level but not at the .05 level. It was suggested that the 4 or 8 gram level would give more accurate results in sheep.

In Arizona work, McCann and Theurer (1967) compared four rumen-fistulated steers with an equal number of intact steers in two trials designed to study chromic oxide recovery and the estimation of fecal production. A 1:2 mixture of chromic oxide:solka floc (a purified wood cellulose product) was administered in gelatin capsules at 7 a.m. with a single daily fecal grab sample being taken at the same time. The steers were confined in individual concrete floored pens. Total collection was initiated on the fourth day of the 12-day experiment and continued for the remaining nine days.

The average daily recovery of chromic oxide for combined and individual trials in the Arizona work is presented in Table 4. Percent recovery of chromic oxide was about the same for both groups of steers within trials, although individual daily values of 52 to 116 percent recovery were noted. There was a significant ($P < .05$) difference in average chromic oxide recovery for the eight steers between trials (76 vs. 87 percent). Average recovery for individual steers ranged from 70 to 81 percent in Trial 1 and from 83 to 93 percent in Trial 2. Recovery of chromic oxide was fairly constant from the fourth day of administration through the 12th, suggesting that four days of initial administration are adequate for constant recovery of chromic oxide dispersed on solka floc.

At Oregon (Wheeler, 1962), three methods of administering chromic oxide were compared in an experiment utilizing two steers per treatment; all the steers received the same basal ration of meadow hay. Mean recoveries for the treatments: chromic oxide powder given in gelatin capsules, 73 percent; chromic oxide powder mixed with cottonseed meal, 84 percent, and chromic oxide dispersed on solka floc, 81 percent. In a second trial comparing ad libitum vs. limited intake of meadow hay and with three steers per treatment, recoveries were 84 and 85 percent, respectively.

It is evident from the data reviewed above that even under highly controlled conditions, recovery of chromic oxide in the feces can be quite variable, both within experiments conducted by the same investigators within a given set of conditions, and particularly among researchers from different institutions who may be using a wide variety of techniques under differing experimental conditions. Under certain circumstances it appears that good to excellent results can be obtained with chromic oxide. However, these can only be considered superior from the viewpoint of the range nutritionist if similar results can be obtained under range conditions.

Grazing Trials

In New Mexico studies (Kiesling *et al.*, 1969) five steers grazing dormant tobosa were each given three boluses of chromic oxide in shredded paper (13.6 grams total) with a balling gun at 7 a.m. daily for eight days. No chromic oxide was recovered (Table 5) during the first day and recovery was very low for the second day. The average recovery for days 3 through 8 was 71.5 percent, and varied from 64.1 percent on day 7 to 82.0 percent on day 8. None of these differences was significant ($P > .05$). The low recovery may have been caused by loss of some boluses by regurgitation, incomplete collection of feces, or improper sampling. Neither of the losses was noted. Steer differences were significant with recovery ranging from 54.4 to 85.5 percent.

In a similar trial (Table 6), four steers grazing dormant tobosa were given 13.02 grams of chromic oxide in shredded paper packed in gelatin capsules. Recovery of chromic oxide steadily increased until the fourth day and remained fairly constant (82.4 percent average) for days 4 through 9. There were no differences ($P > .05$) among steers and among days 4 through 9.

With steers grazing green tobosa (immature to pre-bloom stage) three boluses of chromic oxide paper (12.9 grams of chromic oxide) were administered at 7 a.m. daily for 10 days. One of the steers, after leaving the corral, was observed to regurgitate several of the paper boluses which dropped to the ground. Data for this steer were excluded from the results. Losses from regurgitation were reduced by keeping the steers confined in the corral for about 30 minutes after administration of the bolus. Recovery was measured on days 2 through 10. Recovery was low on days 2 and 3 (Table 7) but increased to an average of 75.1 percent by day 4. Recovery for one of the steers was low until day 9, and it is suspected that he regurgitated some of the boluses. Differences in percentage recovery of chromic oxide for days 4 through 10 were not significant, although there were some steer differences.

Recovery studies were conducted at two locations in Nebraska (Table 8) with steers (trials 1-64, 2-64) or heifers (trials 1-65, 2-65, 3-65) grazing native summer range and heifers grazing native winter range. Fifteen grams of chromic oxide paper per head per day were administered in gelatin capsules for a 10-day preliminary dosing period and a six-day sampling period, and total collection was made with fecal bags. Mean recoveries were 80 and 96 percent on the winter range and varied from 83 to 95 percent on the summer range. Recovery of chromic oxide obtained from fecal "pats" on the ground corresponding to trials 1-64 and 2-64 gave respective recoveries of 81 to 85 percent (Streeter, 1966). An insufficient preliminary period could have been the cause of low recovery (80 percent) in one trial at Fort Robinson as indicated by a gradual increase in recovery from 72 percent on the first day of collection to 93 percent on the sixth day. The pattern was not found in the second trial at the same location. No explanation was found for the low recoveries at Scotts Bluff (83 to 95 percent).

In other Nebraska work (Rittenhouse, 1969), five ovariectomized heifers and one steer, all fitted with esophageal fistulae, were grazed on native range in two trials. Chromic oxide paper (36 grams Cr_2O_3) was administered in two doses (24 and 12 grams of chromic oxide) at 5 a.m. and 5 p.m. daily for an eight-day

preliminary and a six-day collection period. Mean chromic oxide recoveries were 87.8 and 90.1 percent for Trials 1 and 2, respectively; both differed ($P < .05$) from 100 percent (Table 9). Ranges of individual daily recoveries for the first and second trials were 44.4 to 127.8 percent and 28.2 to 110.8 percent, respectively. It should be noted, however, that some of the low values were identified with incomplete recovery of total feces. In subsequent work to measure the influence of protein and energy supplements on intake and digestibility of winter range forage, only 75 percent recovery of chromic oxide was obtained over four collection periods with a daily morning grab sample.

At Arizona, four rumen-fistulated steers grazing a southern desert grassland-type vegetation were given 10 grams of chromic oxide/head/day mixed in a ratio of 2:1 of solka floc:chromic oxide and enclosed in a gelatin capsule. Total collection and a single daily grab sample (7:40 a.m.) were taken over the seven-day collection period. Chromic oxide recovery for the total collections (Table 10) ranged from 71.4 to 80.1 percent with a mean of 76 percent. The grab samples ranged from 75.8 to 82.2 percent with a mean of 80.8 percent.

Wheeler (1962) utilized six steers in three trials corresponding to stages of maturity (immature, mature, and dried mature) of crested wheatgrass (Agropyron desertorum). Five grams of chromic oxide was administered in a gelatin capsule daily for a five-day preliminary and five-day collection period. During each trial, three steers grazed on one-acre pastures and three were hand-fed clipped crested wheatgrass. Recoveries (Table 11) tended to be high in all cases with treatment means ranging from 101 to 132 percent. Means for the grazing animals were consistently higher than for those hand-fed, and there did not appear to be any pattern in recoveries related to forage maturity.

Two trials conducted at the Squaw Butte Station in Oregon (Pryor, 1966) were designed to study the effects on chromic oxide recovery of forage maturity (immature crested wheatgrass in Trial 1 and mature wheatgrass in Trial 2) and number of dosings (1 or 2/day). Two total collection periods were utilized in each trial: days 3-8 and 13-18 in Trial 1 and days 3-8 and 12-17 in Trial 2. In each trial, three steers were dosed at 8 a.m. with two capsules containing five grams of chromic oxide each mixed with solka floc. Three other steers were given two separate five-gram doses, one at 8 a.m. and the other at 4 p.m. Twice daily fecal samples were taken from all steers following morning and evening dosing times. Recoveries (Table 12) were consistently lower on the immature forage (71.1 to 85.1 percent) than with mature forage (94.9 to 107.5 percent), and a daily single dose generally resulted in less variation than the twice-daily dosing. Means for Trial 2 (mature forage) approached 100 percent in all cases (98.7 to 103.8 percent).

In Wyoming work, yearling steers grazed native shortgrass range (three trials on cured grass and one trial on green grass) and were dosed twice daily with five-gram capsules of chromic oxide. Recoveries ranged from 61.9 to 105.4 percent.

Discussion

Only four stations (New Mexico, Nebraska, Oregon, and Arizona) reported studies with grazing animals, as well as in confinement, which might provide a basis for comparing chromic oxide recovery under the two situations. Such a comparison would require that similar techniques be utilized by the same investigator(s) for both experiments.

At Arizona (Table 4 and 10) recoveries tended to be lower for the grazing trial (71 to 80 percent) as compared to the drylot trials (76 to 88 percent), although there were overlapping values. The range in recoveries and the relatively low values obtained under both situations raise questions regarding the use of chromic oxide and/or the techniques utilized here for studying range intake and digestibility problems.

Even more notable are the differences in recovery at New Mexico and Nebraska. New Mexico workers obtained a recovery of 103 percent (Table 1) for days 3-11 in a drylot trial, but when conducted similar trials with grazing cattle (Tables 5, 6, & 7) mean recoveries of 72 and 82 percent were obtained on mature tobosa and 88 percent on green tobosa. None of the daily means and only one steer mean exceeded 95 percent.

Similarly, the 99 percent recovery in each of two drylot trials at Nebraska exceeded all values obtained in grazing trials with values ranging from 80 to 96 percent (Table 8). Recoveries of 94, 95, and 96 percent might be considered to fall within an acceptable margin of error from 100 percent, but such recoveries were not obtained consistently. Some observed reasons for low chromic oxide recoveries were regurgitation of chromic oxide boluses and failure to obtain complete fecal collection. Other suggested causes were retention of chromic oxide within the animal, improper sampling, faulty analytical techniques, and an inadequate preliminary dosing period.

Unlike most of the other studies which reported low chromic oxide recoveries, Oregon's work (Table 11) gave high values, with 16 to 18 daily individual recoveries exceeding 100 percent. Mean ranged from 101 to 132 percent, and hand-fed steers consistently produced lower recoveries than those that were grazing concurrently. The possibility was mentioned that the chromic oxide might pass through the tract at a faster rate when associated with highly digestible green forage. In support of this hypothesis, a New Mexico grazing trial on green tobosa resulted in higher recovery (88 percent; Table 7) than either of two trials on dormant tobosa (72 and 82 percent; Tables 5 and 6). However, in the Oregon study mentioned above, the immature forage was associated with a mean recovery of 121 percent that fell between those for the two mature stages (190 and 132 percent). In other Oregon work (Pryor, 1966; Table 12), mature forage produced recoveries near 100 percent, whereas those for immature forage were much lower at 77 to 82 percent).

DIURNAL VARIATION

The purpose behind the use of chromic oxide in range forage intake and digestibility studies is to eliminate the need for total fecal collection, a procedure which is laborious, time-consuming, and may alter the grazing pattern of the animal. Consequently, some form of sampling is required, and the concentration of the indicator in the sample can then be utilized to estimate fecal production. One problem arising, and one which has been recognized for some time, is that the excretion of chromic oxide in the feces is not uniform, but varies over a 24-hour period, hence the oft-applied name, "diurnal variation". Efforts to circumvent this problem are generally two-fold: 1) the use of a sustained-release mechanism such as chromic oxide impregnated on paper or a plaster-chromic oxide pellet that will release the indicator at a uniform rate over a period of time; and 2) attempts to determine patterns of excretion which might indicate optimum dosing and/or sampling times for obtaining a representative sample or allow appropriate correction factors to be applied. This section will treat work done on excretion patterns. Methods of indicator administration will be presented in the next section.

Wyoming workers found no definite pattern related to time of chromic oxide excretion in total collection samples or grab samples taken at three-hour intervals from 6 a.m. to 6 p.m. These steers were grazing a native shortgrass range in three trials on cured forage and in one trial on green grass. Chromic oxide was given in five-gram capsules at 6 a.m. and 6 p.m. (Harris *et al.*, 1967).

In three trials at Scottsbluff, Nebraska, a study was made of diurnal variation in the concentration of chromic oxide in the feces. Rectal grab samples were collected at 4:30 and 8:30 a.m. and at 12:30, 4:30, and 8:30 p.m. Animals were allotted so that one sample was collected from one animal at each sampling time. Samples were collected for six days during each trial to provide 30 grab samples per trial.

No differences ($P > .05$) were found among sampling times and no consistent pattern was noted in the variation in chromic oxide concentration. It was concluded that sampling could be done at any time of day provided that sufficient number of animals were sampled for a sufficient number of days to average out the large animal-day variation (C.V. = 20 percent).

Rittenhouse (1969), using regression analysis, concluded that a morning grab sample gave a better estimate of fecal production than one taken in the evening.

Border (1962) gave sustained release pellets (equal parts of chromic oxide and gypsum) to steers over 11 time periods (2 days/period) with grab samples taken four times daily. Data were expressed as a ratio of chromic oxide/dry matter, (Figure 2). Analysis of the data indicated that the 11:30 a.m. grab sample estimated total fecal collection with more precision than a combination of all four times, although this was not great enough to preclude the use of any one of the four periods, used alone, from giving equally reliable estimates of the total feces.

Table 13 shows the diurnal variation in rectal grab samples obtained by New Mexico workers (Nelson and Green, 1969) taken at two-hour intervals for a 48-hour period (see page 2). Each mean represents 12 measurements (2 days with 6 steers per day). The average recovery was 96 percent with no significant differences among collection times, although the recovery at 9 a.m. was 100 percent. There were day differences and a day X steer interaction ($P < .05$) with percentage recovery significantly higher on day 2 for three of the steers. Differences among steers were significant on day 1 but not on day 2.

In another trial (Table 14) a similar sampling pattern was followed with steers receiving chromic oxide only on alternate days (see page 3). The overall average recovery of 68 percent for days of administration was lower ($P < .05$) than the 112 percent recovery for days following administration. Recovery was lower for period 1 (days 10 and 11) than for period 2 (days 14 and 15). Sampling times were different ($P < .01$). On the day of administration, chromic oxide recovery was 103 percent at 7 a.m. and declined steadily until 1 a.m. On the day following administration, recovery was 65 percent at 7 a.m., 99 percent at 11 a.m., and exceeded 100 percent for the remainder of the day.

Another six-day trial to study diurnal variation was conducted following day 21 of the trials mentioned above (see page 3). Because of possible effects of one two-hour sampling on successive sampling times, only two grab samples were taken from each steer daily at different times for the six-day period. The average recovery of 64 percent (Table 15) for days of chromic oxide administration is in close agreement with the 68 percent observed when fecal grab samples were taken from each steer every two hours. Also, the 107 percent recovery on days following administration is comparable to the 112 percent recovery for the two-hour sampling from each steer. The difference between day of and day following administration of chromic oxide was again significant ($P < .01$). The interaction of time of day X administration day (day of administration vs. alternate days) was significant, with significant differences between days on 9 of the 12 sampling dates. Differences between days were not significant at 9 a.m., 11 a.m. and 1 p.m. However, differences among sampling times within days of administration and within days following administration were quite variable. The recovery of 90 percent of the chromic oxide at 7 a.m. on the day of administration with only two fecal samples per steer daily was lower than the recovery of 103 percent when there were 12 fecal samples per steer daily. These observations suggest the feasibility of administering chromic oxide on alternate days and collecting fecal grab samples on the same day in grazing studies with steers.

In a grazing study, New Mexico workers (Kiesling, *et al.*, 1969) obtained grab samples from five steers at two-hour intervals for the last two days of an eight-day trial (see page 7). The results, shown in Table 16, exhibit rather low recoveries with the highest average recovery (79.5 percent) being obtained at 5 p.m. This recovery was significantly higher than the recoveries from 5 a.m. through 3 p.m. Average recovery was lower ($P < .05$) the first 24 hours (65.5 percent) than for the second 24 hours (73.5 percent). Recovery was higher ($P < .05$) for steer E (88.8 percent) and lower for steer A (48.4 percent) than the other steers. Average recovery for steers B, C, and D was 70.4 percent, with no difference among them ($P < .05$).

Day X steer interaction was significant ($P < .05$) with recovery being higher during the second 24 hours for steers C and D; there were no significant differences between 24-hour periods for the other three steers. Day X hour interaction was also significant ($P < .05$) with recovery being higher during the second 24 hours at 5 and 7 p.m. than during the first 24 hours. There was no significant difference among hours during the first 24 hours, but considerable differences existed during the second 24 hours.

Two trials were conducted by Oregon worker (Pryor, 1966) to study the excretion patterns of chromic oxide. In one trial (Figure 3), two five-gram capsules of chromic oxide were given to each of two steers fed meadow hay ad lib. The dose was given at 8 p.m. and grab samples were taken every two hours for three days starting at 6 a.m. the morning after dosing with chromic oxide. Appreciable chromic oxide appeared in the feces within 10 hours. Maximum concentration was between 18 and 26 hours, reductions to levels with near the limit of analytical accuracy in three to five days.

In the second trial, four bred heifers fed meadow hay were used to observe the excretion pattern of chromic oxide given with cellulose (solka floc). There were two treatments, one a single dosing with two five-gram capsules at 7:45 a.m. for a six-day preliminary period and a five-day collection period. The second treatment was dosing with one five-gram capsule at 7:45 a.m. and another at 4 p.m. with preliminary and collection periods as above. Grab sampling was done every four hours, with the first sample taken after the morning dosing. The results (Figures 4 & 5) indicate a more uniform excretion pattern from animals dosed at 7:45 a.m. than those dosed twice daily. It was suggested that an additional day for the preliminary period (7 days vs. 6) would be optimum for the conditions of this experiment.

METHOD OF CHROMIC OXIDE ADMINISTRATION

The problem of diurnal variation in chromic oxide excretion has stimulated a search for a means of administering the marker which will produce a consistent excretion pattern.

One of the simplest methods of administering chromic oxide is in the powder form, either in a gelatin capsule given orally with a balling gun or mixed with a feed supplement. Both methods have been used by Oregon workers in a confinement trial with two steers per treatment. Chromic oxide given in a capsule form resulted in considerably higher estimates of fecal output (138 percent) when compared to measured output than did either chromic oxide mixed with cottonseed meal (119 percent) or chromic oxide mixed with solka floc (123 percent).

The chromic oxide-solka floc mixture used at Oregon (Pryor, 1966) was prepared with air-dried solka floc and air-dried chromic oxide in the ratio of 1:2.33. They were mixed with water to form a slurry, dried at 100°C for 24 hours, broken up, oven dried for another 24 hours, and then thoroughly mixed again and allowed to equilibrate with the atmosphere. Five samples analyzed for chromic oxide had to be within two percent of the mean for the batch to be accepted. This mixture was then weighed into gelatin capsules. In the work

reported by Wheeler (1962), the mix consisted of approximately 60 percent chromic oxide, 39 percent solka floc, and one percent Al_2SO_4 . The aluminum sulfate was used as a mordant to insure adherence of the chromic oxide onto the fibers.

Workers at Arizona (McCann and Theurer, 1967) also used a solka floc: chromic oxide (2:1) mixture administered in gelating capsules or wrapped in filter paper. Dosing was either oral administration with a balling gun or by placing the dose directly in the rumen through a fistuala.

Workers from New Mexico (Kiesling *et al.*, 1969; Nelson and Green, 1969), Nebraska (Rittenhouse, 1969; Streeter, 1966), and Utah (Border, 1962) have used chromic oxide impregnated on paper and enclosed in gelatin capsules. The work in Utah was with sheep; the others were with cattle. The use of paper as a carrier for chromic oxide was originally proposed by Corbett *et al.* (1958).

Border (1962) utilized a sustained-release pellet encased in a gelatin capsule containing equal parts by weight of chromic oxide and gypsum as described by Pigden and Brisson (1957).

Work at Idaho (Robertson, 1966) was directed towards the development of a satisfactory sustained-release pellet. Fourteen trials were conducted to study the breakdown rates of sustained-release pellets containing chromic oxide, and the excretion pattern of chromic oxide released from such pellets. Breakdown was evaluated by suspending pellets enclosed in nylon bags in the rumen of each of three fistulated heifers in five trials, two heifers in one trial, and one heifer in one trial. Excretion was studied both by total fecal collection and grab sampling, using three heifers for one trial, five steers for five trials, and seven steers for one trial.

Three types of pellets were manufactured. A dental cast plaster-chromic oxide pellet, contained 4.05 ± 1.3 percent chromic oxide, had a diameter of 2.2 centimeters and a length of 6 centimeters, and had a specific gravity of 1.60 ± 0.03 . A kaolin clay-chromic oxide pellet was made in a mold and contained approximately 10 percent chromic oxide, had variable dimensions and had a specific gravity of 4.37 ± 0.20 . The third type was a dental plaster-chromic oxide pellet made by pressure that contained 17.49 ± 0.47 percent chromic oxide, had a diameter of 2.2 centimeters and a length of 1.5 centimeters, and had a specific gravity of 2.28 ± 0.01 .

Breakdown studies on the cast pellet gave the prediction equation:

$$\hat{Y} = 26.20 + 17.58X - 1.35X^2, s_{y.x} = 1.65$$

where \hat{Y} is the predicted percentage loss in weight of the pellet and X is the number of days in the rumen. Difficulties with regurgitation were experienced when this type of pellet was administered to cattle. There were significant differences ($P < .01$) in the excretion pattern of chromic oxide from cast pellets between animals, between days, and between times of sampling.

Kaolin clay-chromic oxide pellets were found to be insoluble in the rumen and no chromic oxide was detected in the feces of animals fed the pellets.

When pressure formed pellets were used, the prediction equation obtained for breakdown was: $\hat{Y} = 2.01 + 4.58X$, $S_{y.x} = 1.30$ where \hat{Y} is the predicted percentage loss in weight of the pellet and X is the number of days in the rumen. The predicted time required for complete dissolution of the pellet, when in a nylon bag, was about 21.5 days. The rate of breakdown was significantly different ($P < .01$) between trials, and it was suggested that the dissolution of the pressure formed pellet used was caused mainly by a physical process depending on the fiber content of the ration.

Excretion of chromic oxide from pressure formed pellets allowed predictions to be made of fecal output on the second and third days after administration when animals were on an ad libitum hay diet. There was no significant difference between the means of the estimated and actual outputs of five steers. When animals were on pasture, excretion of chromic oxide from the pressure formed pellet used was too low and too variable to have any value as an estimator of fecal output.

The possibility of foliar application of chromic oxide was investigated at Oregon using five steers in a total collection digestion trial on crested wheat-grass range. The trials were conducted during the first part of August on an area that had not been grazed that season. A three-acre plot of crested wheat-grass was sprayed with chromic oxide in combination with a polyethylene adhesive material and a wetting agent in a water solution. The weight of dry forage per acre was estimated and chromic oxide was applied to make up 0.3 to 0.7 percent of the dry weight of the forage. However, percent of ground cover (or density of the grass stand) was overestimated and the chromic oxide content of the forage was actually less than 0.1 percent. This is below the desirable concentration for chromic oxide when used for an indicator. Spraying was not difficult and the material adhered well to the foliage. The concentration of chromic oxide, although below the expected, was uniform across the plot.

Digestion trials were conducted with a seven-day preliminary period and a five-day collection period. Total fecal collections were made. Forage samples during the collection period were obtained by both clipping and by the rumen evacuation method. Samples obtained by both methods were similar with respect to chromic oxide and nitrogen; therefore, the average of all samples was used in calculating digestibility. The apparent dry matter, nitrogen digestibility for each animal, average daily fecal output, and calculated intake are shown in Table 17.

This technique may be applicable under some conditions but needs further investigation. If the grass stand is adequate to give good ground cover, it could be practical, but even then may be limited to mature forage. If applied during the "fast growth" part of the season, a dilution factor for the chromic oxide consistent with the increase of total forage would need to be calculated daily.

ESTIMATION OF FECAL OUTPUT AND FORAGE INTAKE

The ultimate test of an experimental technique is how well it performs the job for which it is employed, or in this case, how accurately can fecal production or forage intake and digestibility be determined using chromic oxide as an external indicator? Applicable data came from results of studies reported by Arizona, Oregon, Nevada, and Nebraska workers.

Fecal output was over-estimated by chromic oxide concentration in a single daily fecal grab sample in two of three trials (Table 18) reported by Theurer (1969). Fecal dry matter production estimates in Trial 1, a confinement trial, over-estimated measured output by 11 percent, and in Trial 3, a grazing trial, by 24 percent. Measured production was accurately estimated by grab sampling in Trial 2 (99.7 percent of measured).

Wheeler (1962); compared measured fecal dry matter output with estimates obtained from three chromic oxide values. Table 19 shows estimates obtained from composite chromic oxide, composite grab samples, and from grab samples taken at 6 a.m. and 4 p.m. for steers in two treatments (grazing or hand-fed with three steers per treatment) and in three trials (immature, mature, and dried mature forage).

To study the effects on fecal output estimation by different methods of administering chromic oxide, the indicator was given with cottonseed meal as a carrier, encapsulated as a powder, or encapsulated as a chromic oxide-solka floc mixture. Estimation of fecal output using chromic oxide exceeded measured values by 19, 38, and 23 percent, respectively, for the three methods (Table 20).

A comparison of the effects of ad libitum and restricted feed intake on fecal output resulted in over-estimations of 19 and 17 percent, respectively. Limited intake was approximately 82 percent of ad lib. There were, however, no differences ($P > .05$) in measured fecal output (4.36 pounds/day for ad lib vs. 4.08 pounds/day for limited). Estimated output was higher ($P < .05$) for ad lib intake (5.18 pounds/day) than for limited (4.79 pounds/day) and approached significance at the 1 percent level.

In two trials, corresponding to immature and mature forage, of two collection periods each (see Page 10), Pryor (1966) compared estimated fecal dry matter output from steers receiving a single daily dose of chromic oxide or an equivalent amount of chromic oxide given in a morning and an evening dose. A value of unity in Table 21 indicates completely accurate prediction. The estimates and errors are largest in the first collection periods, most noticeably in the group dosed at 8 a.m. only. Errors were generally larger in Trial 1 than Trial 2, and for the group dosed once compared to that dosed twice daily.

In Nevada work (Lesperance and Bohman, 1963) grab fecal samples using chromic oxide for estimating fecal excretion were compared with total fecal collection. Grab sample technique gave a 0.4 percent over-estimation of measured total collection. Individual correlation between the two methods was $r = .920$.

Chromic oxide standards were prepared in the presence of fecal ash. Determinations based on chromic oxide standards prepared without the presence of fecal ash over-estimated total fecal excretion by 14 percent. This experiment was conducted using four rumen-fistulated steers in a 4 X 4 latin square design. A seven-day collection period followed a 10-day preliminary period, and chromic oxide and grab samples taken at 8 a.m. and 5 p.m. daily.

Rittenhouse (1969) compared fecal production and intake as estimated by composite and grab sample chromic oxide to that measured and estimated from total collection. Data for only the last of a six-day collection period were available for comparisons. Fecal production measured by total collection (grams/kilograms of body weight 0.75) was 24.0 compared to that estimated by composite chromic oxide of 27.2 grams and for grab sample chromic oxide of 27.5 grams. There was no statistical difference between the two chromic oxide estimates, but the total collection was lower ($P < .05$). Small differences ($P > .05$) were found between composite chromic oxide estimates of intake (80.7 grams/kilograms of body weight 0.75) and those for grab samples (77.4 grams), but larger differences ($P < .01$) were found when compared to estimates derived from total collection (71.8 grams/kilograms of body weight 0.75). Figure 6 shows variation in fecal production estimates as determined by total collection, composite chromic oxide, and grab chromic oxide in two trials.

DISCUSSION AND CONCLUSIONS

The diversity of experimental conditions embraced by the work reported herein, and the disparity of results obtained from the same make an accurate evaluation of the combined data difficult. However, the consistency, or lack of it, provide information as to the suitability of chromic oxide as an indicator for range nutrition studies. Consideration has been given to a comparison of confinement and field trials with respect to chromic recovery. To illustrate the distinction between the two experimental conditions, workers from one station concluded at the end of a confinement trial, "These studies show that chromic oxide impregnated in paper can be administered daily or every other day with nearly complete recovery of the indicator within three days...in studies with steers fed day in drylot." At the conclusion of a series of grazing trials, the tone was somewhat different---"Recovery of chromic oxide was considerably less than 100 percent and was highly variable among steers. Apparently, administering this indicator impregnated in shredded paper is no better than other methods of administration and results in wide variation among animals within a trial and between trials." Consequently, emphasis will be placed on work actually performed under grazing conditions in evaluating results derived from this regional project.

The "diurnal variation" reported here appears to be just that--variation in the excretion pattern of chromic oxide throughout the day, as well as from day to day. Work done at Wyoming, Nebraska, Utah, and New Mexico suggested no consistent pattern that could be utilized to improve the estimation accuracy by a particular sampling scheme. In one Nebraska study, it was felt that a morning

sample improved the estimating ability as compared to an evening sample, although this is probably related also to the dosing time. Of these studies, only the work in Utah was done in confinement.

Some drylot work at New Mexico did indicate the possibility of dosing on alternate days and taking grab samples on the morning of dosing. This was not tested (or if so, was not reported) with grazing animals, however, leaving the technique in doubt as to its usefulness for range studies.

Only three methods of chromic oxide administration were employed in grazing trials. The sustained-release dental plaster pellet used at Idaho were found to be unsuitable for range studies. In other work, paper and solka floc appear to be comparable as carriers for chromic oxide. The former was used in range work at Nebraska and New Mexico, and the latter at Oregon and Arizona. Although there were differences in recoveries, variation was comparable within experiment. Between experiment variation appears to be attributable to factors other than method of indicator administration, as supported by the observation that experiments conducted by the same workers at different times and possibly slightly differing conditions (Table 12) have given widely differing results.

When grab sample chromic oxide was actually used to estimate fecal production or forage intake, the disparities between measured and estimated values suggest that good agreement between any given measured and estimated quantity is fortuitous, rather than the result of superior technique. Under- and over-estimates of fecal production ranged from -31 to +21 percent (Table 18) and -16 and +87 percent (Table 21) at Oregon. Arizona work gave a 24 percent and Nebraska work at 14 percent over-estimation of fecal output. Intake at the latter location exceeded by 8 percent the estimate obtained using total fecal collection.

At Nevada, good agreement was obtained between measured and estimated fecal output when chromic oxide standards were prepared in the presence of fecal ash. Otherwise, the estimated output value exceeded measured output by 14 percent.

Chromic oxide has been analyzed by the method of Bolin *et al.* (1952) at Nevada and Oregon, and by the method of Kimura and Miller (1957) at Nebraska, Arizona, Utah, and New Mexico. Both these methods require the use of nitric acid, perchloric acid, and sodium molybdate. Problems were experienced at Arizona in chromic oxide work (not related to these projects) in which during the wet-ashing procedure (boiling in nitric acid), several samples were heated simultaneously on a large hotplate. Measured chromic oxide was lower for samples placed nearer the outer edge of the hotplate where the heat was less intense than those nearer the center, although there did not appear to be any difference in the solutions. At the Squaw Butte Station in Oregon, a set of chromic oxide-containing samples from a digestion trial was sent to a second laboratory for analysis, with subsequent erratic experimental results derived from the chromic oxide values obtained. When the same samples were analyzed by the station

laboratory technician, one with considerable experience working with chromic oxide, the results were entirely different and much more credible. It might be revealing to learn of the problems experienced (and those which have gone undetected) by other workers in this field.

The use of chromic oxide as an external indicator in range nutrition studies necessarily will be limited in view of the work reported herein, unless several problems can be resolved. The reasons for low or high recoveries and erratic excretion patterns of chromic oxide need to be better understood and means of correcting them developed. The approach to be taken to resolve these difficulties is not readily apparent.

RECOMMENDATIONS

On the basis of studies of this regional project, the following recommendations are offered where chromic oxide is to be used in range nutrition studies:

1. Results obtained under drylot conditions should not be presumed to apply similarly to grazing trials.
2. Chromic oxide should be used only for estimating forage intake and/or digestibility on a comparative, not an absolute, basis.
3. Comparisons should be limited to one trial, and comparisons between trials or experiments conducted at different times or under differing conditions should not be compared.
4. Animals utilized should be as nearly alike as possible, in keeping with good experimental technique.
5. Chromic oxide can be administered with either paper or solka floc as a carrier (although not verified by these studies, other work suggests that chromic oxide given in powder form results in a more erratic excretion pattern than when given impregnated on paper.
6. There is some indication that a twice daily dosing will produce a more uniform excretion pattern than once daily, although this is not conclusive.
7. A minimum four-day preliminary dosing period should be employed when either paper or solka floc is used as a chromic oxide carrier, and this period may need to be longer under some conditions.
8. Sampling can be done at any time of the day, but provisions should be taken to insure that a sufficient number of samples be taken to average out the day and steer variation.

9. Great care should be taken when analyzing chromic oxide samples to insure that for a given trial, all samples (a) be tested under uniform analytical conditions, (b) be compared to the same standards, (c) be analyzed by the same person, and (d) be analyzed as a group at the same time insofar as feasible.

LITERATURE CITED

- Bolin, D. W., R. P. King and E. W. Klosterman. 1952. A simplified method for the determination of chromic oxide (Cr_2O_3) when used as an index substance. *Science* 116:634.
- Border, J. R. 1962. Development of the chromic oxide indicator technique for estimating feces output by ruminants. Unpublished Ph.D. dissertation, Utah State University, Logan, Utah.
- Corbett, J. L., J. R. D. Greenhalgh and A. P. MacDonald. 1958. Paper as a carrier of chromium sesquioxide. *Nature* 182-1014.
- Harris, L. E., G. P. Lofgreen, C. J. Kercher, R. J. Raleigh and V. R. Bohman. 1967. Techniques of research in range livestock nutrition. *Utah Agr. Exp. Sta. Bull.* 471.
- Kiesling, H. E., H. A. Barry, A. B. Nelson and C. H. Herbel. 1969. Recovery of chromic oxide administered in paper to grazing steers. *J. Animal Sci.* 29:361.
- Kimura, F. T. and V. L. Miller. 1957. Improved determination of chromic oxide in cow feed and feces. *J. Agr. Food Chem.* 5:216.
- Lesperance, A. L. and V. R. Bohman. 1963. Use of fistulated and intact cattle for predicting digestibility. *Proc. West. Sec. Am. Soc. Animal Sci.* 14:XXXIV.
- McCann, C. P. and B. Theurer. 1967. Evaluation of lignin ration and chromic oxide indicator methods. *Proc. West. Sec. Am. Soc. Animal Sci.* 18:249.
- Nelson, A. B. and G. R. Green. 1969. Excretion of chromic oxide administered in paper to steers fed prairie hay. *J. Animal Sci.* 29:365.
- Pigden, W. J. and G. W. Brisson. 1957. Note on a chromium oxide pellet to provide uniform release of this indicator in the rumen of cattle. *Can. J. Animal Sci.* 14:289.
- Pryor, W. J. 1966. Some techniques for determining fecal output and digestibility of range forage by cattle. Unpublished Ph.D. dissertation, Oregon State University, Corvallis, Oregon.

- Rittenhouse, L. R. 1969. The influence of supplemented protein and energy on intake and digestibility of winter range forage. Unpublished Ph.D. dissertation, University of Nebraska, Lincoln, Nebraska.
- Robertson, J. B. 1966. Observations on the dissolution of sustained release pellets and the excretion pattern of chromium sesquioxide liberated from such pellets. Unpublished M.S. thesis, University of Idaho, Moscow, Idaho.
- Streeter, C. L. 1966. Methods of estimating the digestibility and voluntary intake of range forage consumed by grazing cattle. Unpublished Ph.D. dissertation, University of Nebraska, Lincoln, Nebraska.
- Theurer, Brent. 1969. Chemical indicator techniques for determining range forage consumption. USDA Misc. Publ. 1147.
- Wheeler, R. R. 1962. Evaluation of various indicator techniques in estimating forage intake and digestibility by range cattle. Unpublished Ph.D. dissertation, Oregon State University, Corvallis, Oregon.

Table 1. Recovery (%) of chromic oxide by days after start of administration

| Day | Total collection fecal samples | Grab fecal samples |
|--------------------------------|-----------------------------------|-----------------------|
| | Mean ^a | Mean ^a |
| 1 | 22 | |
| 2 | 65 | |
| 3 | 112 | |
| 4 | 110 | |
| 5 | 104 | |
| 6 | 106 | |
| 7 | 101 | |
| 8 | 92 | |
| 9 | 99 | |
| 10 | 97 | |
| 11 | 102 | |
| Mean ^b , days 3-11 | 103 | |
| 14 | 104 | 101 |
| 15 | 101 | 97 |
| 16 | 92 | 90 |
| 17 | 99 | 89 |
| 18 | 88 | 89 |
| Mean ^c , days 14-18 | 97 | 93 |

^aMean of 6 values.

^bNo differences ($P > .05$).

^cOnly the days X steers interaction was significant ($P < .05$).

Table 2. Recovery (%) of chromic oxide by days after start of every other day administration

| Day | Chromic oxide administered ^a | Total fecal | Fecal |
|----------------|---|--------------------|-------------------|
| | | collection samples | grab samples |
| | | Mean ^b | Mean ^b |
| 1 | + | 30 | |
| 2 | - | 85 | |
| 3 | + | 86 | |
| 4 | - | 100 | |
| 5 | + | 77 | |
| 6 | - | 96 | |
| 7 | + | 69 | |
| 8 | - | 110 | |
| 9 | + | 73 | |
| Mean, days | | | |
| 2, 4, 6, 8 | - | 98 | |
| Mean, days | | | |
| 3, 5, 7, 9 | + | 76 | |
| 15 | - | .. | 127 |
| 16 | + | 78 | 81 |
| 17 | - | 119 | 124 |
| 18 | + | 74 | 79 |
| 19 | - | 114 | 104 |
| 20 | + | 75 | 83 |
| 21 | - | 122 | .. |
| Mean, days | | | |
| 16, 17, 19, 21 | - | 76 ^c | 81 |
| Mean, days | | | |
| 15, 17, 19, 21 | - | 118 ^{c,d} | 118 ^e |

^aPositive = chromic oxide administered; negative = no chromic oxide administered.

^bMean of 6 samples.

^cDifference among steers within each group of days ($P < .05$).

^d $P < .01$ for differences between odd- and even-numbered days.

^e $P < .05$ for differences between odd- and even-numbered days.

Table 3. Chromic oxide recovered (%) per level of chromic oxide fed^a

| Treatment level Cr ₂ O ₃ fed | Recovery of Cr ₂ O ₃ fed |
|---|---|
| Grams | % |
| 1 | 94.6 |
| 2 | 97.0 |
| 4 | 100.0 |
| 8 | 100.5 |

^aMeans of 4 animals X 14 days (or seven 2-day periods).

Table 4. Average daily recovery of chromic oxide

| Treatment | Trial 1 ^a | | Trial 2 ^b | | Average | |
|------------|----------------------|-----------------|----------------------|-----------------|---------|----|
| | Grams | % | Grams | % | Grams | % |
| Intact | 8.3 | 76 | 8.4 | 88 | 8.4 | 82 |
| Fistulated | 8.3 | 76 | 8.3 | 86 | 8.3 | 81 |
| (Average) | 8.3 | 76 ^c | 8.4 | 87 ^c | 8.4 | 82 |

^a10.88 gm Cr₂O₃ administered daily.

^b9.59 gm Cr₂O₃ administered daily.

^cMeans differ (P < .05).

Table 5. Chromic oxide recovery (%) from steers grazing dormant tobosa

| Day of collection | Steer | | | | | Average |
|---------------------|-------------------|-------------------|--------------------|--------------------|-------------------|---------|
| | A | B | C | D | E | |
| 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2 | 47.1 | 39.4 | 33.9 | 48.3 | 62.1 | 46.2 |
| 3 | 79.6 | 66.7 | 64.4 | 71.1 | 83.1 | 73.0 |
| 4 | 63.1 | 78.1 | 82.0 | 60.5 | 70.6 | 70.8 |
| 5 | 32.5 | 98.8 | 66.8 | 67.1 | 84.3 | 69.9 |
| 6 | 54.3 | 88.6 | 59.3 | 91.5 | 51.0 | 68.9 |
| 7 | 45.7 | 68.1 | 55.7 | 61.9 | 89.2 | 64.1 |
| 8 | 51.1 | 112.9 | 78.0 | 71.7 | 96.2 | 82.0 |
| Average of days 3-8 | 54.4 ^b | 85.5 ^a | 67.7 ^{ab} | 70.6 ^{ab} | 79.1 ^a | 71.5 |

^{ab}Steer means with different letter superscripts differ ($P < .05$).

Table 6. Chromic oxide recovery (%) by steers grazing dormant tobosa

| Day of Collection | Steer | | | | Average |
|---------------------|-------|------|------|------|---------|
| | A | B | C | D | |
| 2 | 24.9 | 43.0 | 41.7 | 44.7 | 38.6 |
| 3 | 54.6 | 65.5 | 75.3 | 69.1 | 66.1 |
| 4 | 80.0 | 90.6 | 77.4 | 82.2 | 82.5 |
| 5 | 78.3 | 73.2 | 79.0 | 76.6 | 76.8 |
| 6 | 82.7 | 77.7 | 79.6 | 77.5 | 79.4 |
| 7 | 83.3 | 85.5 | 92.2 | 84.2 | 86.3 |
| 8 | 78.3 | 96.0 | 99.1 | 81.3 | 88.6 |
| 9 | 82.3 | 88.6 | 71.4 | 82.2 | 81.1 |
| Average of days 4-9 | 80.8 | 85.3 | 83.1 | 80.6 | 82.4 |

Table 7. Chromic oxide recovery (%) by steers grazing green tobosa

| Day of collection | Steer | | | | Average |
|----------------------|--------------------|--------------------|-------------------|-------------------|---------|
| | A | B | C | D | |
| 2 | 43.7 | 4.2 | 40.3 | 47.6 | 33.9 |
| 3 | 63.1 | 23.1 | 58.4 | 81.9 | 56.6 |
| 4 | 87.2 | 90.7 | 39.3 | 83.3 | 75.1 |
| 5 | 96.8 | 108.2 | 21.6 | 82.2 | 77.2 |
| 6 | 105.9 | 127.6 | 50.4 | 78.4 | 90.6 |
| 7 | 91.6 | 114.9 | 70.5 | 104.7 | 95.4 |
| 8 | 103.7 | 92.3 | 79.5 | 90.1 | 91.4 |
| 9 | 82.7 | 93.0 | 97.1 | 96.1 | 92.2 |
| 10 | 85.7 | 110.4 | 94.4 | 87.2 | 94.5 |
| Average of days 4-10 | 93.3 ^{ab} | 105.4 ^a | 64.7 ^c | 88.8 ^b | 88.0 |

^{abc}Steer means with unlike superscripts are different ($P < .05$).

Table 8. Recovery of chromic oxide from steers and heifers grazing native summer or winter range

| Location ^a | Trial | Diet ^b | No. Animals | Recovery, % |
|-----------------------|-------|-------------------|-------------|-------------|
| SB | 1-64 | SR | 3 | 94 |
| SB | 2-64 | SR | 3 | 86 |
| SB | 1-65 | SR | 5 | 83 |
| SB | 2-65 | SR | 5 | 91 |
| SB | 3-65 | SR | 5 | 95 |
| FR | 1-66 | WR | 5 | 80 |
| FR | 2-66 | WR | 4 | 96 |

^aSB = Scotts Bluff; FR = Fort Robinson.

^bSR = native summer range; WR = native winter range.

Table 9. Daily recovery of chromic oxide (%) from heifers and steers grazing native range

| Animal | Day | | | | | | | Mean ^a |
|---------|-------------------|-------------------|-------------------|-------|-------------------|-------------------|-------|-------------------|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | |
| Trial 1 | | | | | | | | |
| 1 | 66.0 | b | 85.0 | 80.9 | 69.3 | 87.7 | 97.6 | 81.1 |
| 2 | 85.4 | 45.3 ^c | 55.1 ^c | 88.3 | 44.4 ^c | 80.8 | 127.8 | 95.6 |
| 3 | 114.5 | 82.6 | 93.0 | 105.2 | 69.0 | 73.8 | 114.5 | 93.2 |
| 4 | 80.5 | 74.7 | 49.3 ^c | 65.5 | 58.2 ^c | 50.2 ^c | 55.4 | 73.6 |
| 5 | 100.3 | b | 95.1 | 96.2 | 93.6 | 79.4 | 85.8 | 91.7 |
| 6 | 91.2 | 86.8 | 90.7 | 96.7 | 117.0 | 85.4 | 74.9 | 91.8 |
| Average | | | | | | | | 87.8 ^d |
| Trial 2 | | | | | | | | |
| 1 | 73.4 | 79.3 ^c | 95.6 | 80.1 | 76.2 | 92.5 | 83.4 | 83.5 |
| 2 | 28.2 ^c | 110.8 | 103.8 | 82.0 | 74.0 | 96.0 | 66.0 | 87.1 |
| 3 | 109.2 | 95.5 | 93.0 | 92.0 | 100.4 | 97.4 | 91.8 | 97.0 |
| 4 | 99.9 | 98.6 | 34.9 ^c | 95.1 | 87.0 | 84.5 | 87.5 | 92.1 |
| 5 | 83.5 | 100.1 | 89.2 | 96.7 | 94.9 | 106.9 | 91.9 | 94.7 |
| 6 | 89.7 | 74.4 | 98.6 | 90.8 | 92.0 | 89.6 | 68.6 | 86.2 |
| Average | | | | | | | | 90.1 ^d |

^a Computed only on known values.

^b Missing sample.

^c Incomplete total collection.

^d $H_0 : X = 100\%; P < .05.$

Table 10. Chromic oxide recovery and estimation of fecal output from fecal grab sample chromic oxide^a

| Steer number | Cr ₂ O ₃ recovery | | Daily fecal output | |
|--------------|---|-------------------|-----------------------|--|
| | Total collection % | Grab samples % | Collection bags kg | Grab sample Cr ₂ O ₃ kg |
| 1 | 80.1 | 82.2 | 3.04 | 3.07 |
| 2 | 71.4 | 75.8 | 3.03 | 4.00 |
| 3 | 77.0 | 87.0 | 2.76 | 3.18 |
| 4 | 75.5 | 78.3 | 2.87 | 3.66 |
| Average | 76.0 | 80.8 | 2.93 | 3.63 |

^aCr₂O₃ administered once daily; fecal grab sample taken once daily.

Table 11. Chromic oxide recovery in feces from steers grazing or hand-fed crested wheatgrass^a

| Trial No. | Animal No. | Treatment | % Cr ₂ O ₃ recovered | |
|----------------------------|------------|-----------|--|-----|
| 1 immature herbage | 1 | grazing | 126 | |
| | 3 | | 124 | |
| | 5 | | 112 | |
| | | Mean | | 121 |
| | | 2 | hand-fed | 103 |
| | | 4 | | 109 |
| | | 6 | | 94 |
| | Mean | | 102 | |
| 2 mature herbage | 1 | grazing | 102 | |
| | 3 | | 112 | |
| | 5 | | 114 | |
| | | Mean | | 109 |
| | | 2 | hand-fed | 102 |
| | | 4 | | 99 |
| | | 6 | | 102 |
| | Mean | | 101 | |
| 3 mature-dry herbage | 1 | grazing | 138 | |
| | 2 | | 132 | |
| | 3 | | 127 | |
| | | Mean | | 132 |
| | | 2 | hand-fed | 130 |
| | | 4 | | 128 |
| | | 6 | | 112 |
| | Mean | | 123 | |

^a5 grams of Cr₂O₃ in 10 gram gelatin capsule given once daily for 5 days.

Table 12. Chromic oxide recovery from feces of steers grazing immature or mature crested wheatgrass^a

| Collection period ^b | Animal ^c | Chromic oxide recovery | |
|--------------------------------|---------------------|------------------------|---------|
| | | Trial 1 | Trial 2 |
| | | % | % |
| 1 | 1 | 81.6 | 107.6 |
| | 2 | 71.1 | 97.8 |
| | 3 | 77.1 | 94.9 |
| | Mean | 76.9 | 100.1 |
| | 4 | 83.4 | 95.6 |
| | 5 | 78.8 | 101.9 |
| | 6 | 85.1 | 104.3 |
| 2 | Mean | 82.4 | 100.6 |
| | 1 | 78.0 | 103.5 |
| | 2 | 71.9 | 100.3 |
| | 3 | 79.2 | 107.5 |
| | Mean | 76.7 | 103.8 |
| | 4 | 85.4 | 95.6 |
| | 5 | 78.0 | 99.8 |
| 6 | 84.3 | 100.8 | |
| | Mean | 82.5 | 98.7 |

^aTrial 1 conducted on immature forage; Trial 2 conducted on mature forage.

^bPeriods of 5 days each.

^cAnimals 1-3 were dosed with 10 grams of chromic oxide at 8 a.m. daily; animals 4-5 were dosed with 5 grams at 8 a.m. and 4 p.m.

Table 13. Diurnal variation in percent recovery of chromic oxide administered daily, two-day average

| Time | Mean recovery | Standard error |
|-------------------|-----------------|----------------|
| 7 p.m. | 93 | 6.0 |
| 9 p.m. | 98 | 3.4 |
| 11 p.m. | 98 | 5.3 |
| 1 a.m. | 99 | 1.9 |
| 3 a.m. | 92 | 2.2 |
| 5 a.m. | 99 ^a | 3.5 |
| 7 a.m. | 88 | 4.1 |
| 9 a.m. | 100 | 2.7 |
| 11 a.m. | 96 | 2.0 |
| 1 p.m. | 98 | 1.6 |
| 3 p.m. | 97 | 4.3 |
| 5 p.m. | 99 | 5.5 |
| Mean ^b | 96 | 1.1 |

^aThis value represents only one day and was not included in the overall mean or in the statistical analysis.

^bDifferences between days and a day X steer interaction ($P < .05$).

Table 14. Diurnal variation in recovery (%) of chromic oxide administered every other day (across steers and periods)

| Time | Day of Cr ₂ O ₃ administration | | Day following Cr ₂ O ₃ administration | |
|---------|--|------|---|------|
| | Mean* | S.E. | Mean* | S.E. |
| 7 a.m. | 103 ^a | 5.3 | 65 ^e | 5.5 |
| 9 a.m. | 93 ^b | 5.9 | 76 ^e | 7.7 |
| 11 a.m. | 91 ^b | 5.1 | 99 ^d | 8.6 |
| 1 p.m. | 80 ^c | 6.6 | 108 ^d | 13.3 |
| 3 p.m. | 70 ^d | 5.2 | 119 ^c | 8.6 |
| 5 p.m. | 64 ^d | 5.8 | 130 ^{abc} | 8.3 |
| 7 p.m. | 55 ^e | 5.2 | 136 ^a | 6.4 |
| 9 p.m. | 50 ^e | 4.9 | 132 ^{ab} | 5.7 |
| 11 p.m. | 49 ^e | 4.3 | 125 ^{abc} | 3.4 |
| 1 a.m. | 45 ^e | 4.2 | 125 ^{abc} | 3.8 |
| 3 a.m. | no sample | ... | no sample | ... |
| 5 a.m. | 52 ^e | 3.9 | 120 ^{bc} | 4.2 |
| Mean | 68 | 6.2 | 112 ^{**} | 7.0 |

* Means of 4 days X 6 steers.

** Recovery was higher ($P < .01$) on days following administration of chromic oxide.

abcde Time means within a column and having different letter superscripts were different ($P < .05$).

Table 15. Diurnal variation in recovery (%) of chromic oxide administered every other day with only two fecal samples daily from each steer

| Time | Day of Cr ₂ O ₃ administration | | Day following Cr ₂ O ₃ administration | | Difference |
|---------|--|------|---|------|------------------|
| | Mean ^a | S.E. | Mean ^a | S.E. | |
| 7 a.m. | 90 ^b | 8.1 | 66 ^e | 3.4 | 24 [*] |
| 9 a.m. | 90 ^b | 6.1 | 94 ^{cd} | 10.6 | 4 |
| 11 a.m. | 71 ^{bc} | 8.0 | 89 ^{de} | 2.6 | 18 |
| 1 p.m. | 90 ^b | 13.1 | 110 ^{bcd} | 8.5 | 20 |
| 3 p.m. | 55 ^{cd} | 9.3 | 91 ^{cd} | 8.4 | 36 ^{**} |
| 5 p.m. | 67 ^{bc} | 7.9 | 116 ^{bc} | 15.0 | 49 ^{**} |
| 7 p.m. | 47 ^{cd} | 7.3 | 117 ^{bc} | 5.1 | 70 ^{**} |
| 9 p.m. | 53 ^{cd} | 2.1 | 134 ^b | 5.6 | 81 ^{**} |
| 11 p.m. | 52 ^{cd} | 13.3 | 115 ^{bc} | 2.0 | 63 ^{**} |
| 1 a.m. | 65 ^c | 6.2 | 126 ^b | 5.4 | 61 ^{**} |
| 3 a.m. | 39 ^d | 4.4 | 117 ^{bc} | 3.1 | 78 ^{**} |
| 5 a.m. | 53 ^{cd} | 2.7 | 106 ^{cd} | 15.1 | 53 ^{**} |
| Mean | 64 | 5.1 | 107 | 5.4 | 43 ^{**} |

* P < .05.

** P < .01.

^a Each value is the mean based on 2 samples taken from 3 steers on 3 different days.

^{bcd} Time means within the same column and having different letter superscripts are different (P < .05).

Table 16. Diurnal variation in excretion of chromic oxide (%) by steers grazing dormant tobosa

| Item | 24 hr. | 24 hr. | Difference | Average |
|-----------|-------------------|----------------------|-------------------|--------------------|
| Hour | | | | |
| 7 a.m. | 71.1 | 65.3 ^d | 5.8 | 68.2 ^g |
| 9 a.m. | 63.4 | 65.3 ^d | 1.9 | 64.4 ^g |
| 11 a.m. | 62.8 | 68.2 ^{bcd} | 5.4 | 65.5 ^g |
| 1 p.m. | 70.0 | 66.2 ^{cd} | 3.8 | 68.1 ^g |
| 3 p.m. | 65.5 | 73.6 ^{bcd} | 8.1 | 69.5 ^g |
| 5 p.m. | 69.2 | 89.8 ^a | 20.6 [*] | 79.5 ^f |
| 7 p.m. | 61.9 | 79.8 ^{ab} | 17.9 [*] | 70.9 ^{fg} |
| 9 p.m. | 64.4 | 79.0 ^{abc} | 14.6 | 71.7 ^{fg} |
| 1 a.m. | 61.6 | 72.1 ^{bcd} | 10.5 | 66.8 ^g |
| 3 a.m. | 66.0 | 77.6 ^{abcd} | 11.6 | 71.8 ^{fg} |
| 5 a.m. | 65.1 | 71.2 ^{bcd} | 6.1 | 68.1 ^g |
| Steer | | | | |
| A | 48.6 ^k | 48.3 ^l | 0.3 | 48.4 ^z |
| B | 65.6 ^j | 70.0 ^k | 4.4 | 67.8 ^y |
| C | 62.5 ^j | 78.7 ^j | 16.2 [*] | 70.6 ^y |
| D | 64.3 ^j | 79.3 ^j | 15.0 [*] | 71.8 ^y |
| E | 86.7 ⁱ | 90.0 ⁱ | 4.2 | 88.8 ^x |
| \bar{X} | 65.5 | 73.5 | 8.0 | 69.5 |

abcd Hour means within the same 24-hour period with different superscripts are different ($P < .05$).

fg Average hour means with different superscripts are different ($P < .05$).

ijkl Steer means within the same 24-hour period with different superscripts are different ($P < .05$).

xyz Average steer means with different superscripts are different ($P < .05$).

* $P < .05$ for differences between 24-hour periods.

Table 17. Apparent dry matter and nitrogen digestibility, daily fecal dry matter, and dry matter intake for each animal, calculated with chromic oxide foliar application

| Animal No. | Apparent digestion coefficients | | Daily fecal dry matter | Daily dry matter intake |
|------------|---------------------------------|------------|------------------------|-------------------------|
| | Nitrogen | Dry matter | | |
| | % | % | kg | kg |
| 1 | 47.1 | 57.8 | 2.81 | 4.88 |
| 2 | 45.8 | 52.8 | 3.32 | 6.30 |
| 3 | 46.1 | 53.4 | 3.06 | 5.75 |
| 4 | 41.4 | 52.8 | 2.42 | 4.59 |
| 5 | 39.6 | 51.4 | 3.28 | 6.39 |
| Mean | 44.0 | 53.6 | 2.98 | 5.58 |

Table 18. Estimated fecal output from chromic oxide concentration in single daily fecal grab samples^a

| Trial | Treatment | Number of steers | Measured output ^b | Estimated | |
|-------|---------------|------------------|------------------------------|---------------------|---------------------|
| | | | | Output ^b | Percent of measured |
| | | | gm/day | gm/day | % |
| 1 | Hay, hand fed | 8 | 2148 | 2413 | 111.4 |
| 2 | Hay, hand fed | 8 | 3168 | 3196 | 99.7 |
| 3 | Native range | 4 | 2926 | 3634 | 124.2 |

^aChromic oxide mixed in a 2:1 ratio with cellulose and administered once daily.

^bDry matter basis.

Table 19. Average fecal dry matter output as measured by total collections and estimated by three chromic oxide values from fecal samples from steers hand-fed or grazing crested wheatgrass

| Trial* | Treatment** | Fecal dry matter output | | | Difference |
|--------|-------------|-------------------------|------|-----------|---------------|
| | | Measured | | Estimated | from measured |
| | | lb/day | | lb/day | % |
| 1 | hand-fed | 4.6 | A*** | 4.3 | -6.5 |
| | | | B | 5.0 | 8.7 |
| | | | C | 5.0 | 8.7 |
| | grazing | 6.1 | A | 5.1 | -16.4 |
| | | | B | 5.1 | -16.4 |
| | | | C | 5.7 | -6.6 |
| 2 | hand-fed | 6.8 | A | 6.0 | -11.8 |
| | | | B | 7.6 | 11.8 |
| | | | C | 7.4 | 7.4 |
| | grazing | 8.8 | A | 7.3 | -17.0 |
| | | | B | 9.8 | 11.4 |
| | | | C | 9.7 | 10.2 |
| 3 | hand-fed | 7.4 | A | 5.3 | -28.4 |
| | | | B | 8.0 | 8.1 |
| | | | C | 7.4 | 0.0 |
| | grazing | 9.1 | A | 6.3 | -30.8 |
| | | | B | 9.8 | 7.7 |
| | | | C | 9.4 | 3.3 |

* Trials 1, 2, and 3 correspond to immature, mature, and dry-mature forage respectively.

** Three steers per treatment.

*** A = estimated from composite fecal samples.
 B = estimated from mean of all grab samples.
 C = estimated from grab samples taken at 6 a.m. and 4 p.m.

Table 20. Comparison of three methods of administering chromic oxide to estimate fecal output

| Treatment | Steer | Fecal output ^a | | Estimated as percent of measured |
|--|-------|---------------------------|-----------|----------------------------------|
| | | Measured | Estimated | |
| | | 1b | 1b | % |
| Cottonseed meal | 1 | 4.0 | 5.0 | 124 |
| | 2 | 4.7 | 5.4 | 114 |
| | Mean | 4.4 | 5.2 | 119 |
| Cr ₂ O ₃ powder in capsules | 3 | 3.7 | 4.7 | 126 |
| | 4 | 2.8 | 4.2 | 150 |
| | Mean | 3.2 | 4.4 | 138 |
| Cr ₂ O ₃ -solka floc in capsules | 5 | 3.7 | 4.7 | 127 |
| | 6 | 3.2 | 3.8 | 119 |
| | Mean | 3.4 | 4.2 | 123 |

^a Dry matter per day.

Table 21. Fecal dry matter output ratio (grab^a Cr₂O₃ estimated/measured) for steers grazing crested wheatgrass

| Trial ^b | Collection period | Dosing time ^c | Estimated/measured | |
|--------------------|-------------------|--------------------------|-------------------------|--------------------------------|
| | | | Mean \pm S.E. a.m. | Mean \pm S.E. a.m. & p.m. |
| 1 | A | a.m. | 1.87 \pm 0.16 | 1.49 \pm 0.11 |
| | | a.m. & p.m. | 1.26 \pm 0.02 | 1.38 \pm 0.03 |
| | B | a.m. | 1.36 \pm 0.06 | 1.50 \pm 0.08 |
| | | a.m. & p.m. | 1.16 \pm 0.04 | 1.39 \pm 0.03 |
| 2 | A | a.m. | 0.95 \pm 0.07 | 1.10 \pm 0.11 |
| | | a.m. & p.m. | 0.92 \pm 0.05 | 1.05 \pm 0.04 |
| | B | a.m. | 0.84 \pm 0.04 | 0.97 \pm 0.04 |
| | | a.m. & p.m. | 0.95 \pm 0.02 | 0.96 \pm 0.02 |

^aFecal grab samples taken at 8 a.m. and 4 p.m. daily.

^bTrial 1 was conducted on immature and Trial 2 on mature crested wheatgrass.

^cThree steers per treatment (dosing time).

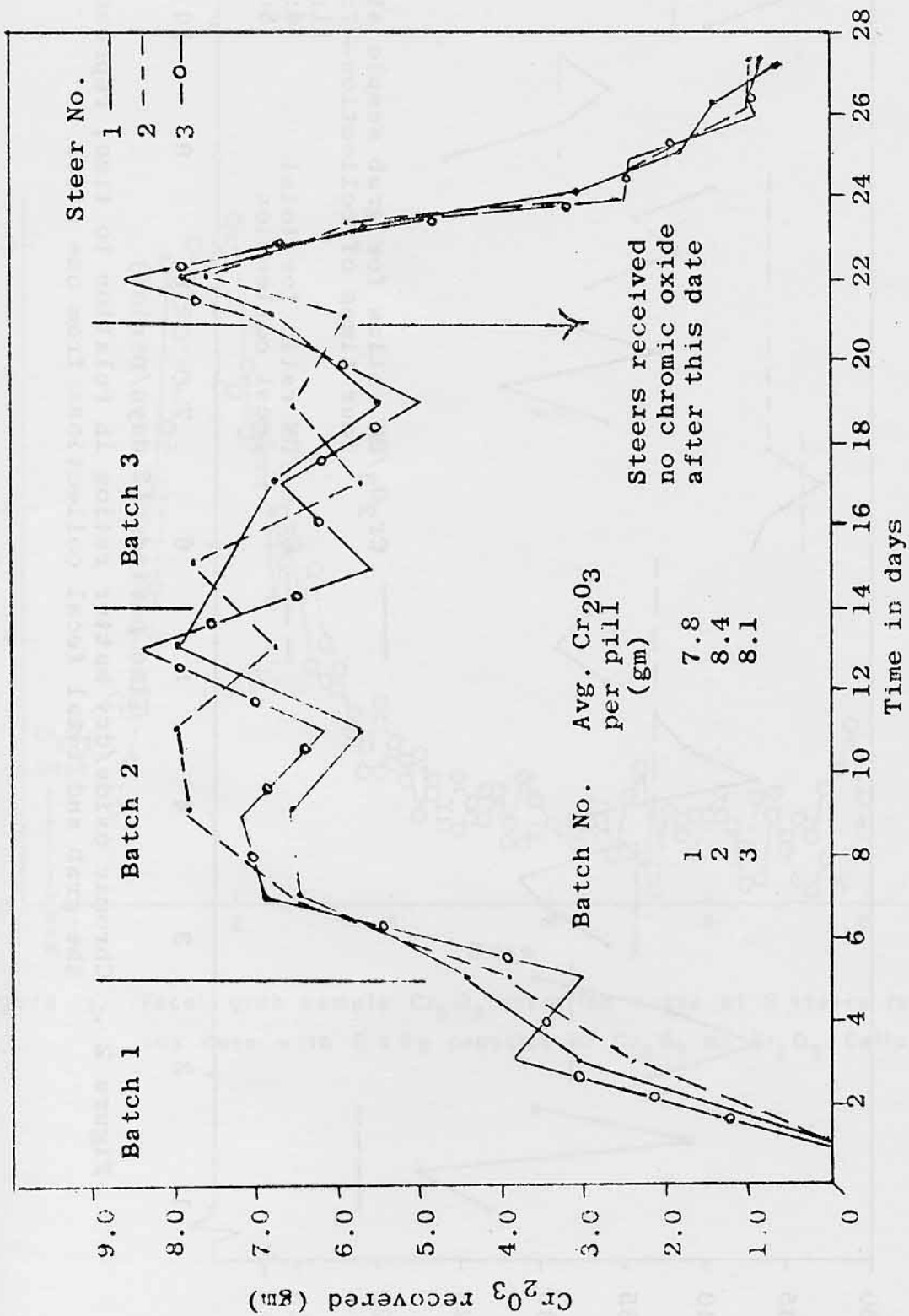


Figure 1. The average daily recovery of chromic oxide for three steers.

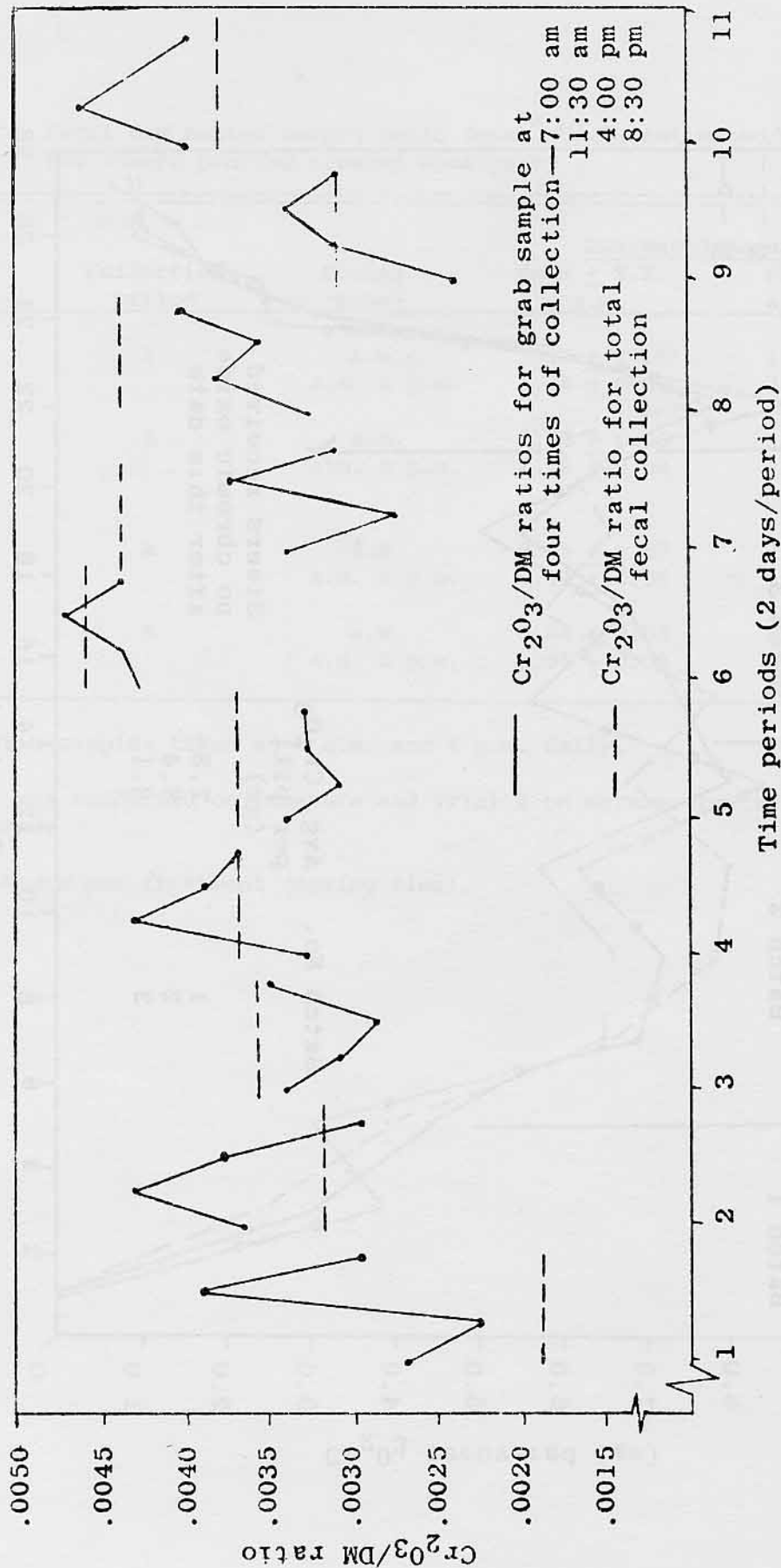


Figure 2 . Chromic oxide/dry matter ratios in relation to time, representing the grab and total fecal collections from one steer.

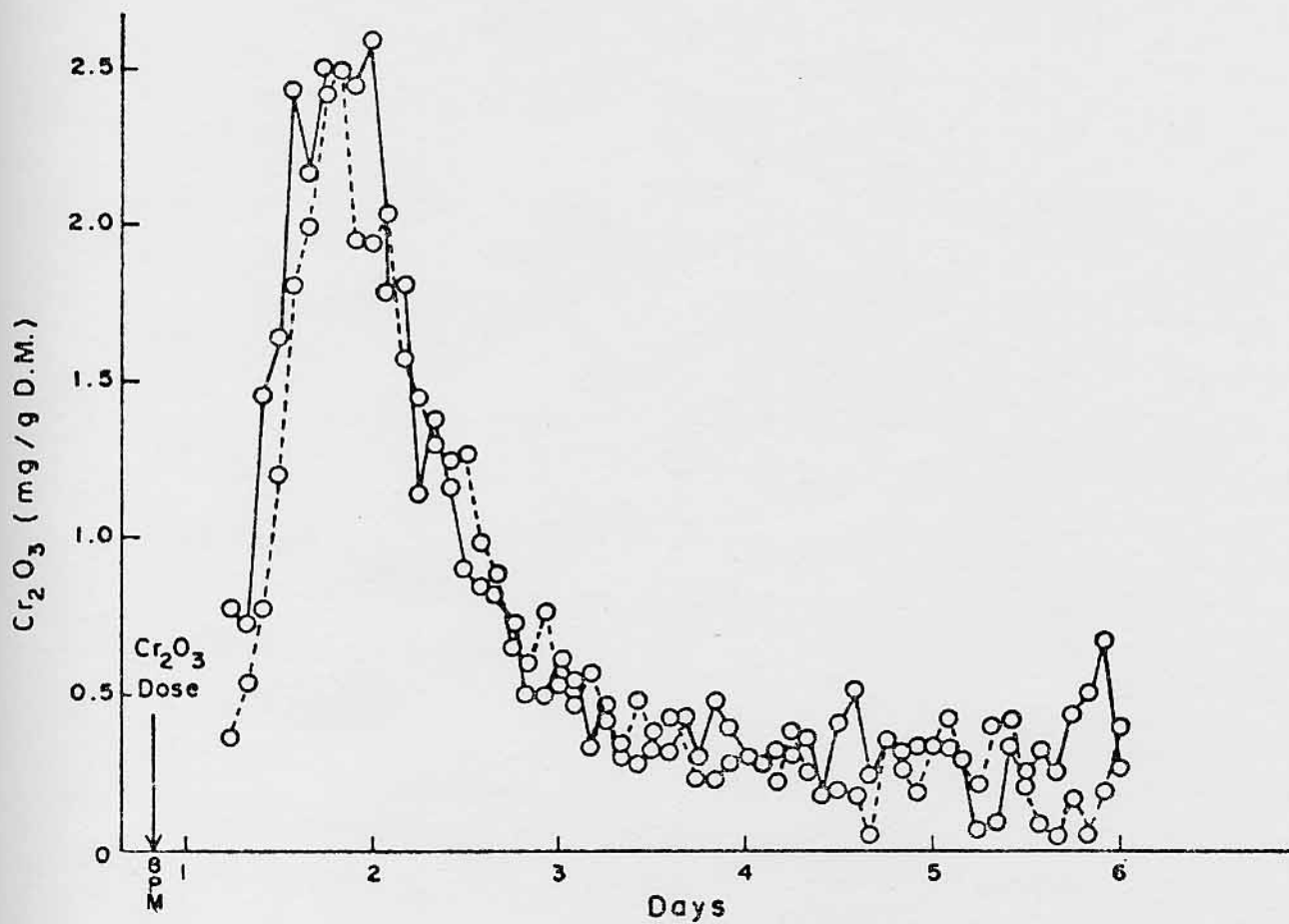


Figure 3. Fecal grab sample Cr_2O_3 excretion curve of 2 steers following one dose with 2 x 5g capsules of Cr_2O_3 as Cr_2O_3 -Cellulose.