DETERMINATION OF THE USEFULNESS OF CHROMIUM-51 IN DIGESTIVE STUDIES OF THE WHITE-TAILED DEER

Thesis for the Degree of M. S.

MICHIGAN STATE UNIVERSITY

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1967

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ABSTRACT

DETERMINATION OF THE USEFULNESS OF
CHROMIUM-51 IN DIGESTIVE STUDIES
OF THE WHITE-TAILED DEER

by William W. Mautz

The usefulness of chromium-51 in digestive studies of the white-tailed deer (Odocoileus virginianus) was determined. Labeling foods with a constant level of Cr-51 for two successive days and collecting fecal samples on these and one additional day permitted the determination of the degree to which the food was digested and the length of time required for that digestion. The Cr-51 method yielded similar but less variable results than the conventional total collection method and eliminated much of the tedious and unpleasant work associated with the latter.

On a lamb-finisher diet, a rapidly rising amount of the isotope was observed in the feces during the period from 8-12 hours after its first ingestion. A maximum level of Cr-51 in the feces occurred 18-20 hours after its ingestion. During a period corresponding to that of continuous isotope feeding, the concentration of indicator in the feces became stable. After food free of the isotope was substituted for the chromium labeled diet, a regular percentage decrease in

the amount of fecal Cr-51 occurred per unit of time. The logarithms of isotope concentration graphed against time indicated the average percentage of rumen contents which were removed per hour. For deer on the lamb-finisher diet, this was 7.56 per cent per hour. The chromium indicator disappeared from the feces 30-40 hours after ingestion. The average digestibility of the lamb-finisher diet was 59.35 per cent of the dry weight.

In a single feeding trial of white cedar (Thuja occidentalis) browse, it was found that this food was passed through the digestive tract less rapidly and was digested to a lesser extent than the lamb-finisher pellets.

Combining data from bomb-calorimeter determinations with passage rate and digestibility percentages yielded values related to food energy. Each dry-weight gram of the lamb-finisher diet yielded 2,734.4 calories to the animal. The corresponding figure for white cedar browse was 2,603.8 calories. The hours required to defecate 95 per cent of a meal of each food were 24.3 hours and 31.5 hours respectively.

As by-product information, three experimental animals defecated an average of 12.6 pellet groups per 24-hour period with no differences between nocturnal and diurnal rates of defecation. Other digestive research which could be aided by the use of Cr-51 includes changes in digestibility of foods resulting from changed conditions, the length of time

required to 'adjust' an animal to a new diet, and the degree of absorption occurring in different areas of the alimentary tract.

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By

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A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Fisheries and Wildlife

1967

ACKNOWLEDGMENTS

Gratitude is expressed to my wife, Sue, who offered encouragement and support throughout the research and writing of this thesis.

These studies at Michigan State University were supported under United States Atomic Energy Commission Contract number At(11-1)-1534, with the cooperation also of the Division of Research, Michigan Department of Conservation.

Dr. G. A. Petrides of the Fisheries and Wildlife Department, served as principal advisor. I wish to thank him and the other members of my advisory committee, Dr. P. I. Tack, Chairman of the Department of Fisheries and Wildlife and Dr. R. H. Baker, Director of the University Museum, for many helpful suggestions and for cirtically reading the manuscript. Dr. W. S. Osburn of the Atomic Energy Commission aided greatly in planning phases. Gary E. Duke, doctoral candidate at Michigan State University, while working with pheasants on a similar project, participated in frequent discussions and made many helpful suggestions.

Dr. D. E. Ullrey of the Animal Husbandry Department served as a consultant throughout the study. Drs. L. D. Fay, R. I. Blouch and C. T. Black of the Michigan Department of

Conservation made experimental animals and some equipment and facilities available. Dr. Fay contributed useful ideas and assisted in handling the test animals.

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INTRODUCTION

Animal nutritionists are confronted with the need to measure food values in various rations. The extent to which a food is digested and the length of time required for this digestion are important factors in determining the food energy available for survival and growth of the consumer organism.

There have been numerous studies to determine digestive data for livestock, but only a limited number of similar studies in wild animals. Of the wild animals studied, the several deer species have received considerable attention. However, much more information is needed on the nutritional values of various food plants and on the physiological processes of digestion in the deer. Simplified procedures for attaining these ends are also valuable.

Digestion trials are normally concerned with either the passage rate of food or the extent to which a food is digested. Passage rate may be defined as the time required for the residues of a given quantity of food to pass through the digestive tract. The extent to which a food is absorbed by an animal is called the digestibility coefficient.

The objective of this study was the development and evaluation of a technique for readily obtaining passage rate,

digestibility, and related data for the white-tailed deer (*Odocoileus virginianus*).

PREVIOUS RESEARCH

Passage Rate:

The rate of food passage normally is determined by measuring the time required for indigestible markers to be defecated. These indicators must be recognizable as such after passing through the digestive tract and should be passed at the same rate as food residues.

A number of different substances have been used in the past as food labels in passage rate studies. Lambourne (1957) determined the food passage rate for sheep by using monastral blue and stained hay. Rubber rings and ferric oxide (Fe_2O_3) were used by Moore and Winter (1934) in studying passage rates in the bovine. Hoelzel (1930) made use of rubber particles, cotton thread, beads, seeds, aluminum, gold, silver, and steel in his study of passage rates in the rabbit, dog, cat, guinea pig, rat, mouse, hen, pigeon, monkey, and man.

Radioactive chromic oxide (Cr_2O_3) was used by Brandt and Thacker (1958) in a study of coprophagy in rabbits. Odum (1961) and Odum and Golley (1963) are among those who employed radioactive tracers in the investigation of metabolic rates and energy transfer. Petrides (1964, 1967) undertook pilot studies on the values of Cr-51 as a food

label in studies of the cotton rat (Sigmodon hispidus), opossum (Didelphis virginiana), bobcat (Lynx rufus) and other animals.

As end points in the determination of the period of food passage, Castle (1956) suggested using the times at which five and 95 per cent of the marker items had been defecated. This convention eliminates some of the problems and uncertainties of indicator defection at low levels, thus has been adopted in this study. These times, as well as the mean retention time, are derived from percentage excretion curves which are constructed by plotting the accumulated percentage of indicator defecated against time. The mean retention time is found by summing the times of excretion of 10 per cent units of the marker, between five per cent and 95 per cent, and dividing the total by 10 (Castle, 1956). This datum along with the times of first appearance, last appearance, and of five and 95 per cent defecation are used in passage rate comparisons in the present study.

Digestibility Coefficient:

The digestibility coefficient is the percentage of a particular food which is absorbed by an animal during its passage through the digestive tract. The conventional method of determining the digestibility coefficient for a particular diet is the total collection method. With this method, the coefficient is normally calculated as $(1 - \frac{\text{feces/time}}{\text{food/time}}) \times 100$.

The period of study must be sufficiently long to enable calculation of the average rates of consumption and defecation. The chief disadvantages of this method are the time and the labor required to collect and handle all food and fecal materials.

A more recent procedure is the indicator or ratio method. With this method, a known concentration of an indigestible substance is added to the food eaten. After the concentratration of the indicator in the feces reaches a stable level, digestibility coefficients may be obtained by comparing the amount of indicator in equal quantities of food and feces. By the ratio method, the amount of indicator originally present per unit weight of food is divided by the indicator present per unit weight of feces. The quotient subtracted from one and multiplied by 100 is the percentage of dry matter digested. Using an indicator, the total collection of feces is not necessary.

Chromic oxide has been the most widely used indicator in ratio type digestion trials. Investigators employing this material have included Lloyd et al. (1956) and Elam et al. (1962) working with sheep and Bradley et al. (1956), Davis et al. (1958), and Clanton (1962) studying cattle.

Suitable results from ratio-type digestion trials require that a stable concentration of indicator be reached in the feces. There are varying reports in the literature on

the length of time required for this constant level of indicator to be obtained. Crampton and Lloyd (1951) using Cr_2O_3 in sheep recommended that fecal samples should not be taken before the fifth day. Border et al. (1963) found that a constant indicator level was not reached in the feces of the lamb until the sixth day after first administration.

METHODS

In this study, chromium-51 as chromic chloride ($CrCl_3$) was applied as the food marker in passage rate and ratio-type digestibility studies. Chromium, in its trivalent form, normally is inert in the gastro-intestinal tract (Roche *et al*. 1957). Using this indicator, most of the time and labor of fecal analysis was eliminated. Chromium-51 disintegrates with the emission of gamma rays, permitting its ready detection and quantification. The half-life of Cr-51 (27.8 days) is neither dangerously long nor inconveniently short. According to Foster (1963), "chromium-51 is considered one of the least hazardous radionuclides."

The isotope, ordered from the New England Nuclear Corporation, had a specific activity of 57.9 to 181 microcuries per milligram. A Nuclear-Chicago Well Scintillation Detector System (DS-202 V) with an 8725 Analyzer/Scaler served as the main radiation detection device. Caloric values were obtained by using a Parr Oxygen Bomb Calorimeter.

The three deer used in this research were supplied by the Michigan Department of Conservation and penned at their Rose Lake Wildlife Research Station near East Lansing, Michigan. All were yearling males, weighing about 40 kilograms each at the beginning of the trials.

One deer at a time was studied. A pen, like those used by Crampton and Lloyd (1951) and Castle (1956), was constructed so that the urine of the test animals was separated from the feces (Figure 1). The floor was three-fourth inch flattened expanded steel. A fine screen beneath this mesh floor deflected the fecal pellets while a sheet of polyethylene film immediately beneath the screen directed the urine into a trough and collecting pan.

A device was built which collected the droppings and separated the fecal groups from each other according to the time of each defecation. Based on the design of Petrides (1964), the mechanism merely involved a roll of freezer paper which was unrolled at a constant rate as it wound on a motor-driven spindle. The time of each defecation could be determined from the position of the feces on the unrolled paper.

The animals in this study were maintained on a diet of Farm Bureau Milling Company lamb-finisher pellets which had a guaranteed analysis of 12.00 per cent minimum crude protein, 2.25 per cent minimum crude fat, 16.00 per cent maximum curde fiber, and 40 grams per ton of oxytetracycline. A brief study of white cedar (Thuya occidentalis) leaf-sprays was used in a final trial to test the feasibility of the Cr-51 technique with a natural food of the deer. Food and water were presented ad libitum. Accurate daily records were kept of the amount of food and water ingested.

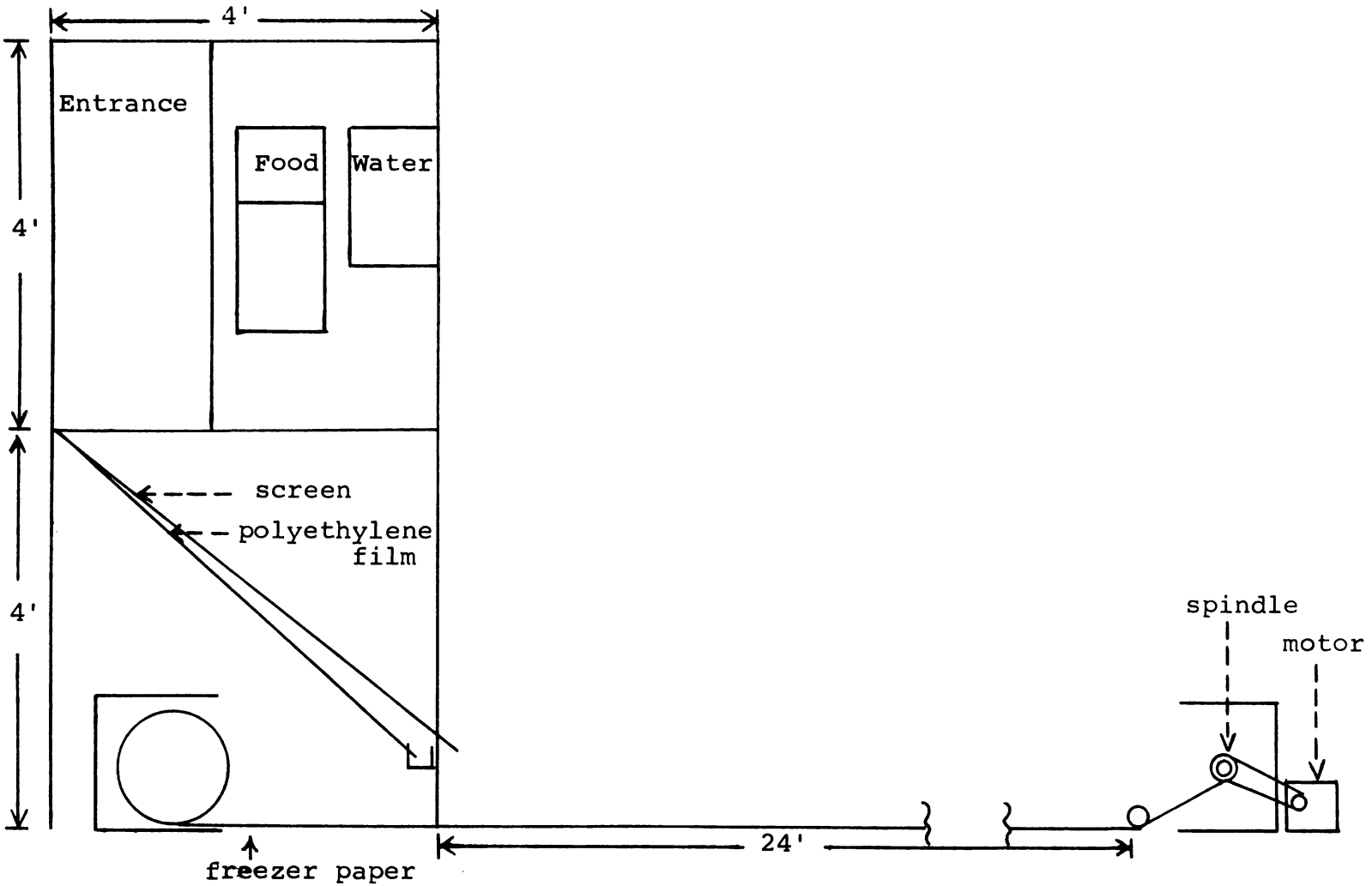

Figure 1. Collection pen used in chromium-51 digestion trials with deer. Rose Lake, Michigan, 1966.

Chromium-51 was administered in two ways. Either (1) a single dose was placed on a small portion of food which was consumed by the deer in one feeding, or (2) the isotope was mixed throughout all food materials eaten over a period of one or more days. These will be hereafter referred to as single-dose and continuous-dose trials, respectively.

In single-dose trials, one-half to two grams of food were labeled with the isotope. Allowing for radioactive decay, diluted samples of stock solution (five millicuries when fresh) were made so that three to six microcuries were present in 15 lambdas (0.015 milliliters) of liquid. One microcurie of isotope on food pellets yielded 4,000 to 5,000 detectable disintegrations per minute. Pipettes with a syringe suction device were used to measure and apply the isotope. The isotope liquid was placed directly on the food and dried thereon with an infrared heat lamp. The labeled food was counted in the detector directly before feeding to confirm the amount of Cr-51 administered.

A simple spray atomizer was used for labeling food to be used in continuous-dose trials. The isotope was diluted to yield an average concentration of 10 to 20 counts (detectable disintegrations) per minute per gram of food when applied. One-half to one milliliter of the isotope solution was placed in a test tube and the atomizer mounted on top. The test tube was counted just before and after

each application to a tray of food to determine the exact amount of isotope applied. Following spraying, the isotope was dried on the glass tray of food, using the infrared lamp. The pelleted food was labeled in 500 gram portions and the cedar in 300 gram portions. Less than one-third of these portions actually came into contact with the spray. Before feeding each portion was thoroughly mixed.

Defecation samples were taken from the time of Cr-51 ingestion until no further radioactive droppings could be detected. Two samples of three or four fecal pellets were taken from each pellet group and sealed in test tubes. The remaining feces of each group were placed in plastic bags. Samples were weighed, completely oven-dried at 100-120 C. and again weighed on a Mettler balance. Each sample was counted and the concentration of indicator per gram of dry feces thus determined. The dry weights of entire defecations were computed as required, based on the wet-dry weight correction factor of samples. The total collection analyses were calculated using the total amounts of food eaten and feces defecated for the entire trial.

RESULTS AND DISCUSSION

Tissue samples including heart, liver, spleen, kidney, muscle, and blood taken during autopsies of the test animals showed no evidence of Cr-51. Frequent urine samples taken during the course of the trials also gave no indication of radioactivity. These findings substantiate the fact that $CrCl_3$ does not leave the digestive tract in any appreciable amount.

Single-dose Trials:

The typical graph of Cr-51 in deer feces following administration of a single dose of the isotope shows a preliminary upward trend (Figure 2). This initial increase in fecal Cr-51 apparently relates to a 'mixing' stage during which food materials to be defecated are removed from the rumen while the incoming labeled food becomes increasingly mixed with the non-labeled materials already present. A peak of Cr-51 concentration, representing its maximum defecation is followed by a regular percentage decrease per hour in the amount of Cr-51 per gram of feces. The terms 'mixing' phase and 'purging' phase were suggested by Duke (1967). For this study, everything present in the stomach at the

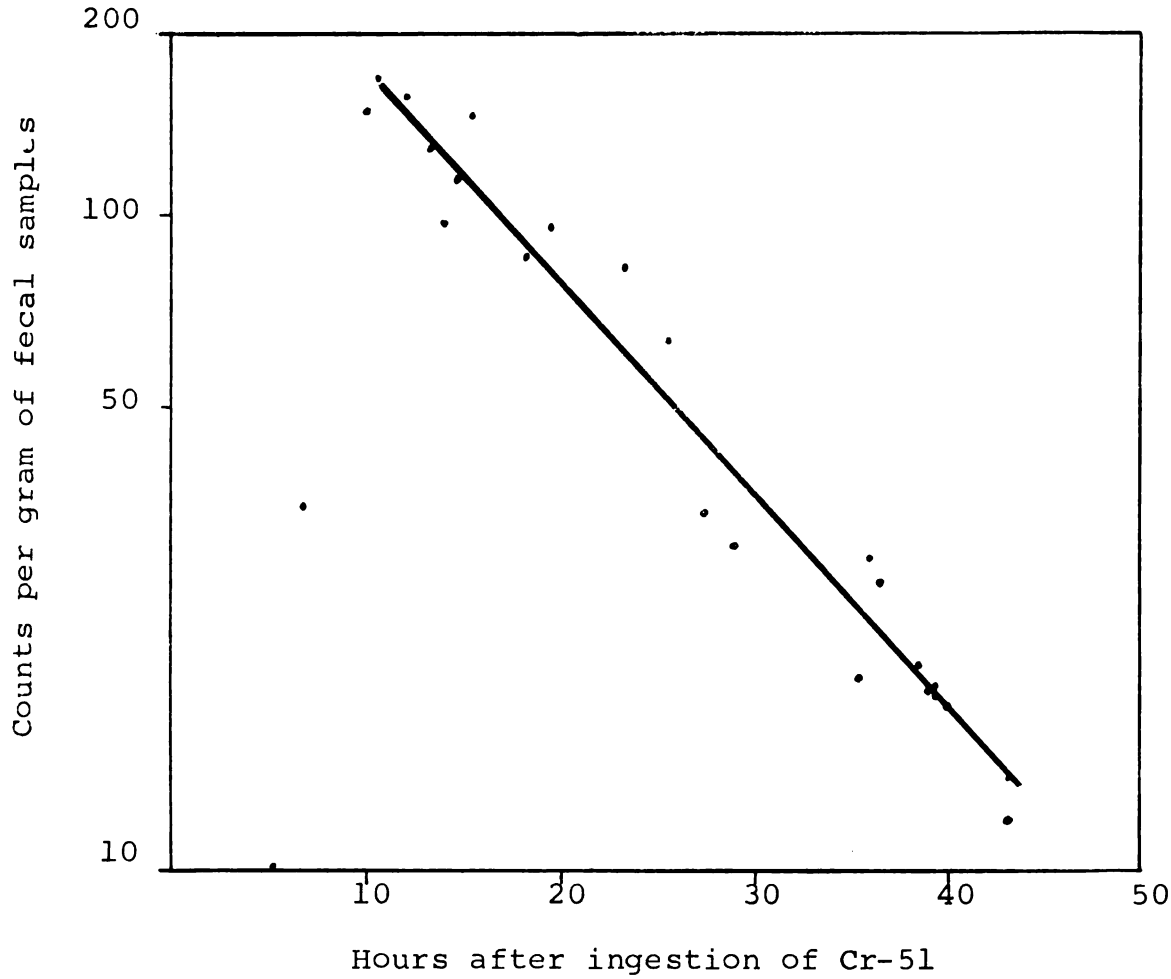

Figure 2. Defecation pattern of chromium-51 for a deer fed a single 9.2 microcurie dose while on a lamb-finisher diet. (Deer 92, single-dose trial 3; Table 1.) Michigan State University, June, 1966.

time the Cr-51 dose is administered is considered to be the test meal.

Based on least squares regression calculations (Ostle, 1965: 164), the average rate at which Cr-51 was purged from deer on the lamb-finisher diet was 7.56 per cent of that remaining in the tract per hour (Table 1).

At least while in the collection pen, the animals were found to defecate as frequently at night as during the day. The weight of the defecations showed no fluctuation from day to night. Over 28 day-long periods, the mean number of defecations for deer on the lamb-finisher diet was 12.6 per day.

A number of problems arise in determining minimum and maximum passage rates of foods. If a constant fraction of the contents is removed from the rumen after complete mixing, an infinitely-divisible amount of indicator should remain behind and never be completely eliminated. However, after an extreme dilution has been reached, it is no longer possible to determine accurately the abundance of an indicator.

In these studies, only those Cr-51 sample counts which were at least twice background were considered. Background levels were normally nine to 12 counts per minute. Usually over 95 per cent of the original meal was passed by the time an 'insignificant' level of Cr-51 was reached.

Table 1. Passage rate data for single-dose chromium-51 trials with deer on a lamb-finisher diet. Michigan State University, 1966.

Animal	Trial	Cr-51 level (microcuries)	First Cr-51 appearance (hours)	95 per cent excretion (hours)	Last Cr-51 appearance (hours)	Hourly percentage of food passage
92	1	3.1	12.0	32.5	47.0	7.21
92	2	6.0	12.0	35.0	38.0	7.31
92	3	9.2	10.0	35.8	40.0	7.25
92	4	4.4	7.5	34.1	37.0	6.42
92	5	3.8	10.5	27.0	38.0	7.95
92	6	3.8	10.5	26.8	34.5	5.14
92	mean	-	10.4	31.7	39.1	6.88
247	1	3.1	12.0	29.8	31.0	9.32
247	2	3.2	8.0	23.2	36.8	7.48
247	mean	-	10.0	26.5	33.9	8.40
255	1	4.2	10.0	35.9	37.8	7.38
255	2	2.0	7.8	22.3	23.0	10.16
255	mean	-	3.9	29.1	30.4	8.77

In three deer on the lamb-finisher diet, the average minimum time for Cr-51 to appear in the feces was 10 hours after ingestion. A peak concentration of indicator was reached 12 to 19 hours after its first administration. Decreasing amounts of fecal chromium were present until approximately 36 hours after ingestion (Table 1).

In sheep, Lambourne (1957) found that Cr_2O_3 and monastral blue with a diet of hay were first defecated five to eight hours after feeding while the peak concentration was reached in 10 to 18 hours. He last detected these indicators 60 to 70 hours after administration.

The 5 and 95 percent excretion times and the mean retention time of a meal were determined for each trial from a percentage excretion curve (Figure 3, Table 2). In continuous-dose trials these curves were constructed from the purging phase data. In most trials the first radioactive defecation contained at least 5 per cent of the indicator. The 95 per cent excretion time, and the mean retention time for these trials were 30 and 18 hours respectively. Castle (1956), working with goats and stained hay, found the 5 per cent and 95 per cent excretion times and the mean retention time to be 16.1, 74.7 and 37.5 hours, respectively.

In three successive trials with the same deer and diet, three, six, and nine microcuries of isotope were administered successively in an attempt to determine the effect

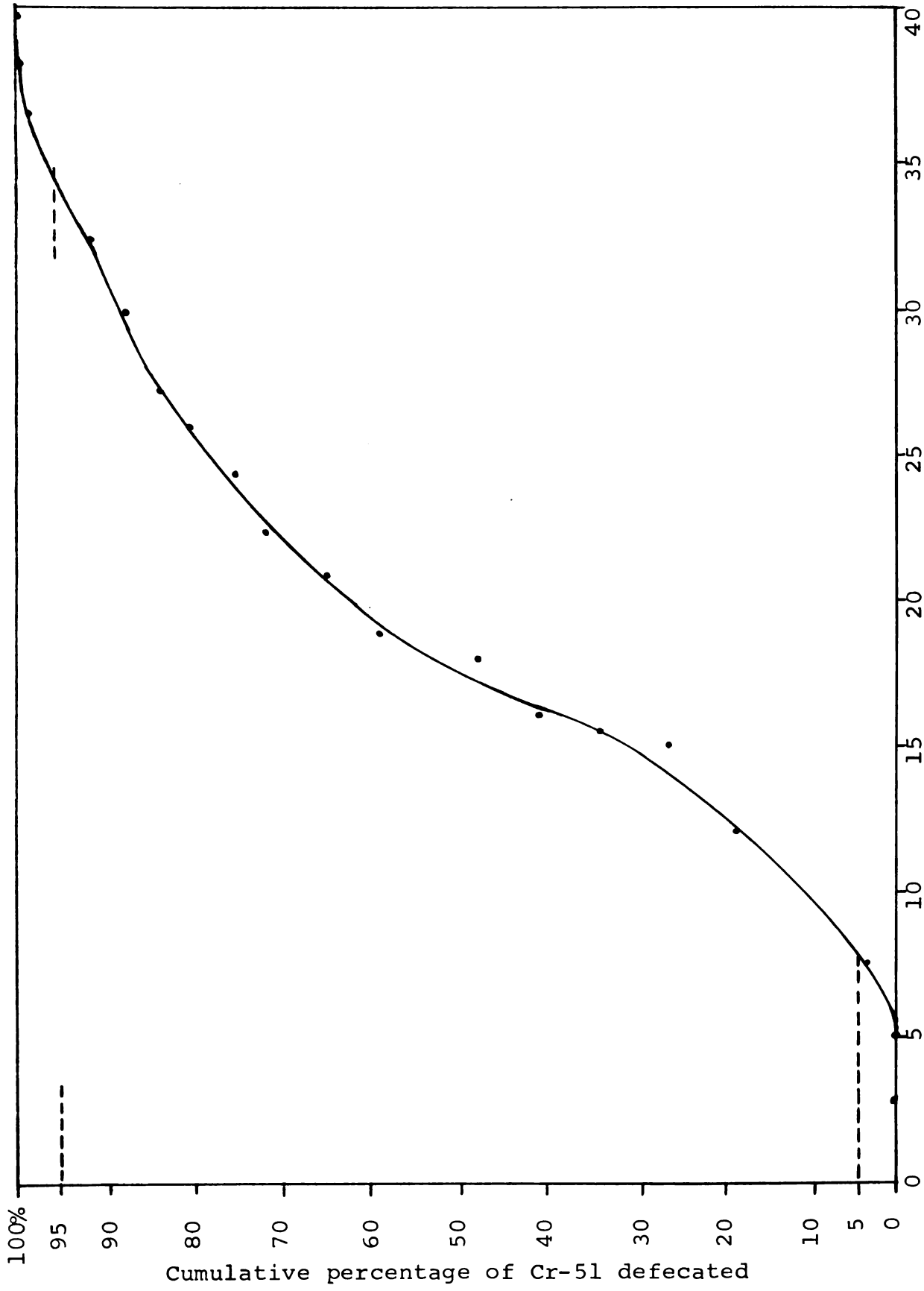

Figure 3. Percentage excretion curve of chromium-51 for a deer on a pelleted lamb-finisher diet. (Deer 92, single-dose trial 4; Table 2.) Michigan State University, 1966.

Table 2. Defecation data for a deer given a single dose of chromium-51 while on a lamb-finisher diet. (Deer 92, single-dose trial 4; Figure 3.) Michigan State University, July, 1966.

Hours since administration	Count per gram fecal Cr-51	Total amount of isotope in defecation	Percentage of isotope in defecation	Cumulative percentage of isotope defecated
0.5	-	-	-	-
2.5	-	-	-	-
3.5	-	-	-	-
5.5	-	-	-	-
7.5	21	342	2.57	2.57
9.5	33	818	6.16	8.73
12.0	45	1278	9.62	18.35
15.0	42	1768	13.31	31.66
15.5	44	1170	8.80	40.46
16.0	38	524	3.94	44.40
18.0	38	1056	7.95	52.35
19.0	55	688	5.18	57.53
21.0	39	608	4.58	62.11
22.5	36	583	4.39	66.50
24.5	23	670	5.04	71.54
26.0	23	508	3.84	75.36
26.5	21	318	2.39	77.75
28.0	17	267	2.01	79.76
30.0	16	741	5.58	85.34
32.5	10	191	1.44	86.78
34.5	16	757	5.70	92.48
37.0	15	651	4.90	97.38
39.0	9	206	1.55	98.93

Note: Size of dose administered: 13,258 counts/ minute.

of dose size on passage rate. At these isotope levels there was no indication that dose rate influenced the observed time of indicator passage (Table 1).

Using 2 to 5 microcurie doses, as much variation in passage rates was found within an individual deer as between different deer of the same age and sex (Table 1). Whether simultaneous trials between different deer would alter these results could not be tested.

Continuous-dose Trials:

The continuous-dose excretion graph (Figure 4) for a deer fed Cr-51 labeled lamb-finisher pellets shows little variation in fecal isotope concentration 17 hours after labeled food was first eaten. For trials of this type a good approximation of the counts per gram of feces, and hence of the digestibility coefficient, can be obtained at the end of the first day (Table 3).

A direct comparison of digestibility coefficients by the Cr-51 ratio and total collection methods was possible in each continuous-dose trial with deer on the lamb-finisher diet (Table 4). The ratio method yielded similar but less variable results than the total collection method.

Natural Foods:

The feasibility of using the isotope technique with natural foods of the deer was given a brief test using white cedar leaf-sprays. A white oak (Quercus alba) acorn diet

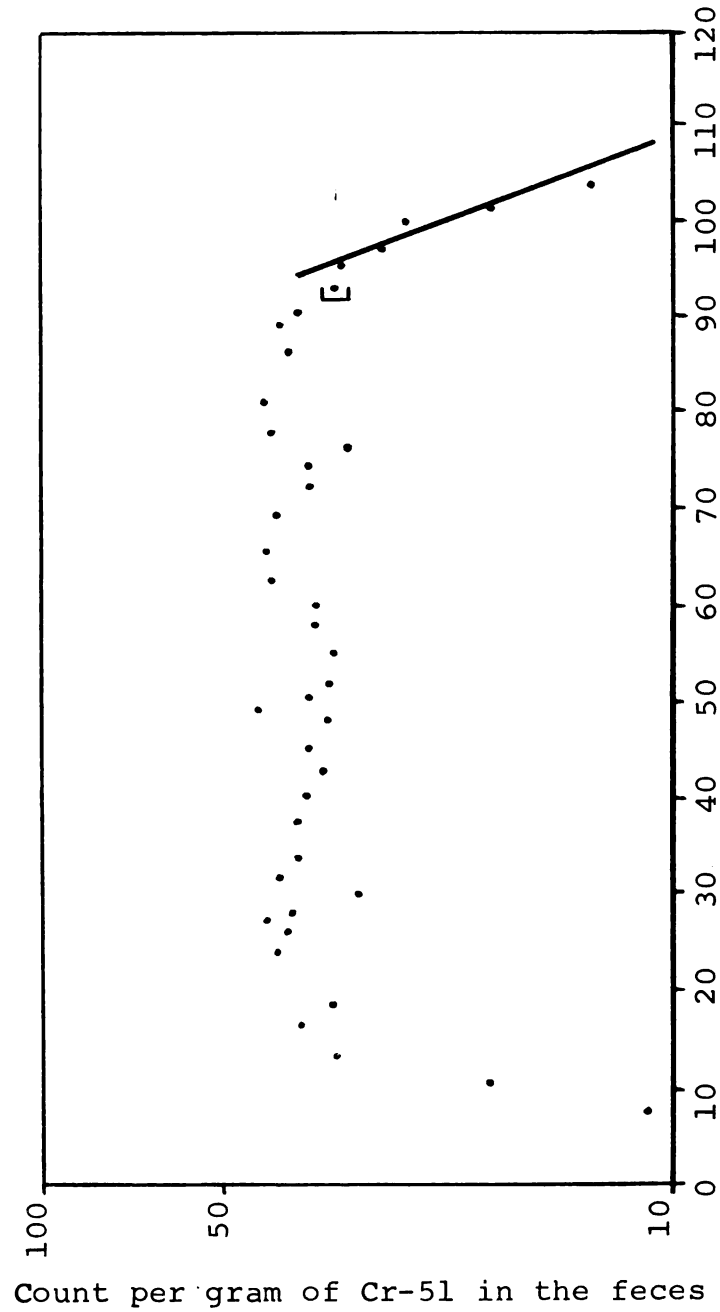

Figure 4. Defecation pattern of chromium-51 for a deer given a continuous dose (17.7 counts per gram) of the indicator while on a lamb-finisher diet. (Deer 247, continuous-dose trial 3; Table 2.) Michigan State University, 1966.

Table 3. Counts of chromium-51 per gram of feces for two deer during continuous-dose trials. Michigan State University, 1966.

Animal	Trial	Day 1 Count per gram	Day 1 Digestibility coefficient	Day 2 Count per gram	Day 3 Count per gram	Day 4 Count per gram	Day 5 Count per gram	Average for total plateau Count per gram	Average for total plateau Digestibility coefficient
92	1	31.1	58.52	33.6	31.8	-	-	32.9	60.79
	2	32.6	62.27	33.4	-	-	-	32.9	62.61
	3	31.0	60.32	-	-	-	-	31.0	60.32
247	1	40.1	58.10	40.2	32.0	37.5	45.5	39.1	57.03
	2	37.3	54.96	39.9	39.6	39.8	-	39.6	57.85
	3	34.8	57.47	-	-	-	-	34.8	57.47

Table 4. Dry matter digestibility coefficients determined simultaneously by ratio and total collection methods. Continuous-dose trials for deer on a pelleted lamb-finisher diet. Michigan State University, 1966.

Animal	Method	Trial 1	Trial 2	Trial 3	Mean	Standard deviation	Number of trial days
247	Cr-51	57.03	57.85	57.47	57.45	.42	Four
	Total Collection	68.43	67.92	27.48	54.61	23.50	
92	Cr-51	60.79	62.61	60.32	61.24	.70	Two
	Total Collection	51.83	63.79	64.35	59.66	4.09	

was also attempted but for unknown reasons the test animal refused to eat acorns. Because of this, the animal used for the cedar study starved for five days prior to the cedar trial.

Single-dose and continuous-dose trials were combined in the cedar test. The first cedar eaten contained a single 4.4 microcurie dose of Cr-51. Non-labeled cedar was then consumed over a 12 hour period and a continuous labeled cedar diet was eaten over the succeeding four days. The isotope was totally defecated by one and one-half days after non-labeled browse was restored.

The excretion graph for this trial (Figure 5) is complex. Apparently the initial single dose of Cr-51 exerted a prominent influence for the first 90 hours, perhaps an indication that food eaten after a fast is held within the empty digestive tract for a longer than normal period. During hours 90 to 133, the concentration of Cr-51 became fairly constant and it is this time through hour 133 that is considered the plateau.

Since the concentration of Cr-51 in the food was 27.1 and in feces 54.3 counts per gram per minute, the dry matter digestibility of the cedar browse was 50.00 per cent. By the total collection method a digestibility coefficient of 52.45 per cent was computed. For cedar the five per cent and 95 per cent excretion times were 13.7 and 31.5 hours respectively. The mean retention time was 23.8 hours.

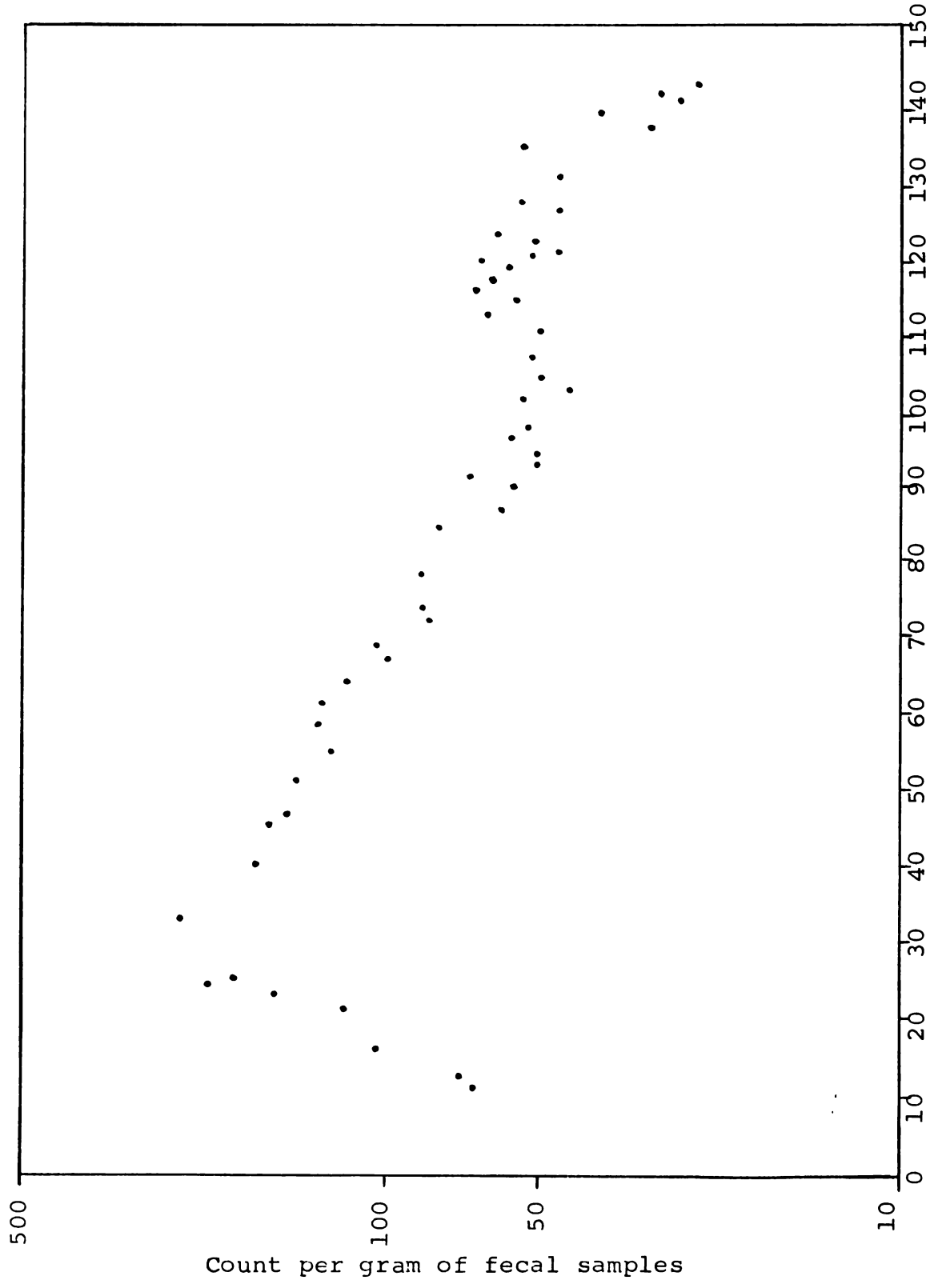

Figure 5. Defecation pattern of chromium-51 from a deer on a white cedar diet fed a combined single and continuous dose. (Deer 92, cedar trial.) Michigan State University, October, 1967.

Three continuous-dose trials with the same deer on the lamb-finisher diet prior to the cedar test gave a digestibility coefficient of 61.24 per cent (Table 4). The times of five and 95 per cent excretion during these trials were 9.6 and 24.3 hours, respectively. The mean retention time for these trials was 16.7 hours.

If 95 per cent excretion times are an accurate measure of the time required for digestion to take place, the animal required 24.3 hours to digest 61.44 per cent of the lamb-finisher diet and 31.5 hours to digest 50.00 per cent of the cedar browse. Thus the deer digested more of the lamb-finisher diet than of the cedar diet and it accomplished this in a shorter time.

Caloric values were determined for food and feces of the deer on both the lamb-finisher and the cedar diets. For the lamb-finisher diet, a gram of food pellets contained 4,363.7 calories per gram while 4,217.5 calories per gram were present in the less voluminous feces. The cedar browse forage yielded 5,207.6 calories per gram while the corresponding feces contained 5,208.5 calories per gram.

As calculated by the formula

$$1-(\text{indig. coef.} \times \frac{\text{cal./g/ feces}}{\text{cal./g. food}}) \times 100,$$

the apparent gross energy digestibility coefficient for the lamb-finisher diet was 62.21 per cent. This constituted 2,737.4 calories obtained by the deer for every gram of food eaten. For white

cedar leaf-sprays, the deer digested 50.01 per cent of the energy consumed, or 2,603.8 calories per gram of food eaten.

The calories per gram derived from the two diets are similar giving the impression that cedar browse is almost as useful from the energy standpoint as the lamb-finisher diet. However, when the time required for passage is taken into consideration, it is seen that the lamb-finisher diet yields much more energy per hour spent in the tract than the cedar. While the two diets yield nearly the same number of calories per gram, a meal of cedar browse requires approximately seven hours longer to be defecated than a meal of lamb-finisher pellets.

The faster passing lamb-finisher diet was consumed to a greater extent (54.0 grams per hour) than the cedar browse (25.3 grams per hour). Thus, while feeding ad libitum the deer obtained 147.4 and 65.8 kilocalories per hour from the lamb-finisher and cedar diets, respectively.

The energy value per unit of food and the percentage digestibility, therefore, must be combined with the food passage and/or consumption rate to yield an accurate comparison of the energy available from different diets. Neither the energy value nor the percentage digestibility alone is an accurate measure of the food's value to an animal.

In this study, the data for the cedar diet were collected only to test the usefulness of the Cr-51 technique

with a natural food. As results of a single test, the values quoted are not known to be representative.

Conclusion:

Chromium-51 is a useful indicator in the study of many digestive characteristics in the deer. A good comparison between different diets is obtained by taking either (a) the amount of food eaten or (b) the passage rate plus the degree of absorption into consideration. If only passage rate data are desired they may be obtained more rapidly by feeding a single dose of Cr-51.

Additional digestive research could be aided by the use of Cr-51, including the length of time required to condition an animal to a new diet, the degree of absorption occurring in different areas of the digestive tract, and the result of adverse conditions on the ability of an animal to digest foods.

SUMMARY

A technique of using the radioactive isotope Cr-51 in the study of digestive characteristics was developed for white-tailed deer. Labeling foods with a constant level of Cr-51 for two successive days and collecting fecal samples by an automated feces-collection device on these and one additional day permitted determination of the degree to which the food was digested and the length of time required for that digestion. Feeding only a single dose of the isotope allowed the determination in two days of passage rate data. Using Cr-51 most of the labor and unpleasantness of fecal analysis for an indicator were eliminated.

The Cr-51 ratio method yielded similar but less variable digestibility coefficients than the conventional total collection method. Some digestive characteristics for the white-tailed deer are given.

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