FOOD PASSAGE RATE STUDIES WITH THE RING-NECKED PHEASANT

Thesis for the Degree of Ph. D.
MICHIGAN STATE UNIVERSITY

Gary E. Duke

1967

This is to certify that the

thesis entitled

CHROMIUM-51 IN METABOLIZABILITY AND FOOD PASSAGE RATE STUDIES WITH THE RING-NECKED PHEASANT

presented by

Gary Earl Duke

has been accepted towards fulfillment of the requirements for

Ph.D. degree in Fisheries and Wildlife

George C. Stiles
Major professor

Date June 2, 1967

O-169

ABSTRACT

CHROMIUM-51 IN METABOLIZABILITY AND FOOD PASSAGE RATE STUDIES WITH THE RING-NECKED PHEASANT

by Gary E. Duke

Two techniques using chromium-51 were developed. In the first, a single-dose of chromium-labeled food was fed to test birds and the passage rates of the food were determined as the times of the first and last appearance of the label. In the second technique a continuous-dose of uniformly labeled food was fed to test birds for one to several days. The ratio of the concentration of Cr-51 in the food to its concentration in the excreta permitted the ready computation of metabolizability coefficients. The weights of materials ingested and defecated also allowed computation of these coefficients by the total collection method. The two techniques were run in succession and passage rates, metabolizability coefficients, and ingestion rates were determined and compared between cocks, between cocks and hens, between adults and chicks, and for three different diets. However, for an unknown reason, the coefficients as determined by the two methods, did not agree. The metabolizability coefficient of a standard commercial diet averaged 63.49 percent

by the total collection method and 53.68 percent by the ratio method for cocks in a controlled environment.

The average minimum passage rate of the standard diet for cocks was 0.6 to 1.6 hours while the average maximum passage time for materials not receiving cecal digestion was nine hours and for materials receiving cecal influence the maximum rate was 38 hours. Cecal excreta are recognizable from rectal excreta and the concentration of isotope in cecal excreta over that in rectal excreta permitted a determination of the extent of cecal digestion. The standard diet provided an average of 3,073 calories of metabolizable energy per gram to the pheasant cocks tested, and an average of 1.8813 grams was eaten per hour. Similar information was obtained for pheasant hens, for chicks of various ages, and for cocks eating whole corn or chokeberries.

The results of feeding trials using chromium-51 showed as much variance within the same male as between males. Metabolizabilities and maximum passage rates were slightly greater for hens than for cocks. Passage rates were faster for chicks up to 42 days of age than for adults, and the level of metabolizability of the standard diet was approximately 5 percent higher for chicks. The metabolizability of corn was higher and of chokeberries lower than that of the standard diet. The passage rates of chokeberries and the standard diet were both shorter than the

passage rate of corn. Considering both metabolizability and passage rate, the standard diet provided a higher average metabolizable energy per day than the other diets. Metabolizability coefficients obtained with cocks on the standard diet by the ratio method were more variable than those obtained by the total collection method. Thus, the ratio technique employing chromium-51 is not considered valid for metabolizability determinations with pheasants. However, the ratio technique is of value in comparing relative daytime to nighttime digestibility and cecal to intestinal digestion. And, passage rate studies using chromium-51 are very useful.

CHROMIUM-51 IN METABOLIZABILITY AND FOOD PASSAGE RATE STUDIES WITH THE RING-NECKED PHEASANT

By

Gary E. Duke

A THESIS

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

DOCTOR OF PHILOSOPHY

Department of Fisheries and Wildlife

1967

ACKNOWLEDGMENTS

This study was supported by U. S. Atomic Energy Commission contract No. AT(11-1)-1534.

I wish to express my appreciation to Dr. George A. Petrides, Department of Fisheries and Wildlife, whose pilot studies on the values of Cr-51 as a food label led to my study. His advice during the project and his careful editing of the manuscript are gratefully acknowledged.

The advice of Dr. Robert K. Ringer of the Poultry Science Department was invaluable. I greatly appreciate his assistance in planning and methods and in physiological interpretation of results. Other members of the Poultry Science Department offered advice and laboratory space in which initial studies were accomplished.

Dr. Leslie W. Gysel of the Fisheries and Wildlife Department and Dr. George J. Wallace of the Zoology Department furnished encouragement throughout the research and critical editing of the final manuscript.

Frequent discussions with William W. Mautz, a fellow graduate student, were extremely helpful throughout the study and during preparation of the manuscript.

My wife, Maryann, deserves much credit for her constant encouragement.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Measurements of passage rate and digestibility of foods are basic to the study of bioenergetics. Livestock and poultry researchers have accomplished many investigations of these phenomena, and although experimental approaches have varied, most recent workers apply insoluble labels to the foods to be studied. The passage rate of food is the time required for a given quantity of that food to pass completely through the digestive tract. The proportion of a diet that is digested and absorbed determines the digestibility of that diet. The ratio of the concentration of a label in food to its concentration in feces from that food, subtracted from unity, gives the digestibility coefficient of that food by the ratio method.

The present study involves the ring-necked pheasant (Phasianus colchicus) as an experimental species and a determination of the usefulness of chromium-51 (Cr-51) as a food label. The pheasant has apparently not been tested in this sort of experiment, and the value of Cr-51 as a label has received only limited study.

Many labels have been tested as indicator substances. Hoelzel (1930) tried rubber, cotton thread, seeds, beads, aluminum, silver, gold, and steel as food markers. Other

labels include those found naturally in plants such as chromogen (Reid et al., 1952) and lignon (Forbes and Garrigus, 1948). In addition, celluloid particles (Mueller, 1956), iron oxide (Bergeim, 1926; Tuckey et al., 1958), ruthenium-106 (Hollis and Thompson, 1958), barium sulfate (Henry et al., 1933), radioactive barium (Imabayashi et al., 1956), oats (Browne, 1922), chromium-51 (Petrides, 1964; Mautz and Petrides, 1967) have been employed. But the most widely used marker is chromic oxide which was first suggested by Edin (1918) for use in digestion trials. It is usually mixed directly with feeds but it is also available in paper pellets or shreds (Corbett et al., 1960; Border et al., 1963; Troelson, 1963) which can be mixed with feeds.

Chromic oxide has been used in nutritional studies of pigs (Schurch et al., 1952; Moore, 1957) man (Krenla, 1947; Irwin and Crampton, 1951), sheep (Elam et al., 1962; Johnson et al., 1964), cattle (Kane et al., 1950; Smith and Reid, 1955), coturnix quail (McFarland and Freedland, 1965), and poultry (Olsson and Kihlen, 1948; Dansky and Hill, 1952; Hill and Anderson, 1958; Edwards and Gillis, 1959; and, Hill and Renner, 1963). Brandt and Thacker (1958) used Cr-51 to study coprophagy in rabbits.

In addition to these chromic oxide studies, Odum (1961) studied excretion rates in two species of terrestrial insects and in a marine isopod using zinc-65. Hoelzel (1930) studied food passage rates in the rabbit, guinea pig, dog,

cat, rat, mouse, monkey, adult female chicken, pigeon, and in himself. Malone (1965) determined the passage rate of certain plankton through mallard ducks while studying the effects of digestion on the plankton. Petrides (1964) determined passage rate and other digestive phenomena in the oppossum (Didelphis virginiana), bobcat (Lynx rufus), cotton rat (Sigmodon hispidus), and other animals using Cr-51.

An alternative method for energy metabolism studies does not use an inert food market. A technique requiring determination of the weight of food eaten and the total weight of feces from that food can be employed. This is called the total collection method for determining a coefficient of metabolizability of a diet. This technique was used by Seibert (1949) to obtain metabolizability information for juncos, white-throated sparrows, English sparrows, blue jays, and field sparrows and by Kendeigh (1949) in studies with the English sparrow. Polyakov (1959) fed chickens at different times and then killed them simultaneously to determine how far food had progressed in the tract and to see how much digestion had occurred in the food. LeFebvre (1964) used D_2O^{18} to estimate CO_2 output, water turnover, and gaseous exchange in pigeons.

Although this review of previous investigations is by no means complete, it is indicative of the extent to which markers have been used in digestion and passage rate

trials, and of the number of different species tested in bioenergetics studies.

Why study bioenergetics? Certainly, for domestic stock the reason is obvious. It is highly desirable to know the energy requirements of the animals one is tending and to know the most economical feeds for satisfying these requirements. The reasons may not be so clear with regard to wildlife. However, if one knows the energy requirements of a species and the usable energy supply available to that species in an area, then the carrying capacity of the area can be predicted or a population on the area can be estimated. Foods of greater energy value can be given more consideration in planting programs if the bioenergetics of the animal species present are known. The level of energy available may be a limiting factor to some species. Zimmerman (1965) suggests that the northward distribution of the dickcissel may be limited by the "magnitude and duration of productive energy available for reproduction." The determination of the nutritive efficiency of various animals with respect to different foods is, of course, important in appraising the usefulness of these species to man, in comparative analysis with domestic stock, and in physiological, ecological, and evolutionary studies. Thus, studies of the energy metabolism of wild species are valuable.

The objectives of this study were to learn whether chromium-51 labeled foods would enable the ready determination of:

1. Patterns of food passage through the digestive tract.
2. The quantities of crop and/or gizzard contents which are digested and replaced per unit time.
3. The dry weight of excreta derived from chromium-51 marked crop or stomach contents.
4. The calorific values of certain foods and of their related excreta.
5. The minimum and maximum durations of food transit in the digestive tract as affected by diet.
6. The patterns and extent of cecal influence on a particular diet.
7. The effects of age and sex on caloric requirements and on nutritional efficiency for a standard diet.
8. An energy budget for the test species.

It was also to be ascertained whether the Cr-51 method could be recommended as an improvement on other current methods of studying metabolizability and food passage rate.

Initial research was directed towards development of techniques for accomplishing the objectives (developmental

feeding trials) while subsequent efforts were aimed at applying the techniques using different individual pheasants and different diets (comparative trials).

METHODS

Materials and Equipment

Browne (1922) showed that soluble dyes pass through the digestive tract of the fowl faster than hard food particles, whereas insoluble markers pass at the same rate. He observed also that a successful marker must be physiologically inert so as not to cross membranes. Certain chromium compounds, especially chromic oxide meet these two criteria. $Cr^{51}Cl_3$ also is nearly insoluble and inert in the GI tract.

Cr-51 has a half-life of 27.8 days and emits gamma rays upon disintegration. The emissions of radioisotopes upon decay can be precisely measured to provide an estimate of the amount of isotope present. Comar (1955) describes the primary advantage of radioisotope use as "the great sensitivity of measurement usually available." An isotope offers advantages in quatitative measurement over the use of rubber pellets, beads, etc., and in simplicity of measurement over chromic oxide and other dyes which must be measured spectrophotometrically.

Foster (1963) describes Cr-51 as "one of the least hazardous radionuclides." This is desirable for both the experimental animal and the experimenter. Chromium is toxic

in high concentrations, but up to 100 parts per million (ppm) as the compound Na_2CrO_4 did not affect the performance of poultry chicks (Romoser et al., 1961). Levels of Cr-51 used in this study were less than this. Ionizing radiations sufficient to sterilize foods do not affect the gross energy, metabolizable energy, or macronutrient content of those foods (Levy et al., 1959).

In mammals, "orally administered $Cr^{51}Cl_3$ was almost totally excreted in the feces at the end of four days. Less than 0.5 percent of the dose was absorbed from the gastrointestinal (GI) tract as indicated by tissue distribution studies. Although the urinary excretion indicated a higher level of absorption, the urine radioactivity was probably due to fecal contamination" (Visek et al., 1953). Roche et al. (1957) demonstrated that "practically negligible" amounts of Cr-51 introduced into the human GI tract were absorbed. Hughes (1966) labeled proteins with Cr-51 in an effort to locate catabolic loci for protein breakdown. His reason for using chromium "lies in the extreme sluggishness of the exchange reactions of chromic complexes." Stacy and Thorburn (1966) stated that "after intraruminal administration (to ewes) very little Cr^{51}-EDTA (ethylene diamine tetraacetic acid) is absorbed from the gut." Hogan (1964) made a similar statement regarding the use of Cr^{51}-EDTA in sheep; however, Downes and McDonald (1964) found that some urine contamination with Cr^{51}-EDTA always occurred after

intraruminal administration "with the maximum amount being 4.7 percent of the dose." Mautz and Petrides (1967) found no detectable urine radioactivity in white-tailed deer (Odocoileus virginianus) which had ingested foods labeled with $Cr^{51}Cl_3$.

In pheasants, too, it appears that $Cr^{51}Cl_3$ is inert. No tissue or blood radioactivity was detectable in pheasants used in the present study. Doses used were considerably smaller than those used in the above studies (0.05 to four microcuries (uc) as compared to 50 to 500 uc) however, making detection of a small tissue contaminant impossible or at least unlikely.

Scintillation detection is the most satisfactory method of measuring the quantity of Cr-51 in a sample (Foster, 1963). This measurement is called "counting" and "counts" are detectable disintegrations registered by the counting equipment. A Nuclear Chicago Well Scintillation Detector System (DS-202V) with an 8725 analyzer scaler was used in this study.

Caloric values of all feeds and the excreta from these feeds were determined with a Parr Oxygen Bomb calorimeter.

During feeding trials, birds were held in test pens 14 x 14 x 14 inches in size. Each pen was raised to allow freezer paper 24 inches wide to pass under it (waxed surface upward) for collecting defecations. A freezer paper roll

was placed behind the pen and was unrolled and pulled under the pen constantly by being attached to a motor placed 12 to 14 feet in front of the pen. Since the paper moved at a rate of 14 inches per hour, it was possible to determine accurately when defecations occurred. A similar system was used by Petrides (1964) in his studies.

Pheasants were maintained both in outdoor enclosures and in small indoor pens. They were put into one of the test pens at least seven days, and usually ten to fourteen days prior to their use in a feeding trial so as to acclimate them to the test conditions. The laboratory used for developmental tests was not fitted for temperature or light control, thus both factors varied somewhat during experiments. The room used for final comparative tests (see beyond) was maintained at a constant temperature of 78°F. The relative humidity was kept at between 35 and 45 percent, and the lights were automatically turned on and off each day to provide an invariable 14 hour period of light. One bird never became accustomed to these conditions and was not used, but the other specimens adjusted remarkably rapidly. The test room normally was visited twice a day at regular times to collect excreta.

Wild pheasants, captured by the Michigan Conservation Department, were used in the development of techniques. Those birds employed in the final comparative trials (see beyond) were all from the same brood though obtained from a

pheasant breeder. Pheasant chicks were also purchased locally.

The primary test ration was Turkey Breeder Pellets made by the King Milling Company of Lowell, Michigan. The guaranteed analysis of this ration was:

Wheat middlings	100 lbs
Yellow corn meal	1,120 lbs
Ground oats	100 lbs
45% soybean oil meal	200 lbs
17/20% dehyalfalfa	100 lbs
50% meat/bone scraps	100 lbs
60% fish meal Menhaden	100 lbs
Dried whey	50 lbs
Brewers dried yeast	40 lbs
Iodized salt	10 lbs
Dicalcium phosphate	20 lbs
Ground limestone, 38% calcium . .	50 lbs
M-4 Vit. trace mineral premix . .	5 lbs
Carbosep	2 lbs
	1,997 lbs

Whole corn and chokeberries (Pyrus melanocarpa) also served as experimental feeds in two trials. All food (Cr-labeled and unlabeled) and water was available ad libitum in all feeding trials.

Suitable Techniques in Developmental Trials

Single-Dose Technique

A single-dose was a single Cr-labeled piece of food (e.g., pellet) fed to a test animal. Initial trials indicated that the time required for complete passage of a single dose through the GI tract varied directly with the level of the Cr-51 dose. Ultimately, however, the minimum dose

level was found which was large enough to make passage rate independent of dose level. Doses were prepared from stock $Cr^{51}Cl_3$ solutions which had specific activities of 57.9 to 181 millicuries (mc) per milligram. Cr-51 levels of 304 counts per minute (cpm) (0.05uc) to 21,252 cpm (3.54uc) were applied to single pellets using Lambda (0.001 milliliter) pipettes in a pipette syringe. Although various levels of the radioisotope were used, approximately ten lambda of solution were used in all cases.

All labeled foods in all tests were fed just after 8:00 a.m. on the first day of the test. Excreta collections were continued until after it was ascertained that all labeled materials had been defecated. After feeding labeled foods, the motor pulling the excreta collecting paper was started. This device was stopped at 8:00 p.m. when hourly excreta samples were placed in tubes. Fresh paper was then attached and the collecting device was restarted. This procedure was repeated daily at 8:00 a.m. and 8:00 p.m. for the duration of the test.

It was necessary to dry excreta samples in test tubes at 110-120°C for 24 hours to reach a constant dry weight. This temperature is higher than those used in most other studies. Manoukas et al. (1964) has suggested that poultry excreta dried at 65°C or higher will suffer a significant loss in gross energy but this situation could be overcome by using fresh excreta in a bomb calorimeter with

N,N-dimethylformamide as a combustion primer. The drying process thus would be eliminated.

Since obtaining a constant dry weight for the excreta samples was desirable, a test was performed to determine the energy loss of excreta dried at 110-120°C. Three fresh defecations were cut in half and one-half of each was put into a tube and dried for 24 hours at 120°C. The other three halves were immediately subjected to calorimetry using N,N-dimethylformamide. Caloric value of the three dried halves was determined after 24 hours of drying. The three dried samples had an average caloric value of 3,195 calories per gram, while the average was 1,012 calories per gram for the three fresh samples. The dry matter content of the moist excreta was 31.5 percent. Thus, the calories per gram of dry excreta converted to a fresh basis (3,195 x 0.315) was 1,006 calories per gram. No significant energy loss was found to have occurred due to the drying technique used. Possibly a different drying procedure (e.g., in a large flat container rather than in a tube) would result in an energy loss as described by Manoukas et al. (1964).

After samples were dried they were weighed. The weight of the dried excreta was measured to the nearest ten-thousandth gram on a Mettler balance (Model H). After weighing, the radioactivity of each sample was counted and the count per minute (cpm) was converted to a per gram basis. All counts were corrected for decay and background error.

Only counts which were twice the background level (usually ten cpm) were used in computations.

The weight of the food eaten was determined for each feeding trial. Samples of each ration were dried in tubes at 110-120°C for 24 hours to determine their dry weight and the weight of all fresh feed eaten by the birds was converted to dry weight. The weights of all materials discussed in this report are on a dry basis unless otherwise specified.

Knowing the total dry weight of food eaten over a several-day period and the total weight of the dried excreta from the food permits the computation of a metabolizability coefficient. Labeling foods with Cr-51 enables determination of the excreta which were derived from those foods. The formula used for this total collection method, as presented by Kleiber (1961, page 254) is:

$$\text{Metabolizability coefficient} = 1 - \left(\frac{\text{total excreta wgt.}}{\text{total food wgt.}} \right) \times 100$$

Continuous-Dose Technique

A continuous-dose is a supply of uniformly-labeled food fed to the test animal over a somewhat prolonged period of time. Preparation of Cr-labeled food for a continuous-dose required dilution of an appropriate aliquot of the stock isotope solution and spraying the diluted preparation onto food with an atomizer. After spraying, samples of the

sprayed food were counted to determine the average cpm per gram of the food used for each trial. The doses used varied from 31 cpm (0.005uc) per gram of food to 184 cpm (0.04uc) per gram. Doses larger than 100 cpm (0.02uc) per gram of food were most satisfactory since detection of radioactivity in very small excreta samples was difficult with smaller sized doses.

The sprayed food was presented to the bird or birds to be tested and the excreta collecting device was started. Subsequently, excreta were collected, dried, weighed, and counted as in the single-dose procedure.

Feeding of a continuous-dose allowed determination of a metabolizability coefficient by the ratio method. The formula normally used (Sibbald et al., 1960) is:

$$\text{metabolizability coefficient} = 1 - \left(\frac{\text{amt. label/gm feed}}{\text{amt. label/gm excreta}} \right) \times 100$$

For Cr-51, the amount present per gram of both food and excreta was measured as cpm.

Knowing the metabolizability coefficient for a diet, and the caloric values of both the diet and the excreta from it, one can determine the metabolizabile energy (M.E.) per gram of the diet.

$$\text{M.E. per gram food} = \text{gross energy per gram food} - \left(\text{nonmetabolizability coefficient} \times \text{gross energy per gram excreta} \right)$$

A common modification of the latter formula includes a factor to correct for the nitrogenous materials in a diet that are retained in the body without being metabolized. The M.E. of a diet is the energy available for metabolism (gross energy minus fecal and urine energy). Thus, the energy of the nitrogenous material in a diet which is retained by the body but does not undergo metabolism in the body should be subtracted from the M.E. of the diet (or added to the excreta energy). The nitrogen correction is made in order to convert all M.E. values of diets to a basis of nitrogen equilibrium for comparative purposes.

The use of a nitrogen correction is not universal hence it was not used in this study. Baldini (1961) stated that in his study, the correction for nitrogen retention did not affect the M.E. values of the diets tested relative to each other. He stated further that "there is some question in the author's mind as to the practical value of such a correction." Swift and French (1954), in regard to the correction, said that "it is difficult to justify the enactment of a penalty from a ration resulting in the storage of 25 calories as protein and 75 calories as fat in comparing it with one resulting in storage of 10 calories as protein and 90 calories as fat." According to Hill et al. (1960), the correction is not uniformly used so it is appropriate to use the term "nitrogen-corrected metabolizable energy" to distinguish values obtained in this way.

Previous investigators (Olsson and Kihlen, 1948; Dansky and Hill, 1952; and Mueller, 1956) have recommended that experiments employing the ratio technique run for several days because of variable indicator concentrations in individual excreta samples. The results of this study showed variation in Cr-51 concentration between hourly excreta samples, between averaged hourly samples from day and night periods, and between daily averages of the hourly excreta samples (Table 1). However, there was also considerable variability in the cpm per gram of the individual food samples used in each trial. Thus, an F-test was used to determine if the variance in the cpm per gram of excreta multiplied by the nonmetabolizability coefficient of the diet, would be significantly different from the variance in the cpm per gram of foods. Only the cpm per gram of intestinal excreta samples occurring during the plateau period (see beyond) were used in the analysis. The cpm per gram of cecal excreta samples were not used because they represented food which was more thoroughly digested and thus had a coefficient of nonmetabolizability different from that of intestinal excreta samples.

The analysis showed in all cases that the variance in the cpm per gram of excreta was not significantly different ($P < 0.05$) from the variance in the cpm per gram of the food from which it came. In other words, the observed variability in Cr-51 concentration between excreta samples was

Table 1. Counts per gram of excreta from continuous-dose feeding trials with pheasants fed Turkey Breeder Pellets, Michigan State University, 1966 and 1967

Expmt. Number	Number of Samples Per Day	Daytime Average For Each Day	Nighttime Average For Each Day	Average of Hourly Samples For Each Day
8a	15	50.4	50.4	50.4
	20	81.5	74.8	78.5
	21	101.8	98.5	100.4
8b	13	74.7	55.6	64.3
	19	74.0	64.9	70.1
8c	20	232.6	224.0	228.3
	23	260.9	197.8	236.2
	21	246.6	194.5	226.8
8d	22	275.3	266.9	271.5
	22	294.4	318.2	304.1
	22	350.1	355.3	351.9
10a	15	547.0	437.0	488.0
	38	582.0	380.0	494.0

due to the variability of indicator concentration in the labeled foods. Some differential digestion apparently took place, however, since a consistent difference in the cpm per gram of excreta between day and night and between cecal and intestinal samples did occur.

Based on these findings, one day trials using the ratio method were deemed justified, but no less than one full day because of differences in Cr-51 concentration between day and night samples. Elam et al. (1962) have

indicated that shorter feeding trials provide more accurate metabolizability results if, as in the present study, total collection of the excreta is accomplished. Perhaps food labeled in the customary manner (i.e., mixed with powdered chromic oxide) or sample collection by "grab samples" would result in higher variability in indicator concentration between excreta and food samples and thus require longer collection periods.

The Ultimate Combination Technique in Comparative Trials

Results of developmental feeding trials indicated that both the single-dose and continuous-dose methods should be used in a combination technique to best meet all objectives. Thus, a 24-hour continuous-dose feeding trial followed by a single-dose trial after sufficient time for foods from the first dose to pass completely through the tract (24 hours was always sufficient) were accomplished. The continuous-dose permitted the determination of metabolizability coefficients by the ratio method. The single-dose provided information on the passage rates of foods. Records of the total amounts eaten and defecated during both trials allowed determination of metabolizability by the total collection method. Approximately 96 hours was required for the performance of this combination technique with time allowed for complete passage of each type of dose.

The combination technique was used in a series of comparative trials to determine the amount of variability that could occur in the findings from trial-to-trial with the same male, with different males, with females or chicks, and with different diets. To determine the extent of variability in passage rate, etc. between males, two birds were tested simultaneously in two separate tests.

The dose levels used in these trials were at least 100 cpm (0.02uc) per gram of food for the continuous-dose and 10,000 cpm (1.67uc) for the single-dose.

RESULTS

Results of Single-Dose Developmental Trials

Collection, drying, weighing, and counting of radioactive waste material following the feeding of a single-dose yielded hourly information on the cpm per gram of excreta (Table 2). Graphs of these data showed the rate and pattern of isotope defecation. The pattern which was typical of all single-dose feeding trials (Figure 1), revealed that the cpm per gram of the second radioactive excreta sample had the highest cpm per gram, with subsequent hourly samples displaying regular decreases in isotope levels.

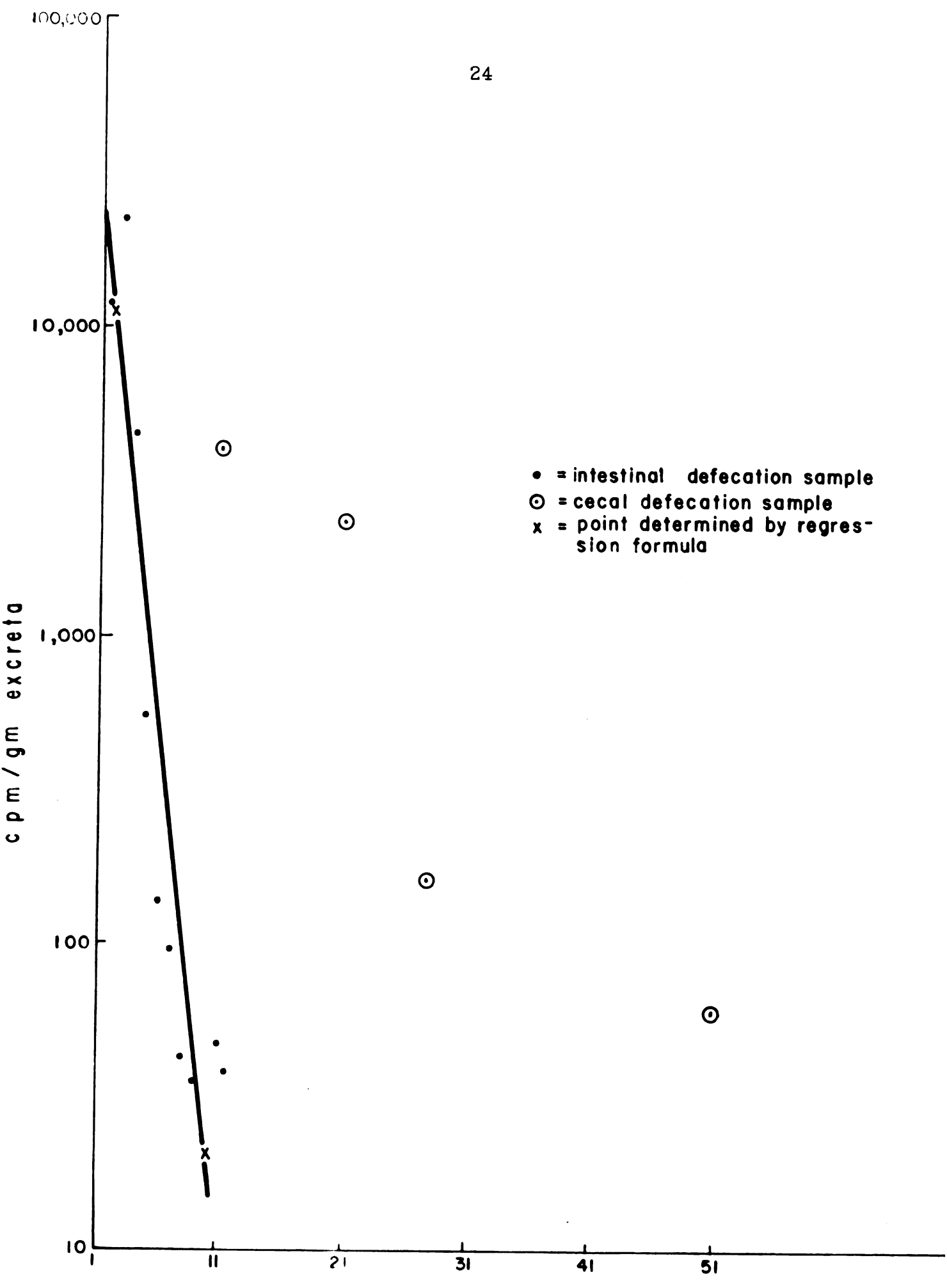
A reasonable explanation of this defecation pattern seems to be that the first sample has a lower Cr-51 concentration apparently because it was passed along the intestinal tract before the label was mixed thoroughly with all materials present in the crop and/or stomach. Thus, this sample contained some unlabeled food materials. The second sample had the highest Cr-51 concentration because the label had become thoroughly mixed with all the crop and/or stomach contents when this sample was passed. Subsequent defecation samples had proportionately lower Cr-51 concentrations as they became mixed with more newly ingested unlabeled digesta. Eventually defecations with no detectable Cr-51 occurred.

Table 2. Data of experiment 4b from which Figure 1 was derived, Michigan State University, 1966 and 1967

Time Elapsed From Ingestion of Cr-51 (hr.)	Excreta Wgt. (gm.)	Cpm of Excreta	Cpm Per Gram of Excreta	Percent of Total Cpm Per Gm.
1	0.0174	...	...	...
2	0.2868	3,402	11,862	24.99
3	0.1866	4,127	22,117	46.60
4	0.4226	1,894	4,482	9.44
5	0.5335	291	545	1.15
6	0.3501	47	134	0.28
7	0.4463	42	94	0.20
8	0.6359	27	42	0.09
9	0.3753	13	35	0.07
11	0.3173	15	47	0.10
11*	0.1079	440	4,078	8.59
21*	0.2612	614	2,351	4.96
28*	0.0962	155	1,611	3.39
51*	0.5489	33	60	0.13
Total			47,458	99.99

*Cecal excreta.

Figure 1. Defecation rate and pattern for a ring-necked pheasant after ingestion of a single-dose of Cr-51 on a Turkey Breeder Pellet. Michigan State University. Experiment 4b, 1966 and 1967.


100,000
10,000
1,000
100
10
cpm / gm excreta
1
11
21
31
41
51
HOURS AFTER INGESTION OF LABELED FOOD
• = intestinal defecation sample
⊙ = cecal defecation sample
x = point determined by regression formula

For convenience, the defecation with the highest cpm per gram is here called the peak defecation. The one or more defecations of the period prior to the peak represents a mixing phase, and the period following the peak is named the purging phase.

A line was fitted statistically to the plotted data by means of a calculation using the least squares regression formula (Dixon and Massey, 1957; page 193). In the computation of this line, the cpm per gram of mixing phase and cecal defecations were not used. This calculation also indicated the proportion of the isotope defecated per hour from the total amount of isotope remaining in the bird after each defecation (i.e., the percentage rate of food passage per hour). The straight line character of the plotted data indicates that a constant proportion of the isotope remaining in the bird is defecated per unit of time. Although Cr-51 seems to be defecated from the ceca in the same proportional manner, the proportion of Cr-51 defecated per hour obviously is much lower (thus the cecal passage rate is slower) than for intestinal defecations. Cecal excreta are readily distinguishable from intestinal excreta in gallinaceous birds (Leopold, 1953).

Perhaps the proportion of isotope passed per hour in the final intestinal defecations is slightly different than that of the initial defecations (Figure 1). A second line could be constructed on the graph for these defecation

samples. However, these samples are of minor significance and of much lower magnitude compared to the initial points so no such calculation was made.

Radioactive wastes also gave information on the rates of passage of foods through the GI tract. For the standard Turkey Breeder Pellet diet, the average minimum passage time was one to two hours, and the average maximum time for digesta not receiving cecal digestion was 8.5 hours. For those materials receiving cecal digestion the average passage time was 35 hours (Table 3).

Metabolizability coefficients also were determined through these tests. By the total collection method the metabolizability of Turkey Breeder Pellets averaged 65.48 percent (Table 3). The extent of cecal digestion (discussed beyond) was also ascertained.

A lower than normal rate of ingestion occurred in three of these developmental trials (experiment numbers 3b, 4a, and 4b; Table 3). This was probably due to the test bird undergoing a brief period of molting since a normal ingestion rate (experiment numbers 6a, 9a, and 9b; Table 3) followed this molting activity. Yet a higher rate of ingestion was expected during molting. Marshall (1961, page 246) shows evidence that the metabolic rate of several species of birds increased during molting, but Davis (1955) concluded that "M.E. values of the English Sparrow (Passer domesticus)

Table 3. Digestibility information gained from Cr-51 feeding trials performed during the development of the single-dose technique with pheasants fed Turkey Breeder Pellets, Michigan State University, 1966 and 1967

Expmt. No.	Bird	Gm/Hr Eaten	Passage Rates (hours) Intestinal Min.	Intestinal Max.	Cecal Min.	Cecal Max.	Dose Size (cpm)	Percent Dose Recov.	Total Coll. Meta. Coef.	Percentage Rate of Food Passage/Hour
1	M-PI	+	1-2	11	5-6	34	+	+	+	+
2	M-PI	+	0-1	10	5-6	29	+	+	+	+
3b	M-PII	0.7165	0-1	6	5-6	44	3,677	96.30	62.95%	72.48%
4a	M-PII	0.7165	1-2	10	5-6	48	15,009	96.04	62.95%	68.26%
4b	M-PII	0.7655	1-2	11	10-11	51	11,425	102.14	69.25%	54.75%
6a	M-PII	2.1651	1-2	8	7-8	47	21,252	97.35	65.24%	67.92%
9a	M-R	2.2796	2-3	5	6-7	23	3,819	80.97	64.48%	80.97%
9b	M-R	2.2462	2-3	7	6-7	6	304	95.00	65.50%	36.62%

TBP = turkey breeder pellets.

+ = records incomplete.

M-PI = adult male, number PI.

before and during molt were not significantly different at comparable temperatures."

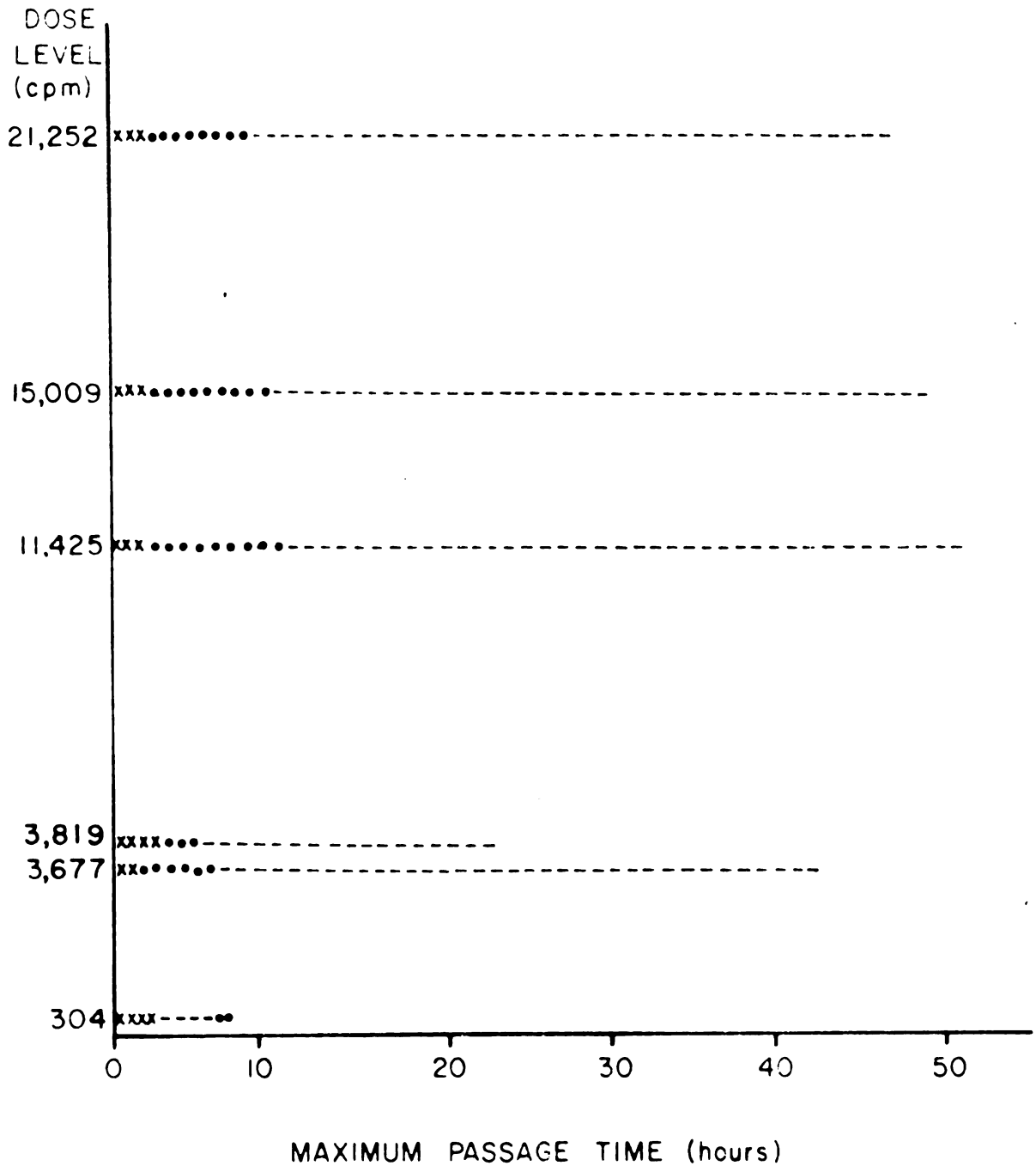
The developmental trials were not conducted in the controlled environment room, so metabolizability information might be expected to fluctuate with the variable environmental conditions.

Varying the Cr-51 dose level affected the measurement of the time of passage directly when the dose was smaller than 10,000 cpm (1.7uc) (Figure 2). If materials are removed from the GI tract in a regular proportional manner (see above), a smaller dose takes longer to be detectable in the excreta and causes the minimum passage time to seem longer. Similarly, a detectable level disappears more quickly from the excreta and makes the maximum passage time appear to be shorter.

Results of Continuous-Dose Developmental Trials

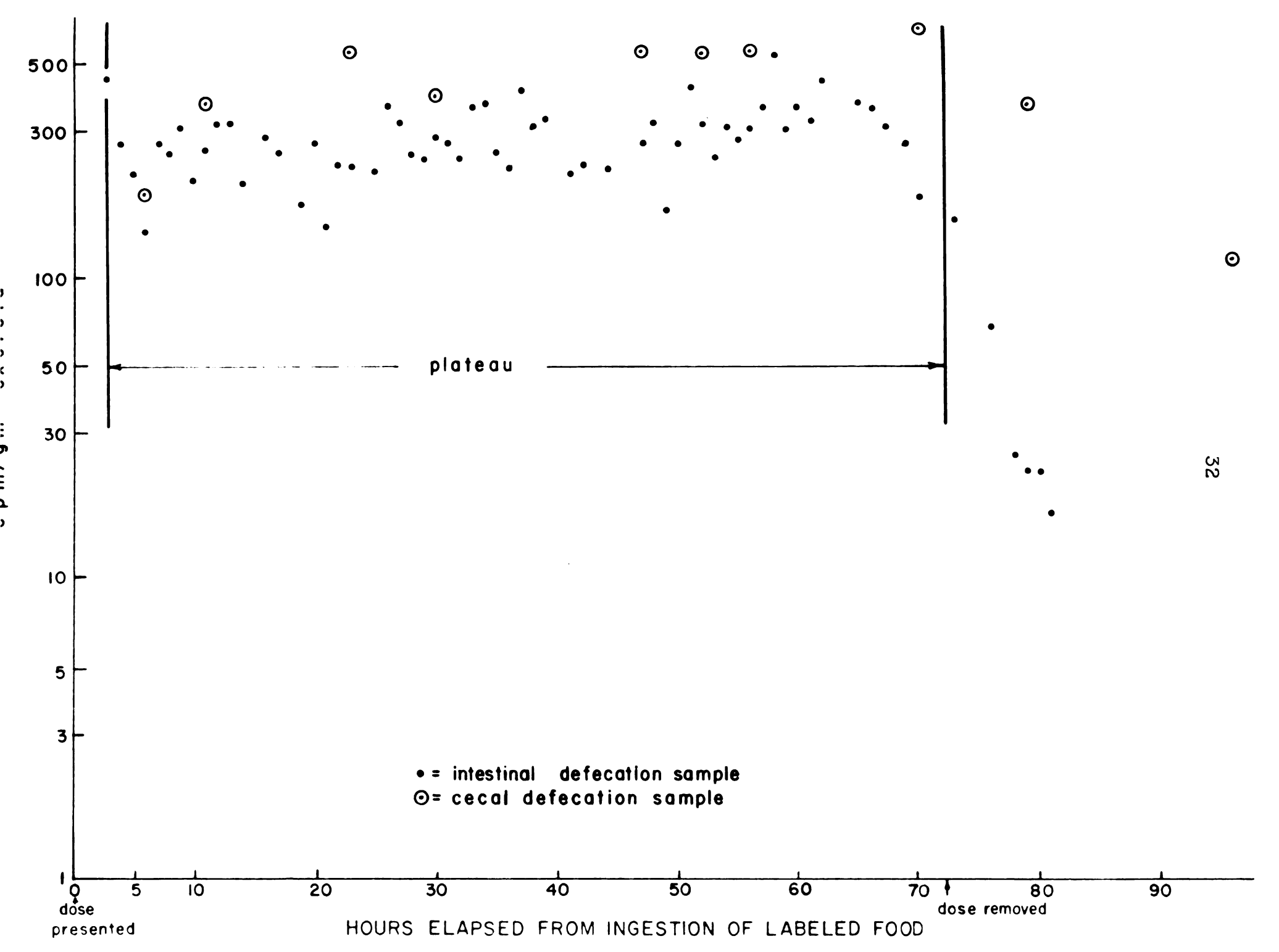
The graphic representation (Figure 3) of the rate and pattern of defecation of the continuous-dose of Cr-51 shows mixing and purging phases, but the peak is replaced by a level plateau. This results from each defecation being thoroughly mixed with approximately the same amount of Cr-51 due the ingestion of a uniformly labeled diet. The plateau persists for a period corresponding to the time that the test bird is eating Cr-51 labeled foods. The average of the cpm per gram of excreta (including cecal defecations) during

Figure 2. Relationship of minimum and maximum passage time to the size of the Cr-51 dose ingested by a pheasant. Michigan State University. 1966 and 1967.



x = minimum intestinal passage time
• = maximum intestinal passage time
- = maximum cecal passage time

Figure 3. Defecation rate and pattern for a ring-necked pheasant after ingestion of a continuous-dose of Cr-51 on Turkey Breeder Pellets. Michigan State University. Experiment 8d, 1966 and 1967.


500
300
100
50
30
10
5
3
1
plateau
• = intestinal defecation sample
⊙= cecal defecation sample
0
dose presented
5
10
20
30
40
50
60
70
dose removed
80
90
HOURS ELAPSED FROM INGESTION OF LABELED FOOD
32

the plateau period is compared to the average cpm per gram of food to determine metabolizability coefficients by the ratio method (Table 4).

The Cr-51 label allows identification of the excreta that are derived from labeled foods. Hence the total weight of ingested materials and their related excreta for the period of a continuous-dose test are known, and a metabolizability coefficient also may be computed by the toal collection method.

Table 4. Digestibility information gained from Cr-51 feeding trials performed during the development of the continuous-dose technique with pheasants fed increasing doses of Cr-51 with Turkey Breeder Pellets, Michigan State University, 1966 and 1967

Expmt. No.	Bird	Grams Eaten	Dose Size (cpm/gm)	Metabolizability Coefficients	
				Total Collection	Ratio
				(%)	(%)
8a	M-RI	1.8280	31	59.62	58.96
8b	M-RI	1.3032	33	62.97	48.97
8c	M-RI	1.8784	118	64.47	51.24
8d	M-RI	2.5927	142	63.21	54.05
10a	M-RI	1.9931	197	58.35	59.96

M-RI = adult male, number RI.

Results of Comparative Trials Using the Combination Technique

Comparative feeding trials were performed in the controlled environment room using pheasants of the same age and lineage. Thus, there should have been only individual and sexual variability between birds. The combination technique permits determination of both passage rates and metabolizability of foods as well as other information such as the amount ingested per hour. Under controlled conditions these factors may be compared between birds, or, since there is no retention of Cr-51 (see above), within the same bird. A graph of hourly isotope defecation was constructed for each tiral by collecting, drying, weighing, and counting radioactive excreta (Figure 4).

The continuous-dose portion of the combination technique permitted the computation of a metabolizability coefficient by the ratio method. For comparisons, this coefficient was also determined simultaneously by the total collection method. Since both methods were measuring the same factor, the coefficients determined by the two methods should have been approximately equal to each other in each test. They were different however, in almost every feeding trial (Table 5) and this situation has no obvious explanation. For cocks on the standard diet, the average total collection metabolizability coefficient was 63.49 percent while this average by the ratio method was 53.68 percent.

Figure 4. Defecation rate and pattern for a ring-necked pheasant during a combination technique feeding trial in which both single and continuous-doses of Cr-51 were fed on Turkey Breeder Pellets. Michigan State University. Experiment 11a, 1966 and 1967.

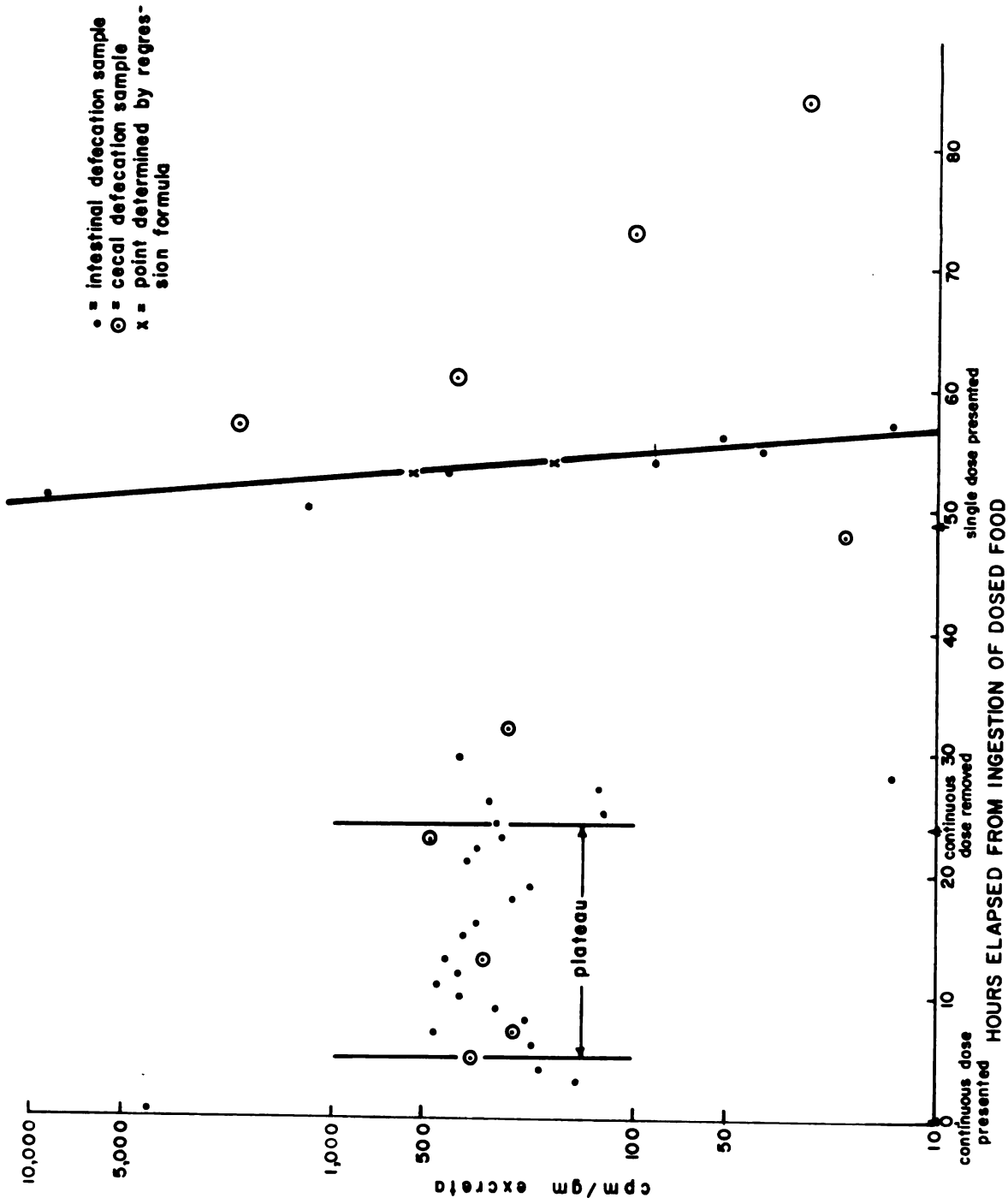

• = intestinal defecation sample
⊙ = cecal defecation sample
x = point determined by regression formula
cpm/gm excreta
10,000
5,000
1,000
500
100
50
10
plateau
continuous dose presented
10
20 continuous dose removed
30
40
50 single dose presented
60
70
80
HOURS ELAPSED FROM INGESTION OF DOSED FOOD

Table 5. Digestibility information gained from Cr^{51} feeding trials using the combination technique, three separate diets, and male and female pheasants, Michigan State University, 1966 and 1967

Expmt. No.	Bird	Diet	Size of Single-Dose (cpm)	Size of Continuous-Dose (cpm/gm)	% of Single-Dose Recovered	Gm/Hr Eaten
11a	M-FI	TBP	11,805	148	89.63	2.0062
11b	M-FI	TBP	13,129	163	86.18	1.8755
11c	M-FI	TBP	13,825	203	100.21	2.0192
11d	F-HI	TBP	22,615	178	98.09	1.8441
11e	F-HII	TBP	14,544	141	90.26	2.4743
11f	M-FI	Choke-berry	12,757	134	95.63	1.0490
11g	M-FI	Whole corn	11,113	82	103.69	1.8795
⊛12a	M-FI	TBP	18,378	142	103.53	2.0690
	M-FII	TBP	12,400	142	98.08	2.0127
⊛12b	M-FI	TBP	10,170	163	98.51	2.2996
	M-SI	TBP	10,270	163	99.80	0.6624
*13a	M-FI	TBP	17,501	...	99.81	2.2133
(*)13b	M-FI	TBP	...	136	...	1.7744

TBP = turkey breeder pellets.

M-FI = adult male, number FI.

F-HI = adult female, number HI.

Total Collect Meta. Coef.	Ratio Meta. Coef.	Calories/Gm Metabolized	Passage Rates (hours)			
			Intestinal		Cecal	
			Min.	Max.	Min.	Max.
63.83	55.15	3,083	1-2	8	9-10	36
61.95	51.34	3,023	1-2	9	9-10	23
61.79	53.87	3,018	1-2	7	9-10	34
65.24	56.16	3,131	1-2	28	7-8	69
67.02	58.65	3,188	1-2	10	8-9	46
48.54	35.89	2,114	0-1	30	10-11	32
80.44	83.06	3,658	1-2	19	10-11	71
62.47	49.28	3,040	0-1	8	11-12	37
63.22	55.49	3,064	0-1	12	12-13	32
65.66	62.61	3,142	1-2	7	11-12	47
65.84	49.38	3,148	1-2	10	10-11	47
64.63	...	3,109	0-1	11	8-9	48
62.02	52.28	3,026	...	..	...	..

⊛ Trials in which 2 birds were tested simultaneously.

* Single-dose test rather than a combination test.

(*) Continuous-dose test rather than a combination test.

The single dose portion of the combination technique allowed determination of minimum and maximum passage rates of foods. For cocks on the standard diet, the average minimum passage rate was 0.6 to 1.6 hours. The average maximum rates were nine hours for materials not receiving cecal digestion and 38 hours for materials which were partially digested in the ceca.

Recovery of the Cr-51 label from single-dose tests ranged from 86.18 to 103.69 percent. Recoveries greater than 100 percent are probably due to normal equipment error occurring both as the prepared dose is counted (before ingestion) and when counting the collected excreta.

Using the caloric values of the standard diet and of the excreta from that diet, the average metabolizable energy (M.E.) per gram of food was 3,073 calories for cocks. The average amount of the standard diet eaten by cocks was 1.8813 grams per hour.

It is possible to obtain valid average results for passage rates, metabolizability, etc. in the comparative trials. However, to learn the differences in results using different diets, it is also desirable to compare the variation in these results within and between cocks and hens in trials using the standard diet.

Cock F-I was used in many of the comparative tests. The total collection metabolizability coefficients for the standard diet for this bird were constant within the rather

narrow limits of 61.79 to 65.66 percent. However, minimum and maximum passage rates and the amount ingested per hour were more variable. Labeled excreta first appeared within the first or second hours after ingestion of the Cr-51 dose. The maximum rate for intestinal excretion ranged from 7 to 11 hours and for cecal excreta from 23 to 48 hours (Table 5). The amount eaten by this bird varied between 1.7744 to 2.2996 grams per hour.

In each of two feeding trials, two birds were tested simultaneously using the standard diet. Total collection metabolizability coefficients, calories of energy obtained per gram of food, and passage rates were all very similar for the two birds in each trial. But all of these measurements differed betweeen the two trials (Table 5).

The amount ingested by the two birds was almost equal in the first trial and extremely unequal in the second. Although one bird ate in excess of three times as much as the other in the second trial, their metabolizability of food and passage rates were almost identical. The bird eating less showed no significant weight change during testing and a normal amount of visceral fat was noted when it was sacrificed at the conclusion of the feeding trial.

The results obtained from trials with hens as compared to those with cock F-I (on the standard diet) showed a small difference in average total collection metabolizability coefficients, viz., 66.13 percent with hens and 63.49

percent with the cock. This is a small difference as is the difference in their ingestion rates; the cock ate an average of 2.0367 grams per hour throughout all feeding trials and the average for the hens was 2.1592 grams per hour during their feeding trials. The minimum passage rates were about equal for the two sexes, but the maximum passage time of the standard diet was considerably longer in hens (Table 5). With a longer passage rate, one would expect to find the higher digestibility which was observed for the hens. The longer passage rate may be in part due to less activity at night by the hens, as shown by fewer defecations. Thus, there was not a great deal of variability between birds tested simultaneously in feeding trials, but there was variability between different birds in different trials and even in trials with the same bird to some degree. There was also some difference between cocks and hens.

A controlled environment was not employed in the developmental trials and test pheasants were from wild stock. Thus, a comparison of the results from those trials with results from the comparative trials can help to determine the effects of the controlled environment. Average values for all cocks undergoing testing on the standard diet in the two types of trials were:

	Total Collection Meta. Coef.	Grams of Food Eaten/Hr.	Food Passage Rates (hours) Intestinal Min.	Intestinal Max.	Cecal Min.	Cecal Max.
Developmental trials	63.54%	1.7768	0.9-1.9	8.5	9.5-10.5	39.4
Comparative trials	63.49%	1.8813	0.6-1.6	9.0	6.1-7.1	38.0

The comparison indicates remarkably little dissimilarity considering the different conditions and pheasant stock used. One developmental test shown in Table 3 was excluded from the average since the dose level used was far below the minimum level found to yield reliable results.

Evaluation of the results of trials in which either chokeberries or whole corn were fed allows some interesting comparisons. Corn had the highest metabolizability of the three diets used, while chokeberries had the lowest (Table 5). Similarly the M.E. per gram of corn was considerably higher than that of the standard diet and almost twice as much as that of chokeberries. The maximum passage rate of corn was much longer than that of Turkey Breeder Pellets or chokeberries. However, chokeberry materials which did not receive cecal digestion took much longer to pass than did such materials in the other two diets. What are the relationships of metabolizability coefficients and the passage rates of food

and how would the relative value of a diet be accessed from these factors?

Of course the standard commercial diet would be rated first among these three diets on the basis of the essential nutrients and vitamins it contained, but on the basis of the M.E., corn is highest. In terms of passage rate, corn would be rated third and chokeberries would be first.

The following equation was used to provide an index to these relationships in this study:

$$\text{Av.M.D. per day} = (\text{M.E./gm} \times \text{gm/hr eaten} \times 24 \text{ hrs}) \left(\frac{24 \text{ hrs}}{\text{max. pass rate}} \right).$$

The product obtained within the first parenthesis tells the M.E. per day if the maximum passage rate of the diet being fed is one day. The term in the second parenthesis changes the M.E. per day to correspond to the actual passage rate of the diet. This computation incorrectly assumes that an equal amount of any unit of food is digested every hour during the passage of that food. Most food is digested very soon after ingestion and only a small proportion requires the full maximum passage time. However, the last 5 percent of a diet to be absorbed may very well be the most beneficial part so should not be ignored. And, the formula yields the <u>average</u> energy available from the food eaten per unit of time.

As an example, the average M.E. provided per day to bird F-I on the three diets tested was:

Diet	Average M.E. Per Day
Turkey Breeder Pellets	87,334.9 cal. (87.3 kilocal.)
Whole corn	55,771.6 cal.
Chokeberries	39,916.5 cal.

These results show the relative value of the diets when passage rate is considered as well as the M.E. of a diet; they show that the standard diet provides more energy per unit of time. Thus, M.E. and passage rates considered together may be more important than either value considered alone.

One of the earliest passage rate studies was accomplished by Ewing and Smith (1917) with the steer. Hillerman et al. (1953) found that marked food materials first appeared in the excreta of chickens and turkeys in less than five hours after feeding. Browne (1922) showed that oats first appeared in the excreta of fasted chicken hens in five to six hours after feeding. Radioactive barium has been used (Imabayashi et al., 1956) to show that approximately one-half of the marked food ingested by poultry was excreted within four to five hours. The first appearance of excreta from food marked with chromic oxide in 21 coturnix quail was from one to two hours after feeding (McFarland and Freedland, 1965). The results of the latter two investigations are most in agreement with the findings of this study with regard to minimum rate of passage.

Study of food passage by means of X-ray shadows of $BaSO_4$-marked food have shown that two ounces of oats were completely removed from the tract of chickens in 16 to 25 hours (Henry et al., 1933). And in the quail study mentioned above, excreta defecated four hours after feeding showed no Cr_2O_3 but it again appeared after five to eight hours indicating the occurrence of cecal evacuation. Thus, the maximum passage rate for chickens appears to be longer than that of pheasants (if cecal influence is omitted), but that of coturnix quail appears to be shorter.

With poultry, the passage rates of pullets and cocks may be slightly faster than for mature hens (Hillerman et al., 1953). In this study, pheasant cocks exhibited somewhat faster maximum passage rates than hens.

In previous studies, values other than M.E., especially productive energy, have been given much attention. Now however, "there is general agreement that the energy value of the diet is best expressed in terms of the metabolizable energy to which it gives rise" (Sturkie, 1965; p. 260). Hill (1964) states that "it has been found that M.E. values determined with chickens are essentially unaffected by the level of food intake, rate of growth or egg production, breed, sex, and wide differences in the nutrient balance of the diet." It has also been shown that there is no significant difference in the M.E. values between the sexes in the English sparrow (Davis, 1955).

In general, the present study is in agreement. M.E. values varied as much in separate trials on the same bird as between wild and game farm birds or between males and females (Table 5). Also, the level of food intake did not appear to affect the metabolizability of the diet (Table 5). The level of metabolizability of the standard diet, however, was about 5 percent higher for chicks than adults. Metabolizability coefficients averaged 68.68 percent for chicks and 63.49 percent for cocks in comparative runs.

Additional Results

Feeding Trials with Chicks

The single-dose technique was used for all experiments with pheasant chicks. Because trials with adults were made concurrently, the excreta collecting device was not available for use in the chick tests. Based on results from feeding trials with adults, however, 24 hours was deemed sufficient to determine minimum and maximum passage rates for materials not receiving cecal digestion. In day-long tests, excreta were collected hourly for at least the first 16 hours. As in adult feeding trials, tests were started at about 8:00 a.m.

The diet for the first five chick trials was Quail Breeder Mash commercially prepared by the King Milling Company of Lowell, Michigan. Chicks were force-fed Cr-labeled mash since ad libitum feeding might have resulted in spillage.

The chicks were accustomed to being handled and the procedure appeared to cause little stress. The guaranteed analysis for Quail Breeder Mash is as follows:

Ground corn	412.5
Soymeal dehulled 50%	370.0
17% Alfalfa meal	50.0
Dried whey	25.0
Meat/bone meal 50%	25.0
Fish meal Menhaden 60%	25.0
Ground limestone ($CaCO_3$)	50.0
Dicalcium phosphate	15.0
Iodized salt	5.0
Vitamin premix 1 Nopco M-4	2.5
Fat	20.0
	1,000.0 lbs.

Turkey Breeder Pellets, the standard ration for adult trials, were used for the final three chick trials.

The amount eaten per hour by the chicks increased fairly regularly until at the age of 78 days their ingestion rate was similar to that of adult birds (Tables 5 and 6). There did not appear to be a correlation between age and the metabolizability of feeds in this study, although Mueller et al. (1956) showed that the metabolizability of all nutrients in the diet of chickens increased slightly from two to four weeks of age and then declined steadily. In this study, the average metabolizability coefficient, 67.53 percent, was slightly higher than that of adult birds.

The minimum passage rate (Table 6) did not appear to change with age during the first 11 weeks, but it was longer for chicks than for adults (Table 5). The maximum passage rate for materials not receiving cecal digestion increased

Table 6. Digestibility and body weight information from Cr-51 single-dose feeding trials with pheasant chicks fed two diets. Michigan State University, 1966 and 1967

Test No.	Bird Age (days)	Bird No.	Body Wt (gm)	Diet	Gm/Hr Eaten (24 hrs)	Passage Rates (hours) Intestinal Min.	Intestinal Max.	Cecal Min.	Cecal Max.	Total Coll. Meta. Coef. (%)	Cal. of M.E.per Gm Food	Percentage Rate of Food Passage Per Hour
7a	7	C-I	24/26*	QBM	0.2553	2-3	5.0	...	...	82.23	+	0.4802
7c	15	C-I	41/48	QBM	0.4105	1-2	5.0	...	...	67.36	+	0.6874
7d	21	C-II	89/103	QBM	0.6470	2.5-3.5	7.5	...	...	48.71	2,344	0.6308
7e	26	C-II	142/145	QBM	1.0125	1.5-2.5	10.5	...	...	75.24	3,155	0.4051
7f	42	C-II	289/284	QBM	0.8718	2-3	6.0	5-6	10	60.66	2,722	0.7615
7g-a	57	C-II	436/435	TBP	1.5513	1-2	24.0	6-7	>24	67.45	3,202	0.4741
7g-b	72	C-II	523/542	TBP	1.5015	2-3	24.0	9-10	>24	72.12	3,241	0.5182
7g-c	78	C-II	582/586	ground TBP	2.3428	1-2	6.0	6-7	>24	66.46	3,102	0.8421

*24/26 = Body wt. at start of test/Body wt. at end of test; for 24 hours.

QBM = Quail Breeder Mash.

TBP = Turkey Breeder Pellets.

+ = Caloric value of excreta was not determined.

slightly up to approximately four weeks of age, but by 57 days the chicks passed these materials at the adult rate.

Cecal excreta were not distinguishable as such, either by their appearance or by the delayed appearance of Cr-51, until the birds were 27 days old. Possibly the development of the ceca or of the cecal flora is inadequate for detectable cecal digestion until after 27 days of age. No previous investigations into this matter were discovered.

In previous studies of passage rates in young and old birds, it was found that the passage of foods through young turkey hens was significantly more rapid than through old turkey hens (Hillerman et al., 1953) and the passage of feed through juvenile chickens was faster than through adults (Thornton et al., 1956). However, Dorozynska (1962) found that "the speed of passage of food down the alimentary canal increases as geese grow."

At the age of 57 days, the chicks were switched from Quail Breeder Mash to Turkey Breeder Pellets and an immediate increase in the maximum length of intestinal passage was noted. Neither the minimum passage rate nor the metabolizability coefficient appeared to be affected however, by the pelleted diet (Table 6). When ground Turkey Breeder Pellets were fed, the metabolizability remained about the same as with whole pellets but the length of the maximum intestinal passage decreased to the level observed with a mash diet.

Possibly the gizzards of the chicks were not sufficiently developed to grind the pellets quickly.

Four weeks is the recommended age at which to start feeding pellets (Warden, 1962), but this may be too early. The metabolizability of pellets evidently is as high as for ground pellets, so a chick receives the same amount of energy per gram from either form of the diet. But a chick gets less energy per hour with pellets, since they are passed more slowly. McIntosh et al. (1962) and Reddy et al. (1962) have previously shown that the M.E. content of a diet is not changed by being fed as pellets or mash. And, it should be noted that slower passage did not result in a more complete digestion of food as might be expected (Table 6).

A difference in passage rates was not observed when fasted young chickens were fed pellets or ground pellets marked with chromic oxide (Jensen et al., 1962). The initial appearance of marked excreta was two hours after feeding either mash, pellets, or ground pellets, and the last appearance of marked excreta was at approximately ten hours for all three diets. These passage rates agree well with those of pheasant chicks in the present study except for the pelleted diets which had longer rates in pheasant chicks. Other passage rate determinations for young chickens showed minimum passage times to be approximately 1.5 to 2.5 hours depending upon the test conditions employed (Tuckey et al., 1958).

Chickens have a metabolic rate which is low at hatching but which increases until about 30 days of age. It then decreases to the adult level by 70 to 80 days of age (Kibler and Brody, 1944; Crandall and Smith, 1952). Apparently none of the data of this study can be adapted to indicate the metabolic rate of pheasant chicks at various ages. Both body weight and ingestion rate approximately doubled each week for the first four weeks, however, and then continued to increase but at a lower rate.

Cecal Influence on a Diet

The ceca harbor bacteria and function primarily in the microbial decomposition of crude fiber (Suomalainen et al., 1945). The length of the ceca is related to the diet and they are longer in bud-eating birds like grouse than in seed-eaters such as quail and pheasants (Leopold, 1953). Cr-51 is very useful in the study of cecal digestion. The percent of cecal influence can be determined from the data of a single-dose feeding trial by dividing the total cpm recovered in cecal defecations by the total cpm recovered from all excreta (including cecal). A cecal metabolizability coefficient can be determined from the data of the continuous-dose feeding trial by using the average cpm per gram of cecal excreta for the plateau period only in the formula for determining metabolizability by the ratio technique (shown above). The maximum passage rate for materials

requiring cecal digestion is a measure of the length of cecal influence on a meal. The rate of cecal defecations as compared to intestinal (rectal) defecations per day is a further measure of cecal activity.

The percentage of cecal influence on a meal was highest with the whole corn diet. This was expected since corn has a higher concentration of crude fiber than the other diets tested. The average percentage of cecal Cr-51 which was recovered from birds on the standard diet was 10.49 for males and 13.59 for females (Table 7). Accordingly, it is possible that females may depend more on cecal digestion than males and this may explain why total collection metabolizability coefficients were higher for females than for males. The slower passage rate in females, however, also may help to explain their higher metabolizability coefficients on the standard diet.

A portion of a diet which received both cecal and intestinal digestion had a higher metabolizability coefficient than one receiving only intestinal digestion (Table 7). Metabolizability of the standard diet showed an average increase of 11.32 percent for pheasant F-I due to cecal digestion. With the chokeberry diet, the metabolizability coefficient for materials which received both cecal and intestinal digestion was lower than that for materials which received no cecal digestion (Table 7). The only apparent explanation for this last case is that the relatively

Table 7. Degree of cecal influence for adult ring-necked pheasants on various diets, Michigan State University, 1966 and 1967

Test No.	Bird	Diet	Av. No.of Cecal Defec/Day	Percentage Cecal Influence on a Diet	Duration of Cecal Influence	Ratio Method Metabolizability Coefficients		Percent Increase In Digestion*
						Cecal	Intestinal	
						(%)	(%)	(%)
11a	M-FI	TBP	3.25	8.71	36 hr-C 8 hr-I	57.23	55.15	2.08
11b	M-FI	TBP	2.50	4.96	23 hr-C 9 hr-I	56.99	51.34	5.65
11c	M-FI	TBP	2.00	9.66	34 hr-C 7 hr-I	70.23	53.87	16.36
11d	F-HI	TBP	2.75	11.29	69 hr-C 28 hr-I	64.26	56.16	8.10
11e	F-HII	TBP	2.00	15.89	46 hr-C 10 hr-I	70.87	58.65	12.22
11f	M-FI	chokeberry	3.00	17.93	32 hr-C 30 hr-I	30.21	35.89	...
11g	M-FI	whole corn	2.00	30.65	71 hr-C 19 hr-I	93.13	83.06	10.07
12a	M-FI	TBP	2.00	9.51	37 hr-C 8 hr-I	70.17	49.28	20.89
	M-FII	TBP	2.00	9.83	32 hr-C 12 hr-I	65.28	55.49	9.79
12b	M-FI	TBP	2.00	10.88	47 hr-C 7 hr-I	78.69	62.61	16.08
	M-SI	TBP	2.00	19.65	47 hr-C 10 hr-I	66.94	49.38	17.56
13a	M-FI	TBP	2.00	10.71	48 hr-C 11 hr-I	...	...	...
13b	M-FI	TBP	2.00	...	...	59.16	52.28	6.88

*Percent increase in the metabolizability of feed if it received cecal digestion.

TBP = Turkey Breeder Pellets; M-FI = adult male, number FI; C = cecal excreta; F-HI = adult female, number HI; I = intestinal excreta.

undigestible fibrous portion of the diet was diverted to the ceca. This caused the cecal excreta to have a higher proportion of undigestible material than intestinal excreta in relation to the amount of Cr-51 present. The lower cpm per gram of cecal excreta apparently lowered the cecal metabolizability coefficient.

Materials receiving cecal digestion had a longer maximum passage rate than materials not receiving cecal digestion. For all tests with the standard diet, the passage rate averaged 30.00 hours longer for materials receiving cecal influence (Table 7). The average minimum cecal passage rate for the standard diet was 7.9 to 8.9 hours.

The average number of cecal defecations per day was slightly over two for all feeding trials. For specimen F-I for which data were most complete, the ratio of cecal to intestinal defecations on the standard diet was 1 to 24.04, and for this bird on a corn diet the ratio was 1 to 29.4. The ratio of cecal to intestinal evacuations for chicken hens is 1 to 11.5 after ingestion of corn and 1 to 7.3 after feeding barley (Rosseler, 1929). Apparently cecal digestion is less important to pheasants than to chickens. Cecal defecations of pheasants occurred most frequently near 7:00 a.m. and 7:00 p.m. (Table 9).

Caloric and Moisture Content of Feeds and Excreta

Caloric values of the three adult diets used were somewhat similar (Table 8), ranging from 4,239 to 4,485 calories.

The amount of moisture in all excreta was approximately 70 percent (Table 8). Fresh excreta were used in determination of this percentage.

Passage Rate of the Cr-51

The question of whether the Cr-51 label passes through the tract at the same rate as the food on which it is applied was studied briefly. Excreta from chokeberries are characteristically colored dark blue. A Cr-51 labeled chokeberry was fed to a bird whose previous diet was Turkey Breeder Pellets. A pure diet of chokeberries was presented to the bird with the labeled berry. Defecations subsequent to ingestion of this label were monitored and a qualitative estimate of the "blueness" of each defecation was made. Each defecation was also checked for the presence of radioactivity. The first appearance of blue color in a defecation coincided precisely with the first detection of radioacitivity. Blueness increased in the next defecation as did the amount of radioactivity, providing a mixing phase and peak when graphed. Subsequent defecations maintained a constant level of blue color as expected on a continuing berry diet, while the purging phase of radioactivity was observed.

Table 8. Calories per gram and percentage moisture of feeds and excreta from both juvenile and adult pheasants, Michigan State University, 1966 and 1967

Bird Age	Excreta on a Diet of			
	QBM	TBP	Chokeberry	Corn
22 days	3,057 cal 70 %			
42 days	3,026 cal 72 %			
57 days	3,187 cal 70 %			
69 days		3,578 cal 67 %		
76 days		3,391 cal 70 %		
Adult male		3,195 cal 68.5 %	4,608 cal 71 %	4,079 cal 77.5 %
Adult female		3,187 cal 69 %		

	Diets			
	QBM	TBP	Chokeberry	Corn
	3,912 cal 10.4 %	4,239 cal 9.2 %	4,485 cal 68.7 %	4,456 cal 9.1 %

QBM = Quail Breeder Mash

TBP = Turkey Breeder Pellets

It appeared that the label does indeed pass at the same rate as the food on which it is applied. There were other evidences, too, contributing to this conclusion. There was, for example, a consistent occurrence of a regular percentage removal of the isotope from the digestive tract. If the isotope was not associated with the food in the tract, it could pass in a single defecation rather than in proportional amounts in successive defecations. There were also close associations of the isotope with cecal defecations which, if the isotope had passed independently of the food, would not have occurred since the label would not be expected to be divided between the intestine and ceca in any particular pattern.

Daily Cycles of Ingestion, Digestion, and Excretion

The average amount of food eaten by a pheasant during daylight hours in the controlled environment room was 46.51 grams per hour, while only 2.47 grams per hour was eaten at night on the average.

The variation in the digestibility of foods between day- and nighttime was discussed earlier. One would expect nighttime digestibility to be higher than daytime in the diurnal pheasant since less is eaten at night and less is defecated. Therefore passage rate should be slower and digestibility should be greater. Pheasants, however, show a higher digestibility during daylight hours. The reasons for

this could be that because less food is eaten at night, less Cr-51 label is consumed. Thus, each defecation at night has a lower proportion of food material, less Cr-51, and more wastes such as sloughed cells and nitrogeneous kidney wastes. This would give a lower cpm per gram excreta for nighttime defecations and cause nighttime digestibility to appear to be lower than in daytime.

The average number of defecations per hour for pheasant F-I in the controlled environment room on the standard diet was considerably less during darkness than when the lights were on (Table 9). The most active excretory times were just after the automatic lights were turned on and just before they were turned off.

Since the peak appearance of Cr-51 occurs one to two hours after ingestion of a single-dose, the major proportion of the excreta associated with the food that it marks must also appear at this time. When the amount of Cr-51 found in each defecation is expressed as a percentage of the original dose and this percentage is multiplied by the weight of its respective defecation, the sum of the products was found to vary from 0.3 to 3.4 grams. If these sums are assumed to be the total weights of the excreta from the food associated with the Cr-51 label during the mixing phase, then these weights divided by the nonmetabolizability coefficient would indicate the original dry weight of the food with which the label was mixed. The broad range of weights (0.3 to 3.4

Table 9. Defecation rates for pheasant F-I during seven feeding trials in the controlled environment room on the standard diet, Michigan State University, 1966 and 1967

Hour	Average Intestinal Defecations/Hour	Average Cecal Defecations/Hour
0830-0930	3.3	0.0435
0930-1030	1.9	0.0
1030-1130	2.5	0.0
1130-1230	2.6	0.0
1230-1330	2.0	0.0435
1330-1430	2.3	0.0
1430-1530	3.0	0.0870
1530-1630	2.9	0.1304
1630-1730	3.4	0.2174
1730-1830	3.0	0.1304
1830-1930	4.2	0.3043
1930-2030	2.6	0.1304
2030-2130*	3.9	0.2174
2130-2230	0.5	0.0
2230-2330	0.7	0.0
2330-2430	0.9	0.0
2430-0130	1.3	0.0
0130-0230	1.6	0.0
0230-0330	1.4	0.0
0330-0430	2.3	0.0
0430-0530	0.8	0.0
0530-0630	1.6	0.0435
0630-0730**	3.2	0.6087
0730-0830	2.2	0.3478
	54.1	2.3043 = average defecations/day

*Lights turned on daily.

**Lights turned off daily.

grams), however, makes it difficult to determine in which organ mixing actually occurs. This makes the determination of the dry weight of excreta associated with the Cr-51 marked crop or stomach contents (objective three above) of uncertain value.

Autopsy Findings

As each feeding trial was concluded, the bird was usually sacrificed to determine whether remnant Cr-51 could be detected in its body. Although twelve separate tissues and blood were checked in each of four birds, no significant traces of radioactivity were found. Further evidence that Cr-51 is essentially inert physiologically is the high rate of its recovery. An average of 95.96 percent was recovered in 16 single-dose trials in which the standard diet was fed (Tables 3 and 5).

Other birds were sacrificed to determine the distribution of Cr-51 in the alimentary tract after feeding either a continuous- or a single-dose. By examining digesta samples from sacrificed birds which had been fed a continuous-dose, information was sought concerning the region of the tract in which most absorption of nutrients occurs. It was reasoned that the higher the cpm per gram of digesta the greater the removal of nutrients through absorption.

Only two pheasants were subjected to this type of test and the results were not conclusive since radioactivity

could not be detected in some digesta samples (Table 10). If larger doses were used, however, and larger samples were taken from each segment of the tract, this difficulty possibly could be overcome. In the present tests, the cpm per gram of digesta increased at about the middle of the small intestine (Table 10), and much absorption may have occurred in the anterior small intestine.

Six chicken hens were fed single-doses and then killed individually 17, 31, 60, 91, 121, and 148 minutes afterwards, respectively. Their alimentary tracts were removed, opened, and digesta samples were taken. When dried and weighed, the samples were counted to determine the disposition and concentration of Cr-51 in each segment of the tract. (An alternative method would be to arrange the entire tracts on X-ray plates for exposure, so that a photographic indication of the location and amounts of radioisotope in the tract could be gained.)

In the six hens (Specimens A-F) the pattern of cpm per gram of digesta of the gut samples from birds A and E (Table 11) was that expected from the characteristic defecation patterns (Figure 1). The pattern from birds B and C, however, was the opposite of that expected. Birds C and F had more material in their intestines than the other birds and apparently the Cr-51 dose remained in the anterior part of their tracts longer (Table 11). Bird D regurgitated a small amount of labeled material as it was being sacrificed.

Table 10. Cpm per gram of digesta** from various segments of the pheasant GI tract after ingestion of a continuous-dose of Cr-51 as determined by autopsy, Michigan State University, 1966 and 1967

Organ or Tract Segment	Bird F-II	Bird S-I
Average Cpm/Gm of Diet	106	152
Proventriculus	*	empty
Gizzard	*	140
	*	163
Duodenum	*	*
	*	90
	*	*
Small intestine (10-12 cm segment)	*	132
	*	159
	*	265
	49	278
	60	192
	+	*
Ceca	429	431
	380	246
Large intestine	248	*
	285	+
Cloaca	251	*

*No detectable level of radioactivity

**Where the diet contains a uniform concentration of Cr-51, the increase in cpm per gram of digesta is directly related to the degree of absorption.

+ = No sample taken.

Table 11. Distribution of Cr-51 in the tracts of six female chickens on a Turkey Breeder Pellet diet. Samples were taken at intervals following ingestion of a single-dose of Cr-51 fed at about 12:45 a.m., Michigan State University, 1966 and 1967

	Bird A	Bird B	Bird C	Bird D*	Bird E	Bird F
Size of Dose (cpm)	14,118	20,459	14,786	13,521	13,876	15,904
	Percentage of original dose found in each organ for birds killed after:					
Organ or Segment	17 min.	31 min.	60 min.	91 min.	121 min.	148 min.
Pre-crop esophagus	0.00	7.64	1.20	10.47	0.00	9.00
Crop	1.11	21.15	13.32	12.25	0.19	5.67
Post-crop esophagus	1.03	15.82	2.96	7.03	0.00	9.00
Proventriculus	1.95	3.40	0.00	1.09	0.23	6.85
Gizzard	14.19	1.28	0.67	0.45	1.08	1.82
Duodenum	7.33	0.78	0.30	0.80	0.27	1.14
Small intestine:						
0-10 inches	0.33	1.02	0.39	1.56	0.00	1.67
10-20 inches	0.00	0.00	0.05	3.50	0.84	2.48
20-30 inches	0.00	0.00	0.08	0.17	5.11	0.19
30-40 inches	0.00	0.00	0.00	0.00	19.99	3.64
40-50 inches	0.00	0.00	0.00	0.00	**	**
Total ceca	0.00	0.00	0.00	3.41	0.22	0.00
Colon	0.00	0.00	0.00	0.00	57.99	0.38

*Bird regurgitated some labeled material when killed.

**No sample.

Since the Cr-51 concentration in its gizzard was lower than that in its small intestine, the regurgitated material could have come all the way from the gizzard. This would explain a higher Cr-51 level in the crop and esophagus of this bird as compared to birds killed earlier. The general pattern that was expected was observed. The labeled digesta were found further along the tract in birds which were killed longest after ingestion of the Cr-51 dose. A high percentage of the dose was not recovered because an effort was made to avoid scraping the gut walls, hence much digesta was left in each segment of the tract.

Further studies of the distribution of food labeled in this fashion would be desirable.

As an incidental observation, the lengths of the various portions of the pheasant alimentary tract were determined during each autopsy. The averages for four pheasants are:

Entire tract from anterior end of the proventriculus to the anus	109.0 cm
Proventriculus	3.5 cm
Gizzard	4.5 cm
Small intestine	88.3 cm
Large intestine	9.3 cm
Each cecum	14.3 cm
Cloaca	3.4 cm

Energy Budget

Using information on the amount of food eaten and metabolized one can construct an energy budget for an animal. The budget for the three diets with bird F-I in the final comparative trials was:

Average Number of Calories per Day	Turkey Breeder Pellets	Chokeberry	Whole Corn
Ingested	207,206	112,913	201,002
Metabolizable	87,335	39,917	55,772
Excreted	119,871	72,996	145,230

The average calories ingested per day was determined by multiplying the average grams eaten per day times the calories per gram of food. Determination of the average calories of metabolizable energy obtained per day has been described above. The average calories excreted per day was taken to be the difference between the average calories ingested per day and the average calories of metabolizable energy obtained per day.

CONCLUSION

Evaluation of Cr-51 Used in the Ratio Method with Pheasants

Previous investigators have concluded that the use of chromic oxide in the ratio technique for determining feed digestibility was satisfactorily comparable with (Kane et al., 1950; Schurch et al., 1950; Davis et al., 1958; and Elam et al., 1962) or superior to (Dansky and Hill, 1952; and Sibbald et al., 1960) the total collection method. Unfortunately, results with Cr-51 in the present study do not permit a similar conclusion.

So far as is known, radioactive chromium should react physiologically like stable chromium. Furthermore, the identical continuous-dose procedures used in this study were also applied in a white-tailed deer study in this laboratory during the same period with complete success (Mautz and Petrides, 1967).

As has been shown, the average metabolizability coefficient determined by the Cr-51 indicator method was about 10 percent lower than that obtained by the total collection method. The coefficients obtained by the ratio method were not only lower but were more variable (standard deviation = 4.324) than those obtained by the total

collection method (standard deviation = 2.121). The metabolizability coefficients for bird F-I in the controlled environment on the standard diet, for example, varied from 49.28 to 62.31 percent. The ratio technique employing Cr-51, therefore, is not considered valid for determining metabolizability coefficients in pheasants. At the present time there is no explanation for the difference in the metabolizability coefficients obtained by the two methods.

The continuous-dose feeding trial is of value in comparing daytime to nighttime digestibilities of feeds, and in comparing digestibility of foods receiving cecal digestion to foods which do not.

It is hoped that the Cr-51 indicator method can be modified and improved in the future to make it as useful for determining metabolizability as it is for determining passage rates. There should be further efforts to resolve the disagreement between metabolizability coefficients as determined by the total collection and ratio methods.

LITERATURE CITED

Bergeim, O. 1926. Intestinal chemistry IV. A method for the study of food utilization or digestibility. J. Biol. Chem. 70:29-33.

Border, J. R., L. E. Harris, and J. E. Butcher. 1963. Study of the quantitative fecal recovery of chromic oxide when administered to sheep as a component of paper. J. Anim. Sci. 22:1117.

Brandt, C. S. and E. J. Thacker. 1958. A concept of rate of food passage through the gastro-intestinal tract. J. Anim. Sci. 17:218-223.

Browne, T. G. 1922. Some observations on the digestive system of the fowl. J. Comp. Path. and Thera. 35:12.

Comar, C. L. 1955. Radioisotopes in biology and agriculture. McGraw-Hill Book Co., New York, Toronto, London. 481 pp.

Corbett, J. L., J. F. D. Greenhalgh, I. McDonald and E. Florence. 1960. Excretion of chromium sesquioxide administered as a component of paper to sheep. Brit. J. Nutr. 14:289-299.

Crandall, R. R. and A. H. Smith. 1952. Tissue metabolism in growing birds. Proc. Soc. Exp. Biol. Med. 79:345-346.

Dansky, L. M. and F. W. Hill. 1952. Application of the chromic oxide indicator method to balance studies with growing chickens. J. Nutr. 47:449-459.

Davis, C. L., J. H. Byers, and L. E. Luber. 1958. An evaluation of the chromic oxide method for determining digestibility. J. Dairy Sci. 41:152-159.

Davis, E. A. Jr. 1955. Seasonal changes in the energy balance of the English sparrow. Auk 72:385-411.

Dixon, W. J. and F. J. Massey Jr. 1957. Introduction to statistical analysis. McGraw-Hill Book Co., Inc., New York. 488 pp.

Dorozynska, N. 1962. Food intake and defecation in the goose, Anser anser L. Acta Biol. Exptl. 22(3):227-240.

Downes, A. M. and I. W. McDonald. 1964. The chromium-51 complex of EDTA as a soluable rumen marker. Brit. J. Nutr. 18(1):153-162.

Edin, H. 1918. Orienterande försök over en pa led kropp-sprincipen grundad metod att bestämma en toderblundnings smaltburhet. Medd. Nr. 105 Centralanstult. försö ksuüsendef jordbruks.

Edwards, H. M. and M. B. Gillis. 1959. A chromic oxide balance method for determining phosphate availability. Poult. Sci. 38(3):569-574.

Elam, L. J., P. J. Reynolds, R. E. Davis, and R. O. Everson. 1962. Digestibility studies by means of chromic oxide, lignin, and total collection techniques w/sheep. J. Anim. Sci. 21:189-192.

Ewing, P. V. and F. H. Smith. 1917. A study of the rate of passage of food residues through the steer and its influence on digestion coefficients. J. Agr. Res. 10: 53-63.

Forbes, R. M. and W. P. Garrigus. 1948. Application of a lignon ratio technique to the determination of the nutrient intake of grazing animals. J. Anim. Sci. 7:373-382.

Foster, F. 1963. Environmental behavior of chromium and neptunium. In Radioecology. pp. 569-577. Reinhold Pub. Corp. and the AIBS. 1963.

Henry, K. M., A. J. MacDonald, and H. E. Magee. 1933. Observations on the functions of the alimentary canal in fowls. J. Exp. Biol. 10:153.

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

_____, D. L. Anderson, R. Renner, and L. B. Carew Jr. 1960. Studies of the metabolizable energy of grain and grain products for chickens. Poult. Sci. 39:573.

_____, and R. Renner. 1963. Effects of heat treatment on the metabolizable energy value of soybeans and extracted soybean flakes for the hen. J. Nutr. 80: 375-380.

Hill, F. W. 1964. Utilization of energy for growth by chicks. European Assn. for Animal Production, 3rd Symposium on energy metabolism. Troon. pp. 327-332.

Hillerman, J. P., F. H. Kratzer, and W. O. Wilson. 1953. Food passage through chickens and turkeys and some regulating factors. Poult. Sci. 32:332.

Hoelzel, F. 1930. The rate of passage of inert materials through the digestive tract. Am. J. Physiol. 92:466-497.

Hogan, J. P. 1964. The digestion of food by grazing sheep. Aust. J. of Ag. Res. 15(3):384-396.

Hollis, O. L., R. C. Thompson. 1958. Irradiation of the GI tract of the rat by ingested Ru-106. Am. J. Phys. 194:308-312.

Hughes, W. L. 1966. Tracing the transport of proteins with Cr^{51}. Abstract in "Research and Development in Progress - Biol. and Medicine." Issued Apr. 1966 by the AEC Biol. and Medicine Div. page 150, DIC 2451.

Imabayashi, K., M. Kametaka, and T. Hatano. 1956. Studies on digestion in the domestic fowl. Tokyo J. Agr. Res. 2:99. In Sturkie, P. D. 1965. Avian Physiology Cornell University Press. 766 pp.

Irwin, M. I., and E. W. Crampton. 1951. The use of chromic oxide as an index material in digestion trials with human subjects. J. Nutr. 43:77-85.

Jensen, L. S., L. H. Merrill, C. V. Reddy, and J. McGinnis. 1962. Observations on eating patterns and rate of food passage of birds fed pelleted and unpelleted diets. Poult. Sci. 41(5):1414-1419.

Johnson, D. E., W. E. Dinusson, and D. W. Biolin. 1964. Rate of passage of chromic oxide and composition of digesta along the alimentary tract of wethers. J. Anim. Sci. 23:499-505.

Kane, E. A., W. C. Jacobson, and L. A. Moore. 1950. A comparison of techniques used in digestibility studies with dairy cattle. J. Nutr. 41:583-596.

Kendeigh, S. C. 1949. Effect of temperature and season on the energy resources of the English sparrow. Auk 66:113-127.

Kibler, H. H. and S. Brody. 1944. Metabolic changes in growing chickens. J. Nutr. 28:27-34.

Kleiber, M. 1961. The fire of life. An introduction to animal energetics. John Wiley & Sons, Inc., New York, London. 454 pp.

Krenla, M. S. 1947. Absorption of carotene from carrots in man and the use of the quantitative chromic oxide indicator method in the absorption experiments. Biochem. J. 41:269-273.

LeFebvre, E. A. 1964. The use of D_2O^{18} for measuring energy metabolism in Columba livia at rest and in flight. Auk 81(3):403-416.

Leopold, A. S. 1953. Intestinal morphology of gallinaceous birds in relation to food habits. J. Wildl. Mgt. 17: 197-203.

Levy, L. M., L. M. Bernstein, E. Francis, R. S. Harding, H. Krzywicki, V. E. McGary, J. Nuss, J. H. Sellars, and E. Devor. 1959. The metabolizable energy and apparent digestibility of food sterilized by ionizing radiation. (Abstr.) p. 420. In The effects of radiation and radioisotopes on the life process. A.E.C. publication TID-3098 compiled by C. M. Pierce. 1963.

Malone, C. R. 1965. Dispersal of plankton: rate of food passage in mallard ducks. J. Wildl. Mgt. 29(3):529-533.

Manoukas, A. G., N. F. Colovos, and H. A. Davis. 1964. Losses of energy and nitrogen in drying excreta of hens. Poult. Sci. 43(3):547-549.

Marshall, A. J. 1961. Biology and comparative physiology of birds. Vol. II. Academic Press, New York and London. 468 pp.

Mautz, W. W. and G. A. Petrides. 1967. Determination of the usefulness of chromium-51 in digestive studies of the white-tailed deer. Proceedings of the Thirty-second North American Wildlife Conference. In press.

McFarland, L. Z. and R. A. Freedland. 1965. Time required for ingesta to pass through the alimentary tract of coturnix. Amer. Zool. 5(2):242. Abstract #197.

McIntosh, J. I., S. J. Slinger, I. R. Sibbald, and G. C. Aston. 1962. Factors affecting metabolizable energy content of poultry feeds. Poult. Sci. 41:445-455.

Moore, J. H. 1957. Diurnal variation in the composition of the feces of pigs on diets containing chromium oxide. Brit. J. Nutr. 11:273-288.

Mueller, W. J. 1956. Feasibility of the chromic oxide and the lignin indicator methods for metabolism experiments with chickens. J. Nutr. 58:29-36.

_____, R. V. Boucher, and E. W. Callenback. 1956. Influence of age and sex on the utilization of proximate nutrients and energy by chickens. J. Nutr. 58:37-50.

Odum, E. P. 1961. Excretion rate of radio-isotopes as indices of metabolic rates in nature; biological half-life of zinc-65 in relation to temperature, food consumption, growth and reproduction in arthropods. (Abstr.) Biol. Bull. 121(2):371-372.

Olsson, N. and G. Kihlén. 1948. Edins indicator method in digestibility experiments on poultry. Proc. 8th World's Poultry Congress, p. 225.

Petrides, G. A. 1964. Manuscript notes. Inst. Radiation Ecology, Savannah River Atomic Energy Plant, Aiken, So. Carolina.

Polyakov, I. I. 1959. Digestion and the passage of food in chickens. Izvest. Timiryazeusk. Sel'skokhoz. A Kad. 2: 188-200. 1957.

Reddy, C. V., L. S. Jensen, L. A. Merrill, and J. McGinnis. 1962. Influence of mechanical alteration of dietary density on energy available for chick growth. J. Nutr. 77:428-432.

Reid, J. T., P. G. Woolfolk, W. A. Hardison, C. M. Martin, A. L. Brundage, and R. W. Kaufman. 1952. A procedure for measuring the digestibility of pasture forage under grazing conditions. J. Nutr. 46:255-269.

Roche, M., M. E. Perez-Yimenez, M. Layrisse, and E. DiPrisco. 1957. Study of urinary and fecal excretion of radioactive chromium Cr^{51} in man, its use in the measurement of intestinal blood loss associated with hookworm infection. J. Clin. Invest. 36:1183.

Romoser, G. L., W. A. Dudley, L. J. Machlin, and L. Loveless. 1961. Toxicity of vanadium and chromium for the growing chick. Poult. Sci. 40(5):1171-1173.

Röseler, M. 1929. Die Bedentung der Blinddärme des Haushuhnes für die Resorption der Nahrung und Verdauung der Rohfaser. Z. f. Tierz. u. Zücht. 13:281. In Sturkie, P. D. 1965. Avian Physio. Cornell University Press. 766 pp.

Schurch, A. F., L. E. Lloyd, and E. W. Crampton. 1950. The use of chromic oxide as an index for determining the digestibility of a diet. J. Nutr. 41:629-636.

_____, E. W. Crampton, S. R. Haskell, and L. E. Lloyd. 1952. The use of chromic oxide in digestibility studies with pigs fed ad libitum in the barn. J. Anim. Sci. 11:261-265.

Seibert, H. C. 1949. Difference between migrant and non-migrant birds in food and water intake at various temperatures and photoperiods. Auk 66:128-153.

Sibbald, I. R., J. D. Summers and S. J. Slinger. 1960. Factors affecting the metabolizabile energy content of poultry feeds. Poult. Sci. 39:544-556.

Smith, A. M. and J. T. Reid. 1955. Use of chromic oxide as an indicator of fecal output for the purpose of determining the intake of pasture herbage by grazing cows. J. Dairy Sci. 38(5):515-524.

Stacy, B. D., G. D. Thorburn. 1966. Chromium-51 ethylene-diaminetetra acetate for estimation of glomerular filtration rate. Sci. 152:1076-1077.

Sturkie, P. D. 1965. Avian physiology. Cornell University Press. 766 pp.

Suomalainen, H. and E. Arhimo. 1945. On the microbial decomposition of cellulose by wild gallinaceous birds (family tetraonidae). Ornis Fennica. 22:21-23.

Swift, R. W. and C. E. French. 1954. Energy metabolism and nutrition. Washington, D.C. Scarecrow Press. 264 pp.

Thornton, P. A., P. J. Schaible, and L. F. Wolterink. 1956. Intestinal transit and skeletal retention of radioactive strontium in the chick. Poult. Sci. 35:1055.

Troelsen, J. E. 1963. Chromic oxide paper pellets for administration to ruminants. Canadian J. of Ani. Sci. 43(2):389.

Tuckey, R., B. E. March, and J. Biely. 1958. Diet and the rate of food passage in the growing chick. Poult. Sci. 37:786-792.

Visek, W. J., I. B. Whitney, U. S. G. Kuhn III, and C. L. Comar. 1953. Metabolism of Cr^{51} by animals as influenced by chemical state. Proc. Soc. Exptl. Biol. Med. 84:610-615.

Warden, W. K. 1962. Game Birds. Fundamentals of selection, incubation, brooding, nutrition, and disease control. 15 pp. mimeo. M.S.U. Poult. Sci. Dept.

Zimmerman, J. L. 1965. Bioenergetics of the dickcissel. Physiological zool., 38(4):370-389.