THE DETERMINATION OF INTAKE AND DIGESTIBILITY OF HARVESTED AND GRAZED FORAGE BY THE USE OF INDICATORS

By

DAVID EDMOND HOPSON

Bachelor of Science

University of Arkansas

Fayetteville, Arkansas

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Thesis Approved:

Jack E. McCroskey
Thesis Adviser

Irvin T. Omtvedt

D. D. Dunham
Dean of the Graduate College

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TABLE OF CONTENTS

Chapter		Page
I.	INTRODUCTION	1
II.	REVIEW OF LITERATURE	3
	Polyethylene Glycol As an External Indicator	3
	Lignin As an Internal Indicator	6
	In Vitro Dry Matter Disappearance	10
III.	THE USE OF INDICATORS TO DETERMINE FECAL OUTPUT, DIGESTIBILITY AND FORAGE INTAKE	12
	Introduction	12
	Experimental Procedure	12
	Results and Discussion	15
	Summary and Conclusions	23
IV.	THE USE OF INDICATORS TO RELATE VOLUNTARY INTAKE TO CHEMICAL COMPOSITION AND DIGESTIBILITY OF GRAZED BERMUDAGRASS	27
	Introduction	27
	Experimental Procedure	27
	Results and Discussion	29
	Summary and Conclusions	34
LITERATURE CITED		37
APPENDIX		41

LIST OF TABLES

Table		Page
I.	Percent Recovery of Polyethylene Glycol and Lignin.	16
II.	Measures of Fecal Output.	17
III.	Analysis of Variance for Fecal Output Estimated by Polyethylene Glycol	19
IV.	Determination of Percent Dry Matter Digestibility	21
V.	Comparison of Indicators to Measure Dry Matter Intake . . .	22
VI.	Calculated Voluntary Intake of Midland Bermudagrass by Grazing Steers	30
VII.	Chemical Composition and *In Vitro* Dry Matter Disappearance of Hand Clipped and Esophageal Fistula Samples.	31
VIII.	Simple Correlations of Intake and *In Vitro* Dry Matter Disappearance With Chemical Composition of Forage	33

LIST OF FIGURES

Figure		Page
1.	Diurnal Excretion Curve for 50 Gm. PEG Given at 8:00 A.M.	42
2.	Diurnal Excretion Curve for 25 Gm. PEG Given at 8:00 A.M. and at 4:30 P.M.	43
3.	Diurnal Excretion Curve for 100 Gm. PEG Given at 8:00 A.M.	44
4.	Diurnal Excretion Curve for 50 Gm. PEG Given at 8:00 A.M. and at 4:30 P.M.	45
5.	Diurnal Excretion Curve for 150 Gm. PEG Given at 8:00 A.M.	46
6.	Diurnal Excretion Curve for 75 Gm. PEG Given at 8:00 A.M. and at 4:30 P.M.	47
7.	Diurnal Excretion Curve for 200 Gm. PEG Given at 8:00 A.M.	48
8.	Diurnal Excretion Curve for 100 Gm. PEG Given at 8:00 A.M. and at 4:30 P.M.	49
9.	Time Required for PEG to Reach Maximum Concentration in the Feces When Administered Once Daily.	50
10.	Time Required for PEG to Reach Maximum Concentration in the Feces When Administered Twice Daily in Split Doses.	51

CHAPTER I

INTRODUCTION

Voluntary intake and the extent to which the nutrients contained in the forage are digested are two factors which are very important in determining forage quality. The intake and digestibility of harvested forage can be determined directly by the so-called conventional method, but there is no satisfactory direct method for measuring digestibility and intake of grazed forage. However, by the use of indicators it is possible to calculate intake and digestibility of grazed forage indirectly. These methods employ the simultaneous use of an external indicator to estimate fecal output and an internal indicator to determine digestibility.

An external indicator must be an inert material which has no physiological effect on the digestive tract and is not absorbed from or destroyed in the digestive tract of the animal. It must pass through the digestive system at a uniform rate and be easily determined in feed or fecal samples. Some external indicators that have been used in other work include iron oxide, chromic oxide, polyethylene powder and a water soluble compound, polyethylene glycol.

An internal indicator is a naturally occurring plant constituent that can be recovered at a known rate in the feces. Some plant components that have been used as internal indicators include lignin,

cellulose and chromogens (plant pigments). The use of the indicator method requires careful attention to the sampling techniques used.

This thesis reports the results of two studies in which polyethylene glycol (PEG) was used as an external indicator for estimating fecal output, and the lignin-ratio method was employed to measure digestibility. In Experiment I the accuracy of the indicators was determined by comparison of calculated fecal output, digestibility, and intake with measured values determined in a conventional digestion trial. PEG was administered at four levels in either single or split doses. The lignin-ratio and *in vitro* dry matter disappearances methods were compared as measures of digestibility. In Experiment II PEG and the lignin-ratio were used as indicators to calculate voluntary intake of grazed bermudagrass by steers. Voluntary intake was then related to seasonal changes in chemical composition and *in vitro* digestibility of the forage.

CHAPTER II

REVIEW OF LITERATURE

The need for a reliable external indicator has long existed. For many years, research workers have searched for an inert substance which would pass through the digestive tract in such a manner that fecal output and, subsequently, digestion coefficients could be determined indirectly by indicator ratio techniques. Recent interest in the measurement of forage digestibility with the grazing animal has increased the significance of indicators in nutrition work.

Polyethylene Glycol As an External Indicator

Sperber et al (1953) suggested that polyethylene glycol (PEG) of high molecular weight (4,000) could be used as an external indicator. It is a highly polymerized long chain compound of the general formula $HO \cdot CH_2 \cdot (CH_2 \cdot O \cdot CH_2)_n \cdot CH_2 \cdot OH$. Polyethylene glycols with molecular weights above 1,000 are solids, but are water soluble. Hydén (1961) stated that the polyethylene glycols with mean molecular weights of approximately 4,000 are not absorbed from the gut, but still distribute themselves satisfactorily in the digesta. Sperber et al (1953) studied the use of polyethylene glycol as a reference substance in ruminants and found that there was no significant absorption, precipitation, or uptake of PEG-4000 by rumen contents. He also found there was no apparent effect upon the bacteria or protozoa in the rumen when

concentrations of 0.1 to 1.0 gm. per 100 ml. of media were present. Corbett (1956) and Hydén (1956) reported that no PEG could be detected in the urine of cattle after dosing with the indicator.

Unlike other indicators, PEG follows the fluid portion of the digestive tract. Corbett (1959) found that PEG, because of its association with the water in the digesta, was cleared from the reticulorumen much more rapidly than indicators such as Cr_2O_3 that are not water soluble. This gives rise to wider variations in the concentration of PEG in the dry matter of feces.

Workers who have reported variable excretion curves with PEG have used the indicator in small doses. Corbett (1956) used 25 gram doses of PEG on dairy cattle and reported low recoveries (40-70%). He also found that losses of PEG were greater on grass than on winter ration feces. Christie and Lassiter (1958) compared PEG of different molecular weights and reported that PEG 4,000 gave higher recoveries than did PEG 6,000, PEG 9,000, or PEG 20,000. These workers were using a dose level of only 15 gms. per day.

The excretion patterns for PEG have been quite variable as reported by most workers. Corbett et al (1958) reported more variability in excretion of PEG than for chromic oxide in their work with dairy cattle when it was given as 25 gm. doses in grass meal cubes. Christie and Lassiter (1958) observed erratic excretion patterns for PEG and stated that no constant day-to-day excretion of PEG was ever reached. They obtained a repeatable 24-hour excretion pattern with maximum concentration at 11:00 p.m. and minimum at 4:00 p.m. in studies with dairy cows.

The low recoveries of PEG have been studied by several workers. Hydén (1956) stated that PEG was destroyed to a limited extent in the digestive tract. Christie and Lassiter (1958) studied *in vitro* recovery by adding a known amount of PEG to dry and wet fecal samples. They stated that PEG was recovered quantitatively from dry feces, but that recovery from wet feces was incomplete and similar to the low recoveries after passage through the digestive tract. They also added dry feces to an aqueous solution containing PEG and concluded that the loss of PEG from the solution to which dried feces was added was a function of the weight of the feces added. From this they concluded that the probable loss of PEG in the digestive tract was through some sort of adsorption onto the fecal particles. Sinka *et al* (1970) also suggested that low recovery was due to adsorption of PEG on fecal material. Corbett *et al* (1958) suggested that low recoveries may be due to procedural errors in the analyses of feces. He stated that the loss of PEG may be caused by precipitation along with the inorganic sulfates and protein.

The chemical determination which is most commonly used for PEG is the turbidmetric procedure reported by Hydén (1956). The procedure consistently yields *in vitro* recoveries of near 100 percent when PEG is added to dry feces, therefore, the procedure is assumed to be accurate.

The relatively poor performance of the indicator in early work was probably due, in part, to the low levels of PEG administered. According to Sperber *et al* (1953), Corbett *et al* (1956), and Christie and Lassiter (1958), much more accurate results and more complete recoveries of the indicator are obtained when 100 to 500 gm. of PEG are administered daily. This indicates that further study is needed with

administration of larger doses of PEG to test its efficacy as an indicator.

Lignin As an Internal Indicator

Lignin, a naturally occurring constituent of plants, has been used widely as an internal indicator for determining digestibility of harvested and grazed forages. Although the exact structure is unknown, the nucleus is a polyhydroxy aromatic arrangement. Some properties of lignin isolated from young plants have been described by Bondi and Myer (1948). These workers used 0.5N NaOH to extract lignins from barley, clover, peanut hay, and vetch. They described the lignin as a fine brown powder that is soluble in dilute NaOH, ethanol, and acetone. The repeating aromatic structure of lignin contained carbon, hydrogen, oxygen, and nitrogen. They stated that plant lignins contain nitrogen, whereas wood lignins do not. It has been well established that lignin appears in the plant as a functional component of the cell wall.

The value of lignin as an indicator is not well defined because of conflicting reports as to digestibility by ruminants. Using methods which they developed, Crampton and Maynard (1938) reported recoveries of ingested lignin to be 97.8 percent with clipped grass fed to rabbits and 99.3 percent with an alfalfa hay-grain ration fed to a steer. Ellis *et al* (1946) developed a procedure for the determination of lignin and obtained a lignin recovery ranging from 94 to 106 percent with cows, sheep, and rabbits fed various rations. Daily variations in lignin concentration of the feces were small; therefore, it was suggested that three to four day fecal collections should be sufficient. On the basis of these results, they suggested that lignin be used as an

indicator of both digestibility and consumption of pasture herbage. This is the first report in the literature of the possibility of using an indicator to determine consumption. Forbes and Garrigus (1948), using steers and wethers, stated that lignin was neither digested nor absorbed by the animal. They had an average recovery of 102 percent in seven trials with steers and 105 percent in one trial with sheep. They stated that dry matter digestibility of various forages varied inversely with the lignin content. Kane et al (1950) fed alfalfa to crossbred dairy cattle and obtained complete recovery of lignin. These researchers stated that there was no significant difference between digestion coefficients obtained by the use of the lignin-chromic oxide ratio and the total collection procedure. Swift et al (1947) also reported no significant differences in the digestion coefficients obtained by the conventional method and the lignin ratio technique with sheep receiving a roughage ration. Pigden and Stone (1952) reported that lignin was relatively unchanged by passage through the animal in the case of dicotyledonous forages. Cook and Harris (1951) compared the lignin ratio technique and the chromogen method of determining digestibility and forage consumption. These workers found close agreement with the lignin ratio method and calculated dry matter intake commonly accepted for sheep on range pasture.

Various workers have stated that lignin is unacceptable as an indicator because of some type of digestion or degradation in the digestive tract of ruminants. Csonka et al (1930) observed that lignin prepared from corn cobs by the alkali method underwent a loss of methoxyl groups in passing through the animal body. After feeding the isolated lignin to dogs and a cow, they concluded that a 36.7 percent

loss of methoxyl groups occurred when the cow was fed isolated lignin with a mixed ration. It was believed that the degradation of lignin took place in the digestive tract, and was not brought about by bacteria but rather by some other agent, possibly an enzyme present in the gastric juice of the animal. Bondi and Myer (1943) found that lignin digestibility in different forages ranged from 35 to 64 percent. They observed that the methoxyl values were lower for feces lignin than plant lignin, and suggested that lignin was changed during passage through the animal.

In later work, Bondi and Myer (1948) also reported that changes occur in side chains of the aromatic rings of lignin. Kane et al (1953) fed orchard grass hay to dairy cows and obtained low recoveries of lignin which gave lower digestion coefficients than the ones calculated by the total collection method. Ely et al (1953) reported digestion coefficients for lignin to be from 3.8 to 16 percent when orchard grass hay was fed to dairy cattle. Sullivan (1955) fed 36 grasses and 2 legumes to sheep and indicated that lignin was changed during passage through the digestive tract. He obtained digestion coefficients of up to 12.4 percent and concluded that the partial and variable digestibility of lignin renders it unsuitable as a reference substance for determining the digestibility of other constituents. Smith et al (1956) stated that the digestion coefficients of lignin in alfalfa fed to deer ranged from 9.3 to 42.1 percent. Hill et al (1961) also found lignin to be unacceptable as an indicator in his work with heifers.

Several workers have shown that differences in plant species can affect lignin recovery. Davis et al (1947) observed lignin digestibility of 16.2 percent for pea vines and 10.6 percent for lima beans.

Kane et al (1951) found that lignin was not as reliable an indicator in orchard grass as it was in alfalfa fed to dairy cattle. Pigden and Stone (1952) found a difference in degradation of lignin between dicotyledonous forages and monocotyledonous forages.

These differences in digestion coefficients of lignin appear to be mainly due to plant species, stage of plant maturity, and accuracy of lignin determination. Kamstra et al (1958) studied the effect of stage of maturity and lignification on the digestion of cellulose in forage plants. These workers concluded that the increase in lignin content as a plant matures is the primary reason for poor feeding value of mature forages. The results that supported this conclusion were: (1) prefeeding chemical treatment of roughage designed to remove the lignin was shown to increase the digestibility of roughage by ruminants, and (2) microscopic examination of cellulose fibers in the process of being digested, suggests that lignification is a physical barrier to cellulose digestion by cellulolytic rumen microorganisms. Thus it would appear that lignin might influence digestibility by acting as an "encrusting substance" and thus preventing the digestive enzymes of the rumen micro-organisms and of the intestinal juices from acting on the plant nutrients. They obtained negative relationships between lignin content and the in vitro cellulose digestion in the whole plant. Phillips et al (1953) also found that lignin increases with plant maturity.

The method of Ellis et al (1951) has been used for determination of lignin in which a standard 72 percent sulfuric acid was the isolating agent. A recent procedure (Van Soest, 1963) has been used with success in accurately determining forage and fecal lignin content. In

using lignin as an indicator of digestibility, a worker should have some knowledge of recovery rate in the type of forage being studied and seriously evaluate the reliability of lignin in estimating the values required.

In Vitro Dry Matter Disappearance

The study of forage nutritive value using in vitro rumen fermentation techniques began somewhat simultaneously in several laboratories. Kamstra et al (1958) related fairly long term in vitro cellulose digestibility to stage of maturity and lignification of forages. The results of their work showed that separating cellulose from lignin greatly improved its digestibility in vitro. Quieke et al (1959) used an in vitro rumen fermentation technique to study the digestibility of cellulose in seven grass hays and six legume hays. These workers compared the results of the in vitro method with the digestibility of the hay determined by conventional digestion trials with sheep. They stated that there was no significant difference between results obtained in vitro and in vivo with grass hays, but cellulose digestibility determined by these techniques was different in some of the legume hays.

The relationship of in vitro criteria measured to the in vivo criteria being estimated has been studied by several workers. In vitro dry matter disappearance has been found to be well correlated with in vivo digestible dry matter and digestible energy (Johnson and Dehority, 1968). These workers stated that dry matter disappearance was not acceptable as a criteria to predict forage intake. Another in vitro measurement commonly used is cellulose digestibility. Long

term *in vitro* cellulose digestibility values (30 to 48 hours) have been found to relate well to *in vivo* cellulose digestibility and dry matter digestibility but not to intake parameters. Donefer *et al* (1960) and Johnson *et al* (1962) found that *in vitro* cellulose digestibilities during shorter incubation periods proved to be highly related to both digestibility and intake.

The selection of an *in vitro* technique depends upon the parameters to be estimated. One procedure that has been widely adopted because of its ease of adaptation into a standard procedure is that of Tilley and Terry (1963). Since most *in vitro* rumen fermentations were an attempt to simulate only the digestive process in the rumen, these workers carried the simulation one step further and added a proteolytic digestion phase to simulate the digestion of protein in the lower gut. They originally reported regression equations on 146 forages with a standard error of 2.31 for the prediction of *in vivo* dry matter digestibility with *in vitro* digestion by the two stage procedure. The four major variables of the *in vitro* rumen fermentation studies are listed by Johnson (1969) as (1) variations in microbial populations, (2) variation due to storage, grinding and processing techniques, (3) differences in medium, and (4) procedural variation.

CHAPTER III

THE USE OF INDICATORS TO DETERMINE FECAL OUTPUT, DIGESTIBILITY AND FORAGE INTAKE

Introduction

This experiment was conducted to study the accuracy of polyethylene glycol (PEG) in estimating fecal output when administered to steers at four different levels either in single or split doses. A comparison was also made between the lignin ratio technique and *in vitro* dry matter disappearance method as estimators of dry matter digestibility.

Experimental Procedure

Sixteen Hereford steers, weighing approximately 225 kg. initially, were used in four metabolism trials with eight steers in each trial. The steers were placed in metal metabolism stalls and fed 1.8 kg. of chopped bermudagrass hay twice daily. A two week adjustment period to the metabolism stalls and rations was used in each trial. Polyethylene glycol administration was started five days prior to the collection period. It was administered as a drench to the eight steers on each trial in a 4x2 factorial arrangement of treatments. Four levels of PEG were administered either once daily in a single dose, or twice daily in split doses. The indicator was prepared at the time of

administration by dissolving the PEG crystals in 225 ml. of water. All PEG levels were dissolved in the same volume of water. Steers receiving the indicator once daily at the morning feeding were drenched with a similar quantity of water at the evening feeding. The slurry of PEG (or water) was given with a six ounce dose syringe at the morning and evening feeding times. Treatments were as follows: (1) 50 gm. given at 8:00 a.m., (2) 25 gm. given at 8:00 a.m. and at 4:30 p.m., (3) 100 gm. given at 8:00 a.m., (4) 50 gm. given at 8:00 a.m. and at 4:30 p.m., (5) 150 gm. given at 8:00 a.m., (6) 75 gm. given at 8:00 a.m. and at 4:30 p.m., (7) 200 gm. given at 8:00 a.m. and (8) 100 gm. given at 8:00 a.m. and at 4:30 p.m.

A seven day total collection of feces was made and fecal "grab" samples were taken at 8:00 a.m. and 4:30 p.m. each day during the collection period. All grab samples were immediately frozen for future analyses. On the last day of each trial, fecal "grab" samples were taken every two hours to establish a daily excretion pattern for the indicator.

During the third metabolism trial fecal "grab" samples were taken during the entire 12 day digestion trial beginning on the day that PEG administration started. These samples were analyzed for PEG concentration to give an indication of the amount of time required for PEG to reach maximum concentration in the feces.

Laboratory analyses on the hay samples included <u>in vitro</u> dry matter disappearance and acid detergent lignin. Fecal samples were analyzed for acid detergent lignin and polyethylene glycol. Lignin was determined by the 72 percent acid detergent lignin procedure of Van Soest (1963). Polyethylene glycol was determined by the turbidimetric

procedure of Hyden (1956). *In vitro* dry matter disappearance was determined by the two stage method of Tilley and Terry (1963). In the preparation for analyses, all samples were dried and ground through a 1 mm. screen in a Wiley mill.

Calculation of fecal output was by the formula described by Sperber *et al* (1953), which is as follows:

$$\text{Fecal Output (gm. D.M./day)} = \frac{\text{Indicator consumed (gm./day)}}{\text{Indicator in feces (gm./gm. D.M.)}}$$

Estimation of dry matter digestibility was made by two methods: (1) *in vitro* dry matter disappearance and (2) the lignin-ratio technique. The following formula explains the use of lignin for estimating digestibility of forage dry matter:

$$\text{D.M. Digestibility (\%)} = 100 - 100 \frac{\text{\% lignin in forage}}{\text{\% lignin in feces}}$$

Forage intake was calculated using PEG to estimate fecal output and either *in vitro* dry matter disappearance or lignin-ratio to determine digestibility. The formula used to calculate forage intake using lignin to measure digestibility is as follows:

$$\text{Forage Intake (gm. D.M./day)} = \frac{\text{\% lignin/gm. D.M. in feces}}{\text{\% lignin/gm. D.M. in forage}} \times \text{Fecal Output}$$

When *in vitro* dry matter disappearance was the measure of digestibility the following formula was employed:

$$\text{Forage Intake (gm. D.M./day)} = \frac{\text{Fecal Output (gm. D.M./day)}}{100 - \text{\% digestible D.M.}}$$

Calculated fecal output values determined using PEG as an indicator were compared to the values obtained by total fecal collection. The values for estimated dry matter digestibility determined by the *in vitro* technique or lignin-ratio method were also compared to values determined in the conventional digestion trial. Therefore, forage intake was calculated by two indirect methods. Calculated intake values were compared with amounts of forage actually consumed to determine the accuracy of the two combinations of indicators.

Results and Discussion

The percent recovery of polyethylene glycol is shown in Table I. Recovery ranged from 85.8 percent for 200 gm. given twice daily to 100.8 percent for 150 gm. given as a split dose. With the exception of the 200 gm. level, recoveries were higher for all levels given in split doses compared to once-a-day administration. The overall mean recovery of PEG was 92.7 percent. This value is higher than the recovery values reported by other workers (Corbett, 1956; Christie and Lassiter, 1958). These workers suggested that higher dose levels would improve the recovery of PEG from the feces; however, two of the lowest recoveries in this study were at the 200 gm. level (87.2% and 85.8% in single and split doses, respectively).

The most accurate mean estimate of fecal output was obtained with 150 gm. PEG given in split doses, which reflects the accuracy in percent recovery of the indicator. When all treatments were pooled, fecal output as calculated by the PEG method was significantly higher ($P < .05$) than the fecal output determined by the total collection method (Table II). The overestimation of fecal output was due to

TABLE I

PERCENT RECOVERY OF POLYETHYLENE GLYCOL AND LIGNIN

Treatment	PEG[1]	Lignin[1]
50 gm.[2]	86.4	91.1
50 gm.[3]	96.2	89.8
100 gm.[2]	94.8	99.3
100 gm.[3]	95.5	97.8
150 gm.[2]	94.9	98.4
150 gm.[3]	100.8	97.9
200 gm.[2]	87.2	93.6
200 gm.[3]	85.8	95.5
MEAN	92.7	95.4
$s_{\bar{x}}$[4]	1.9	1.3

[1]Mean of four steers per treatment

[2]Given as single dose

[3]Given as split dose

[4]Standard error of mean

TABLE II

MEASURES OF FECAL OUTPUT

Treatment	Fecal Output Gm. DM/Day		
	Actual[1]	Estimated by PEG[1]	Difference
1	1619	1885	266
2	1527	1588	61
3	1598	1689	91
4	1616	1685	69
5	1623	1719	96
6	1694	1710	16
7	1622	1858	236
8	1609	1869	260
MEAN[2]	1614[a]	1750[b]	
$s_{\bar{x}}$	16.1	44.3	

[1]Mean of four steers per treatment

[2]Means with different letters differ significantly ($P < .05$)

incomplete recovery of PEG.

PEG concentrations in fecal samples taken at 8:00 a.m. and 4:30 p.m. were adjusted to the daily mean excretion determined from samples taken at two-hour intervals during a 24-hour period. The diurnal excretion patterns of the eight PEG treatments are shown in Appendix, Figures 1 through 8. The patterns of excretion were similar for all treatments with a low concentration at 4:00 p.m. and the highest concentration occurring between midnight and 6:00 a.m. This excretion pattern is very similar to the one obtained by Christie and Lassiter (1958) and Sandiford (1968). From the excretion patterns obtained using this feeding schedule, it appears that the best times to get a sample representing the mean daily excretion would be at 8:00 a.m. and 9:00 p.m.

In the third metabolism trial grab samples taken at 8:00 a.m. and 4:30 p.m. from the start of PEG administration were used to determine the time required for the PEG to reach maximum concentration in the feces. On all eight treatments the PEG reached maximum concentration in the feces between 72 and 96 hours. This is shown graphically in Appendix Figures 9 and 10. The daily excretion pattern failed to reach a constant level over the ten-day period, which demonstrates the erratic excretion of the indicator on a daily basis.

An analysis of variance of the values for estimated fecal output determined by the eight levels of PEG is shown in Table III. No significant differences in fecal output were observed between the levels of PEG given or the methods of administration. There was a significant difference between animals within a treatment ($P < .05$). This may be the reason for the lack of a significant difference between levels or

methods of administration of PEG.

TABLE III

ANALYSIS OF VARIANCE FOR FECAL OUTPUT ESTIMATED BY POLYETHYLENE GLYCOL

Sources of Variance	df	SS	MS	F
Total	31	1185576	38244	
Trials (animals w/in trts.)[1]	3	244271	81424	3.41*
Methods of administration	1	97461	97461	4.08
Levels of indicator	3	217806	72602	3.04
Levels x Methods	3	125120	41707	1.70
Error	21	500918	23853	

*$P < .05$

[1]Treatments replicated in each trial

Although the differences were not statistically significant between levels or methods of administration of PEG, the data indicate that a closer estimate was obtained by the twice-a-day administration. The most favorable results were obtained from the 150 grams per day given as a split dose. Relatively close estimates were also obtained from 50 or 100 grams per day given in split doses. This is shown from the estimates of fecal output (Table II) and percent recovery of PEG (Table I) on these two treatments.

The use of the lignin-ratio method resulted in a significantly ($P < .05$) lower estimated digestibility when compared to the digestibility determined by total fecal collection (Table IV). This was due to low recovery of lignin from the feces. The reason for the low recovery is not known, but many workers have found that lignin is digested to some extent by the ruminant. The percent recovery of lignin ranged from 89.8 to 99.3, with a mean of 95.4 (Table I). An analysis of variance of the digestion coefficients calculated by the lignin ratio method indicates that the PEG treatments had no effect on the recovery of lignin. The wide range in recovery values and the low mean recovery was due to the variation among animals in this experiment.

Dry matter digestibility determined by the in vitro dry matter disappearance technique (Tilley and Terry, 1963) did not differ statistically ($P < .05$) from dry matter digestibility determined by total fecal collection (Table IV). The standard error of the mean indicates that there was more variation using the in vitro technique, but the mean value more accurately estimates digestibility than the mean value determined by the lignin ratio technique.

Calculated dry matter intake values using indicators are compared with actual measured dry matter intake in Table V. These intake values were calculated by using the fecal output values determined by PEG and dry matter digestibility determined either by the lignin-ratio method or by in vitro dry matter disappearance. Mean intake values calculated using the PEG-lignin or PEG-in vitro methods did not differ significantly ($P < .05$) from the mean measured intake. The fact that there were no significant differences between actual dry matter intake and intakes calculated by the two indicator combinations does not

TABLE IV

DETERMINATION OF PERCENT DRY MATTER DIGESTIBILITY

	Method of Digestibility Determination		
Treatment	Conventional[1]	Lignin Ratio[1]	In Vitro[2]
1	51.42	46.39	46.11
2	53.80	48.06	53.07
3	51.87	50.96	47.32
4	51.15	50.03	48.18
5	51.25	50.49	55.16
6	49.26	48.00	50.19
7	50.13	47.74	51.70
8	50.51	49.16	56.07
MEAN[3]	51.17[a]	48.85[b]	50.98[a]
$\bar{sx}$	0.48	0.56	1.29

[1]Mean of four steers per treatment

[2]Mean of duplicate determinations

[3]Means with different letters differ significantly ($P < .05$)

TABLE V

COMPARISON OF INDICATORS TO MEASURE DRY MATTER INTAKE

	Dry Matter Intake (Gm. D.M./Day)				
Treatment	Actual[1]	PEG[2] Lignin	% of Actual Intake	PEG[2] In Vitro	% of Actual Intake
1	3332	3522	105	3498	105
2	3305	3067	93	3384	102
3	3287	3441	105	3206	98
4	3308	3374	102	3252	99
5	3330	3473	104	3833	115
6	3338	3179	95	3433	102
7	3321	3557	107	3847	116
8	3313	3677	111	4254	128
MEAN[3]	3317[a]	3392[a]	102	3553[a]	108
$s_{\bar{x}}$	6.0	81.7		137.8	

[1]All treatment groups were fed equal amounts of air-dry feed

[2]Mean of four calculated values

[3]Means with different letters differ significantly ($P < .05$)

necessarily denote accuracy of the indicators. The calculated intake values are the result of a combination of two offsetting errors. Average fecal output as calculated by the PEG method was 7.5 percent higher than actual measured fecal output. Dry matter digestibilities determined by the lignin-ratio and *in vitro* methods were 4.5 and 3.7 percent lower, respectively, than digestibility determined by total fecal collection. Therefore, when the high calculated fecal output was combined with a low estimate of digestibility the result was a relatively close estimate of dry matter intake. These results agree with work by Sandiford (1968).

Although the combinations of PEG-lignin or PEG-*in vitro* gave relatively close estimates of dry matter intake in this study, this procedure may not be repeatable, especially if some other type of ration is used.

Summary and Conclusions

Experiment 1 was conducted to study the accuracy of polyethylene glycol in estimating fecal output when administered to steers at four different levels either in single or split doses, and to compare the lignin-ratio technique and *in vitro* dry matter disappearance method as estimators of dry matter digestibility. Comparisons were then made between dry matter intake calculated using PEG-lignin ratio method or PEG-*in vitro* method and actual measured dry matter intake.

Sixteen Hereford steers weighing approximately 225 kg. initially were used in four metabolism trials with one steer per treatment in each trial. The steers were fed 1.8 kg. of chopped bermudagrass hay twice daily in a conventional digestion trial in which a seven-day total

collection of feces followed a two-week adjustment period. Polyethylene glycol was administered as a drench to the eight steers in each trial in a 4x2 factorial arrangement of treatments. Four levels of PEG (50 gm., 100 gm., 150 gm., or 200 gm.) were administered either once daily in a single dose, or twice daily in split doses. The PEG was started five days prior to the total fecal collection period. Fecal "grab" samples were taken at 8:00 a.m. and 4:30 p.m. each day during the collection period and were analyzed for acid detergent lignin and PEG. On the last day of each trial, fecal "grab" samples were taken every two hours for a twenty-four hour period to establish a daily excretion pattern for the indicator.

The percent recovery of PEG varied from 85.8 for 200 gm. in split doses to 100.8 for 150 gm. given in split doses. Average recovery of all eight treatments was 92.7 percent which resulted in a significantly higher ($P < .05$) mean value for calculated fecal output when compared to measured fecal output. With the exception of the 200 gm. level, percent recovery was higher at each level when administered twice daily compared to single dosing. The 50, 100, and 150 gm. levels administered in split doses all gave satisfactory recoveries (96.2, 95.5, and 100.8%, respectively). Recovery of PEG at the 200 gm. level was low for both methods of administration (87.2% and 85.8% for single and split doses, respectively). Results of this study indicate that 150 gm. of PEG given in equal split doses in the morning and evening will give the most accurate estimates of fecal output. It was also found in this study that PEG reaches maximum concentration in fecal samples between 72 and 96 hours after initial dosing.

Low recoveries of lignin in the fecal samples resulted in a significantly ($P < .05$) lower estimated digestibility when compared to the digestibility determined by total fecal collection. Dry matter digestibility determined by the *in vitro* dry matter disappearance technique did not differ statistically ($P < .05$) from dry matter digestibility determined by total fecal collection. Hence, the *in vitro* dry matter disappearance appears to be a better estimator of *in vivo* dry matter digestibility than the lignin-ratio technique.

When actual dry matter intake values were compared to dry matter intake as calculated by the PEG-lignin method or PEG-*in vitro* method, no significant differences were found. This was the result of two offsetting errors; that is, a high calculated fecal output and low calculated digestibility values.

The following conclusions concerning the use of indicators to predict fecal output, digestibility, and dry matter intake may be made from the results of this study:

(1) Percent recovery of PEG administered to steers fed bermudagrass hay is higher for levels ranging from 50 to 150 gm. daily when administered in split doses at feeding times. Administration of these levels as a single dose resulted in lower recoveries.

(2) The *in vitro* dry matter disappearance technique (Tilley and Terry, 1963) is a more accurate estimator of *in vivo* digestibility of bermudagrass dry matter than the lignin-ratio technique due to low and variable recovery of lignin.

(3) The combination of PEG as an external indicator and lignin as an internal indicator to calculate dry matter intake gave a very close estimate of actual dry matter intake due to offsetting errors in

the estimation of fecal output and digestibility.

(4) Polyethylene glycol and *in vitro* dry matter disappearance were better estimators of dry matter intake when PEG was given at levels of 50, 100, or 150 gm. daily in two equal doses.

(5) The variability among animals within a treatment indicates that more animals should be used per treatment in future studies.

CHAPTER IV

THE USE OF INDICATORS TO RELATE VOLUNTARY INTAKE TO CHEMICAL COMPOSITION AND DIGESTIBILITY OF GRAZED BERMUDAGRASS

Introduction

This experiment was conducted to determine the voluntary intake of Midland Bermudagrass by grazing cattle, and to relate intake to seasonal changes in chemical composition and digestibility of the forage. Intake was determined by the use of PEG to estimate fecal output and the lignin-ratio technique to determine forage dry matter digestibility.

Experimental Procedure

Five pasture trials were conducted throughout the growing season from May through October. The dates of the five trials were as follows: (1) Trial 1 - May 14-31; (2) Trial 2 - June 19-28; (3) Trial 3 - July 18-27; (4) Trial 4 - August 18-28; (5) Trial 5 - October 1-10. Eight yearling Hereford steers were placed on a relatively pure stand of Midland Bermudagrass (Cynadon dactylon) in each of the five trials. After an adjustment period of one week, the animals were given PEG at a level of 200 grams per day in split doses of 100 gm. at 8:00 a.m. and at 4:30 p.m. The preparation of the PEG administered was the same as described in Experiment I. Fecal grab samples were taken twice daily (8:00 a.m. and 4:30 p.m.) during the five-day collection period of each

trial. Fecal collection was started after the animals had been on the PEG treatments for a five-day period. Indicator administration continued throughout the fecal collection period.

Forage samples were hand clipped to within two inches of the ground on the first, third and fifth days of the collection period of each trial. Sandiford (1968) concluded that hand clipped or hand plucked samples would give a better estimate of actual change in chemical composition of the forage available throughout the season than would forage samples collected by a fistulated animal. Forage samples were also taken by an esophageal fistulated steer on the days hand clipped samples were taken. No attempt was made to clip forage similar to that collected by the fistulated steer.

Hand clipped and esophageal fistula forage samples were analyzed for acid detergent fiber (ADF) and acid detergent lignin (ADL) according to the procedure of Van Soest, 1963 and in vitro dry matter disappearance (IVDMD) according to the procedure of Tilley and Terry, 1963. Other analyses included crude protein (C. P.) according to A. O. A. C., 1960 and neutral detergent fiber (NDF) using the procedure of Van Soest, 1967. The fecal grab samples were analyzed for PEG (Hyden, 1956) and acid detergent lignin so that dry matter intake could be calculated.

Dry matter intake was calculated using PEG to estimate fecal output and the lignin-ratio technique and in vitro dry matter disappearance to estimate dry matter digestibility. Intake values were calculated using the percent lignin in the esophageal samples by the formula given in Experiment I.

After the dry matter intake was calculated, a pooled correlation was made between intake and the chemical composition of the forage. The *in vitro* dry matter digestibility was also correlated with intake and the chemical composition of the forage. The chemical composition of the hand clipped forage samples was considered to be representative of the forage available to the animals and the forage samples collected by the esophageal fistulated steer were considered to be representative of the forage actually consumed by the animals.

Results and Discussion

Daily dry matter intake was expressed as kg. per day on the basis of metabolic size ($W_{kg.}^{.75}$). The intake values obtained are presented in Table VI. The daily forage dry matter intake declined from May to July, then increased from July to October. When the dry matter intake was expressed on a metabolic size basis (Table VI) the pattern of intake was essentially the same. The intake pattern obtained in this experiment differed from the pattern obtained by other workers (Hardison and Reed, 1953; Sandiford, 1966) studying bermudagrass pasture. These workers obtained a peak intake in June and a decrease from July through October. The difference in intake pattern may partially be explained by the availability of forage. Although no data were collected on forage availability, it was noted that there was a large increase in available forage in the months of August and October due to an unusually large amount of rainfall during these months. The chemical composition and *in vitro* data on hand clipped samples indicate that the quality of the forage decreased, but the amount of forage increased during the last three months of the experiment providing sufficient

forage to permit more selective grazing during August and October.

TABLE VI

CALCULATED VOLUNTARY INTAKE OF MIDLAND BERMUDAGRASS BY GRAZING STEERS

Trial	Date	Intake[1]	
		Kg. D.M./Day	Gm. D.M./$W_{kg.}^{.75}$
1	May 14-31	6.1	88.3
2	June 19-28	6.0	92.1
3	July 18-27	4.3	57.8
4	Aug. 18-28	10.7	152.5
5	Oct. 1-10	13.9	176.0

[1]Each value based on mean of eight steers per treatment

The chemical analyses of the forage and the *in vitro* dry matter disappearance values are shown in Table VII for the hand clipped and esophageal samples. Percent crude protein and *in vitro* dry matter disappearance decreased while the percent lignin, acid detergent fiber and neutral detergent fiber increased in the hand clipped samples throughout the growing season. Chemical composition and *in vitro* dry matter disappearance values for esophageal samples were markedly different from hand clipped samples. Percent acid detergent lignin and acid detergent fiber tended to decrease throughout the growing season

TABLE VII

CHEMICAL COMPOSITION AND IN VITRO DRY MATTER DISAPPEARANCE OF HAND CLIPPED AND ESOPHAGEAL FISTULA SAMPLES[1]

	C.P.		ADL		ADF		NDF		IVDMD[2]	
Trial	H.C.[3]	Esoph.[4]	H.C.[3]	Esoph.[4]	H.C.[3]	Esoph.[4]	H.C.[3]	Esoph.[4]	H.C.[3]	Esoph.[4]
					Percent					
1	12.1	11.3	5.4	5.2	32.4	30.5	68.2	67.7	55.3	52.8
2	14.8	14.5	5.2	5.7	33.2	30.7	65.9	61.8	54.8	55.2
3	9.0	12.4	5.5	7.5	35.4	31.7	73.9	64.2	48.9	56.3
4	9.9	13.6	6.1	4.2	37.0	30.8	73.3	66.5	48.0	58.8
5	9.5	13.2	6.1	3.9	36.0	28.2	73.9	66.0	48.7	61.7

[1]All forage samples are composites of three samples taken on 1st, 3rd, and 5th days of each trial.

[2]Mean of triplicate determinations

[3]Hand clipped forage sample

[4]Esophageal fistula forage sample

while *in vitro* dry matter disappearance values tended to increase in the esophageal samples. The percent crude protein and neutral detergent fiber did not show a definite pattern. These data suggest that although the hand clipped samples may be representative of the quality of forage available to the animal, they were not representative of the forage actually consumed by the animals. It is quite apparent that the animals were able to select a more digestible forage that was lower in lignin and fiber and higher in protein than the hand clipped samples. These results are in agreement with other work (Hardison *et al*; 1954; Weir *et al*, 1959; Lesperance *et al*, 1960; Guthrie *et al*, 1968).

The relationships of voluntary intake and *in vitro* dry matter disappearance with chemical composition of the forage are shown in Table VIII. Since the hand clipped forage samples were apparently not representative of the actual forage consumed by the animals, correlations obtained between intake and chemical composition of esophageal forage should give a more accurate estimate of the relationship between these values. There was a significant ($P < .01$) negative correlation ($r = -.93$) between intake and percent lignin in esophageal forage samples when a pooled correlation was made from the five pasture trials. Although not statistically significant, there was also a negative relationship between intake and percent acid detergent fiber ($r = -.79$).

The correlation of intake with percent neutral detergent fiber, protein and *in vitro* dry matter disappearance was positive, with the latter approaching significance ($P < .05$).

The correlation of *in vitro* dry matter disappearance with chemical composition of the forage (Table VIII) indicates that there is a significant ($P < .05$) positive relationship ($r = +.88$) between percent

TABLE VIII

SIMPLE CORRELATIONS OF INTAKE AND *IN VITRO* DRY MATTER DISAPPEARANCE WITH CHEMICAL COMPOSITION OF FORAGE[1]

	C.P.		ADL		ADF		NDF		IVDMD	
	H.C.[2]	Esoph.[3]	H.C.[2]	Esoph.[3]	H.C.[2]	Esoph.[3]	H.C.[2]	Esoph.[3]	H.C.[2]	Esoph.[3]
Voluntary Intake	-.29	+.37	+.87*	-.93**	+.58	-.79	+.39	+.42	-.47	+.80
IVDMD	+.88*	+.36	-.79	-.60	-.97**	-.80	-.95**	+.28		

[1]Values from five pasture trials

[2]Hand clipped forage sample

[3]Esophageal fistula forage sample

*$P < .05$

**$P < .01$

protein and *in vitro* dry matter disappearance of the hand clipped forage samples. There was a significant ($P < .01$) negative correlation ($r = -.97$) between acid detergent fiber content and *in vitro* dry matter disappearance of hand clipped samples. The percent lignin had a high, but non-significant ($P < .05$), negative relationship ($r = -.79$) with *in vitro* dry matter disappearance on the hand clipped forage. The correlations between the chemical components and *in vitro* dry matter disappearance of esophageal forage samples were not statistically significant ($P < .05$), probably due to the selective grazing by the animals.

These data support work by Hardison *et al* (1954) and Guthrie *et al* (1968), who found that there was a significant negative correlation between percent lignin in forage and dry matter intake of steers. These workers also found a positive relationship between intake and percent protein, and a negative relationship for acid detergent fiber and lignin with both intake and digestibility of dry matter.

Summary and Conclusions

Five pasture trials were conducted throughout the growing season to determine the relationships between chemical composition changes and *in vitro* dry matter disappearance and voluntary intake of grazed bermudagrass forage. Eight yearling Hereford steers grazing a pure stand of Midland Bermudagrass pasture were used in each trial to estimate voluntary forage intake.

Dry matter intake was calculated using PEG to estimate fecal output and the lignin-ratio technique to estimate dry matter digestibility. One hundred grams of PEG was administered as a drench twice daily at

8:00 a.m. and 4:30 p.m. Forage samples were hand clipped on the first, third and fifth days of the collection period of each trial to get a measure of the quality of forage available. Forage samples were also taken by an esophageal fistulated steer on the days hand clipped samples were taken to estimate the quality of forage being consumed.

The daily dry matter intake declined from May to July and increased from July to October when expressed in absolute amounts or in grams per unit of metabolic size.

The chemical composition of the hand clipped and esophageal forage samples were quite different, especially during the latter part of the growing season. The data suggest that hand clipped forage samples were not representative of the forage actually consumed by the animals. Apparently the animals were able to select a more digestible forage that was lower in lignin and fiber and higher in protein than the hand clipped samples.

A pooled correlation for the five pasture trials showed a significant ($P < .01$) negative correlation ($r = -.93$) between intake and percent lignin in esophageal forage samples. There was also a negative relationship between intake and acid detergent fiber of esophageal samples. The correlation of intake with percent neutral detergent fiber, crude protein and *in vitro* dry matter disappearance of esophageal samples was positive, with the latter approaching significance ($P < .05$).

The correlation of *in vitro* dry matter disappearance with chemical composition of hand clipped forage indicated a positive relationship ($r = +.88$) with percent protein and a negative relationship with percent neutral detergent fiber ($r = -.95$) and acid detergent fiber ($r = -.97$). The correlations between the chemical components and

in vitro dry matter disappearance of esophageal forage samples were not significant, probably because of selective grazing by the animals.

Although climatic conditions have a direct effect on the availability of pasture forage to the animal, the conclusions drawn from this experiment should be valid for other pasture studies. The conclusions drawn from this experiment are as follows:

(1) Forage quality decreased throughout the growing season and as the quality decreased animals became more selective in their grazing habits. Furthermore, late in the season the quantity of forage available became more abundant which permitted greater selectivity.

(2) The chemical composition of hand clipped forage samples was found to be an unreliable estimate of the composition of forage actually consumed by the animal.

(3) Of the chemical components studied in esophageal samples, acid detergent lignin was correlated most highly with voluntary intake ($r = -.93$). This suggests that of the components studied, lignin is the most important criterion in the animal's selection of forage.

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APPENDIX

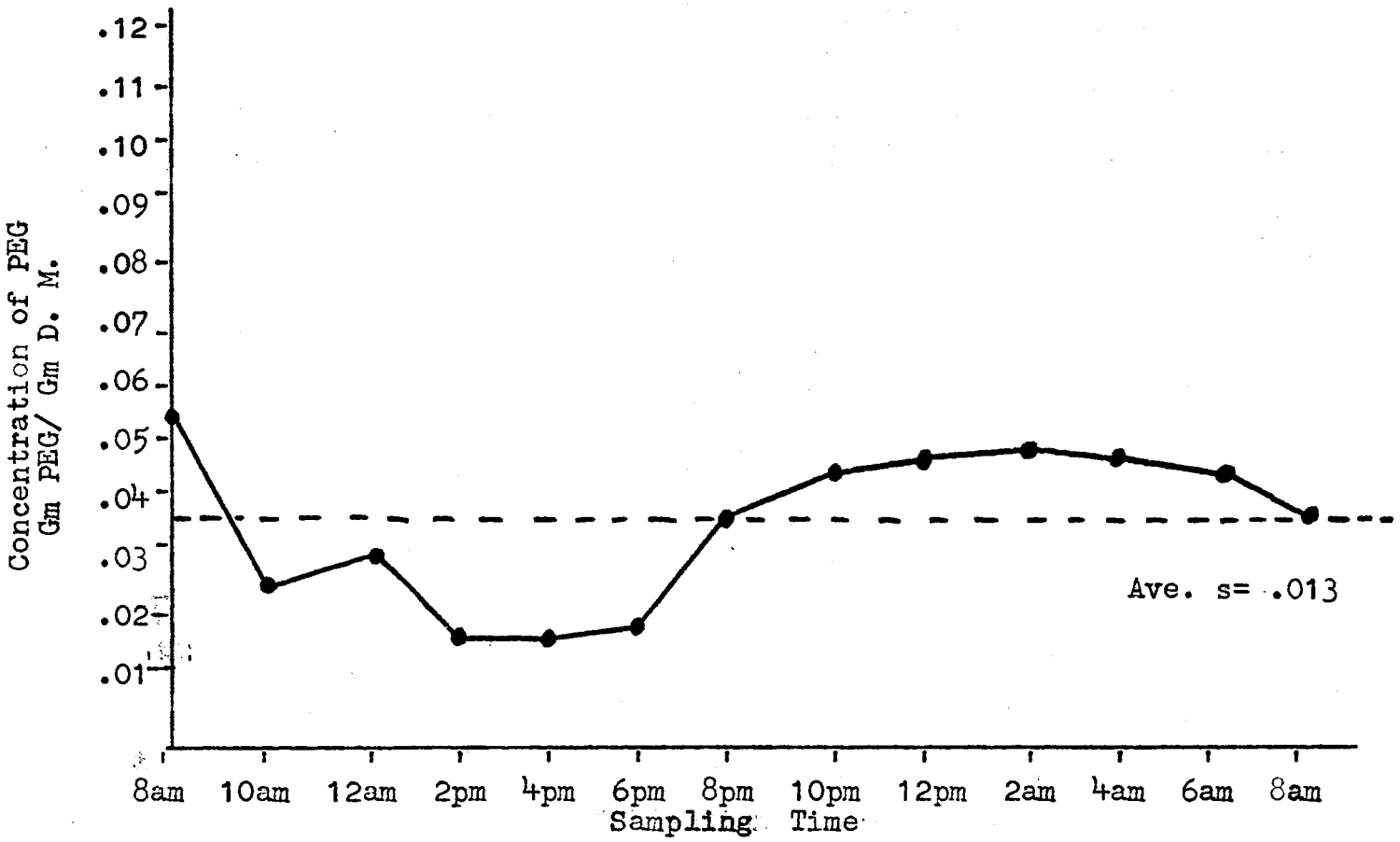

Figure 1. Diurnal Excretion Curve for 50 Gm. PEG Given at 8:00 A.M.

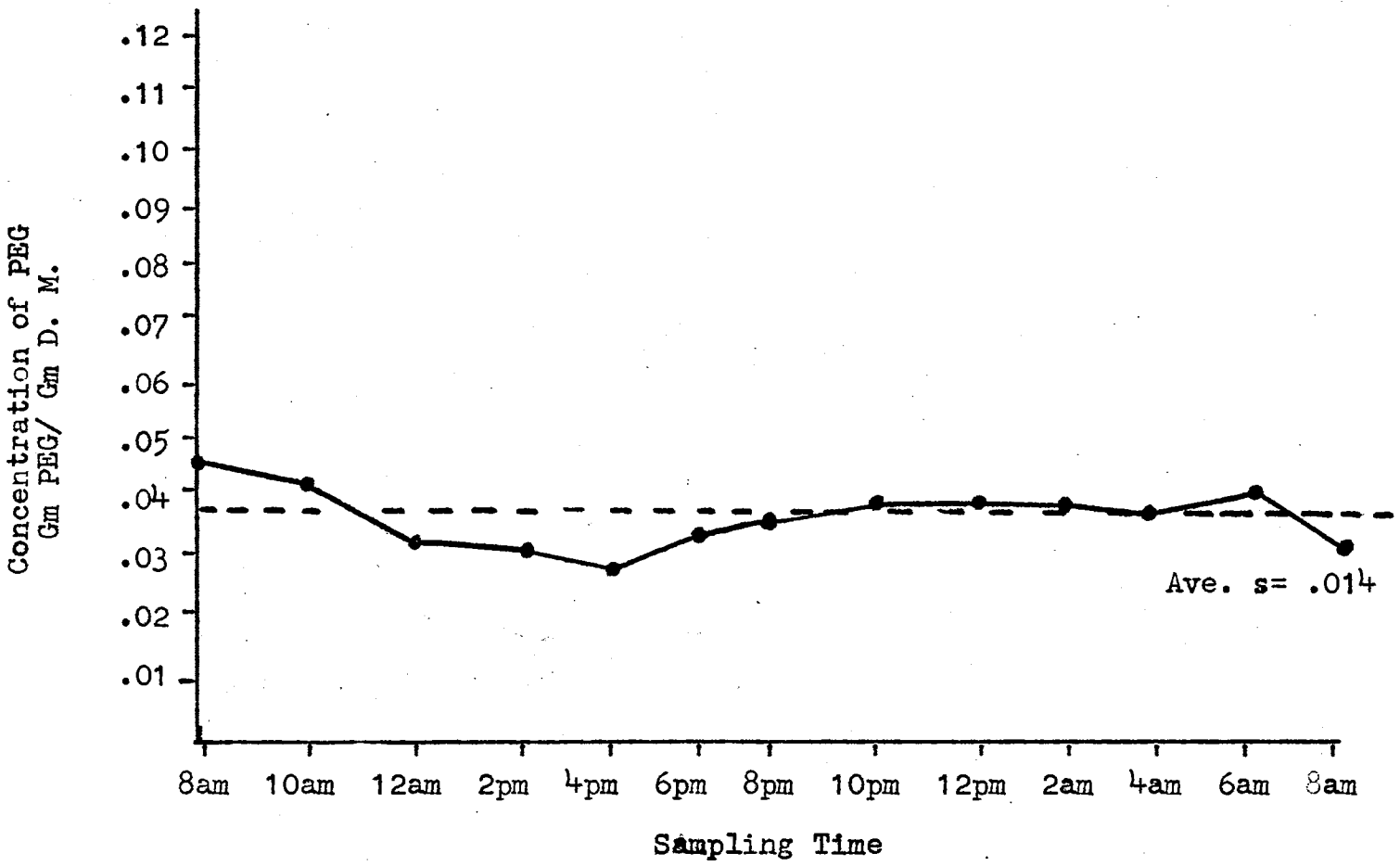

Figure 2. Diurnal Excretion Curve for 25 Gm. PEG Given at 8:00 A.M. and at 4:30 P.M.

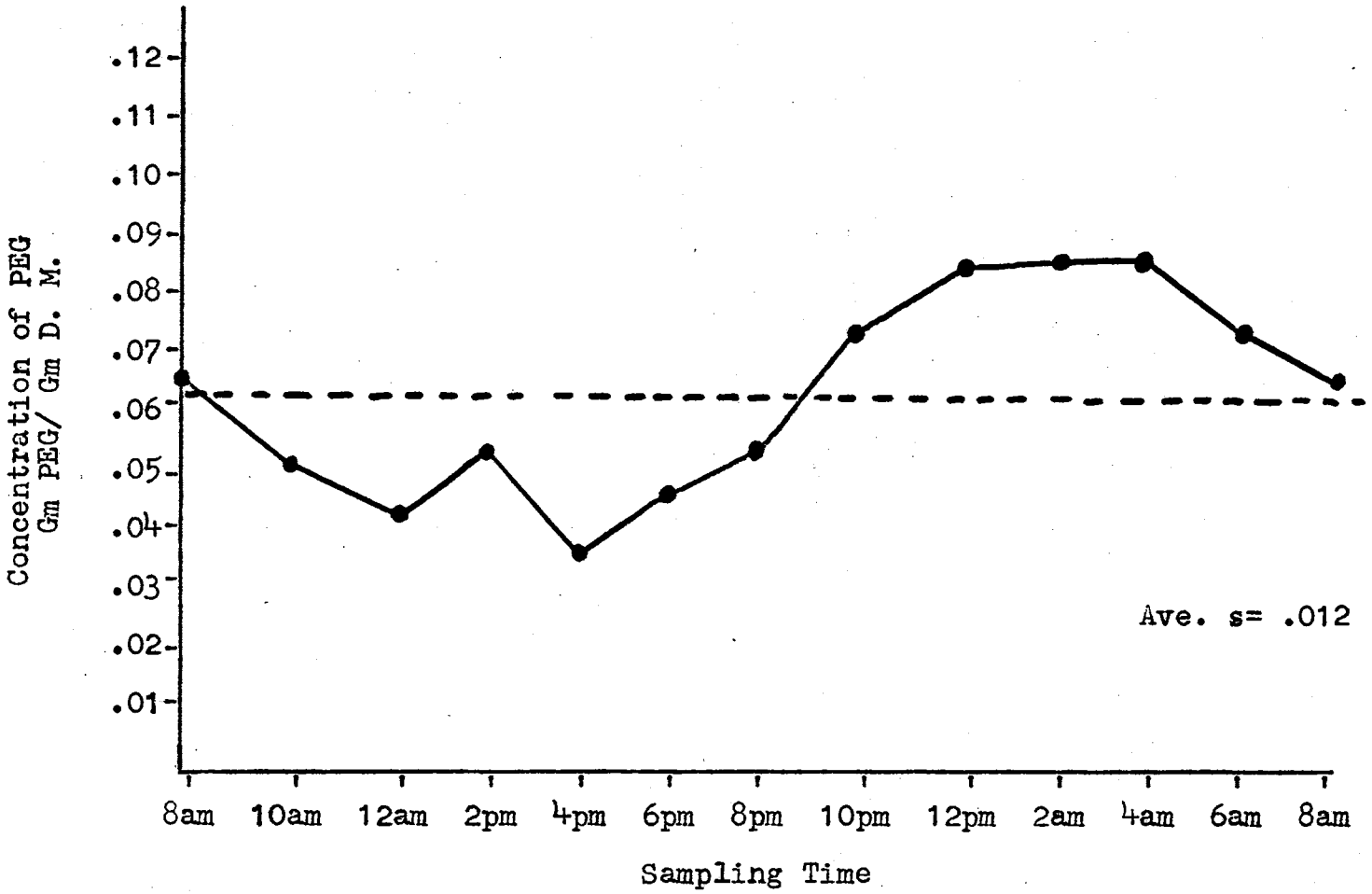

Figure 3. Diurnal Excretion Curve for 100 Gm. PEG Given at 8:00 A.M.

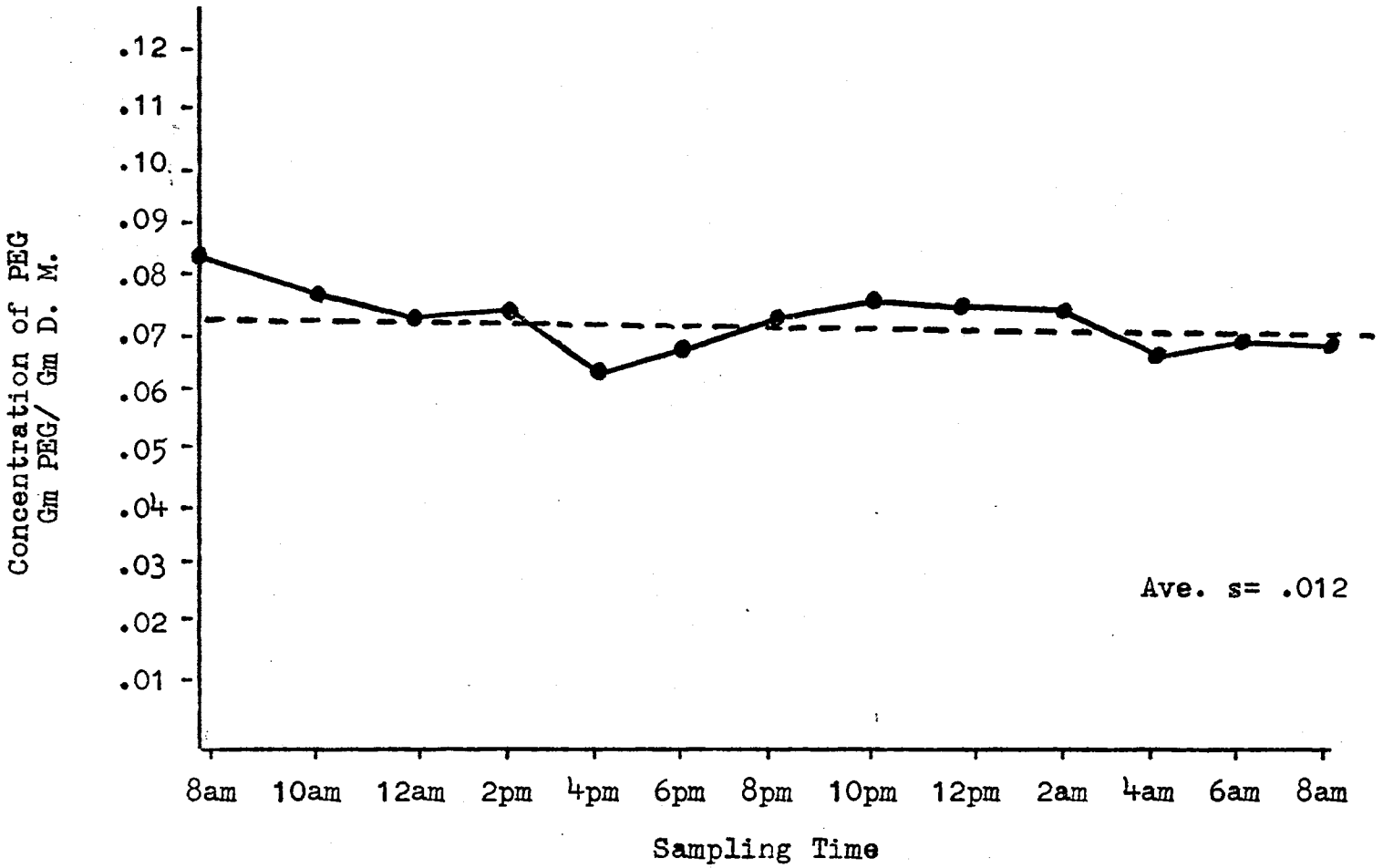

Figure 4. Diurnal Excretion Curve for 50 Gm. PEG Given at 8:00 A.M. and at 4:30 P.M.

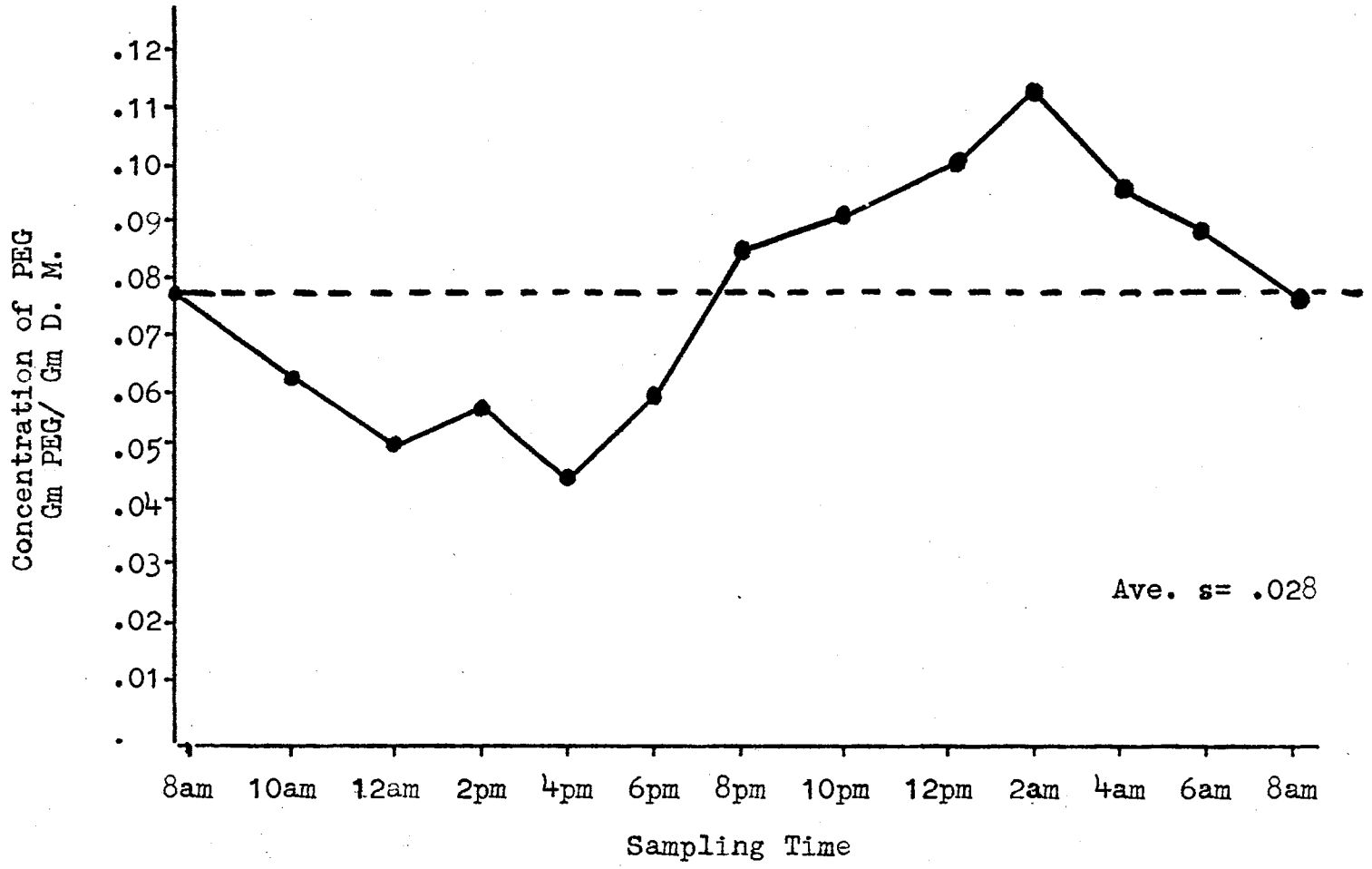

Figure 5. Diurnal Excretion Curve for 150 Gm. PEG Given at 8:00 A.M.

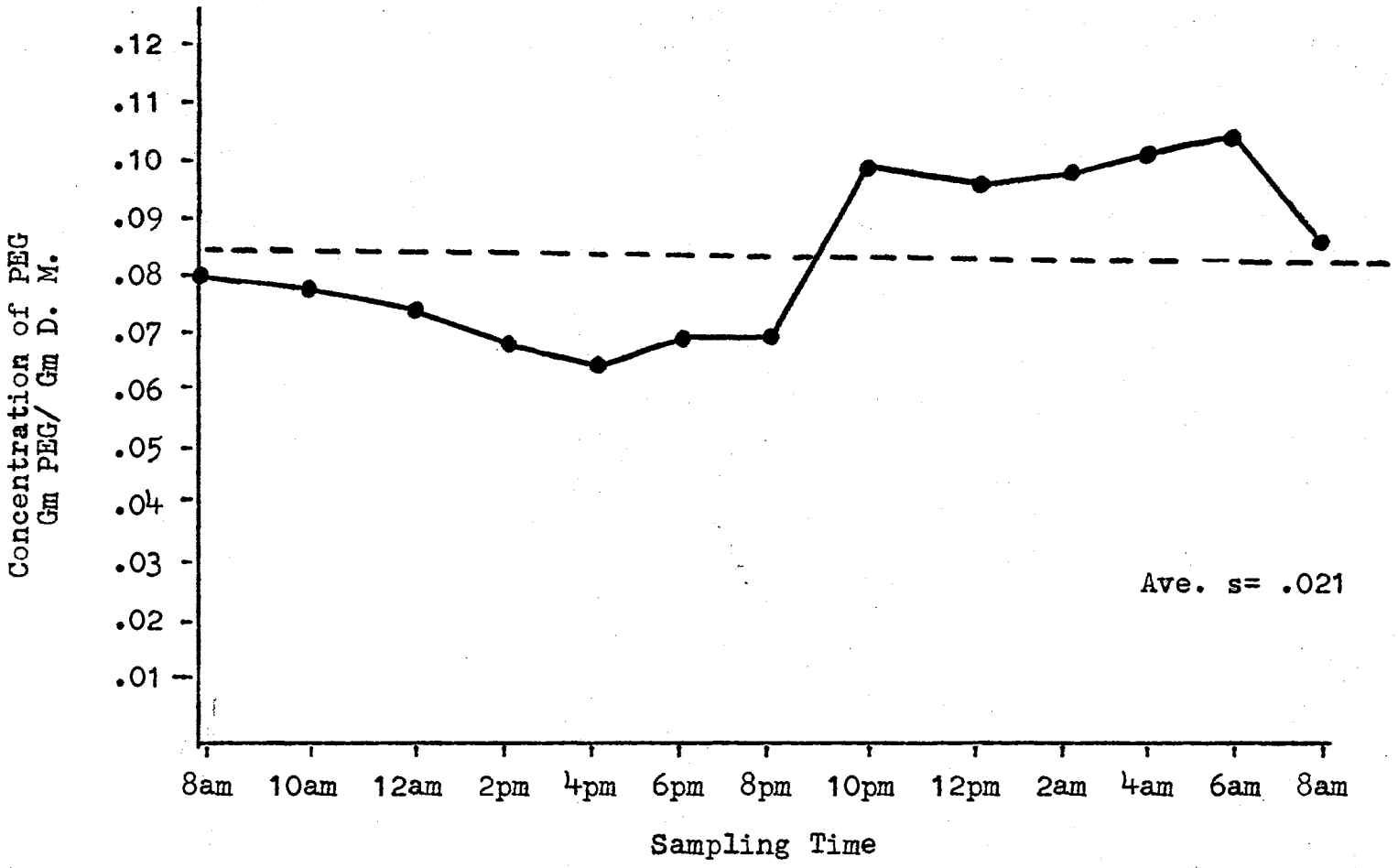

Figure 6. Diurnal Excretion Curve for 75 Gm. PEG Given at 8:00 A.M. and at 4:30 P.M.

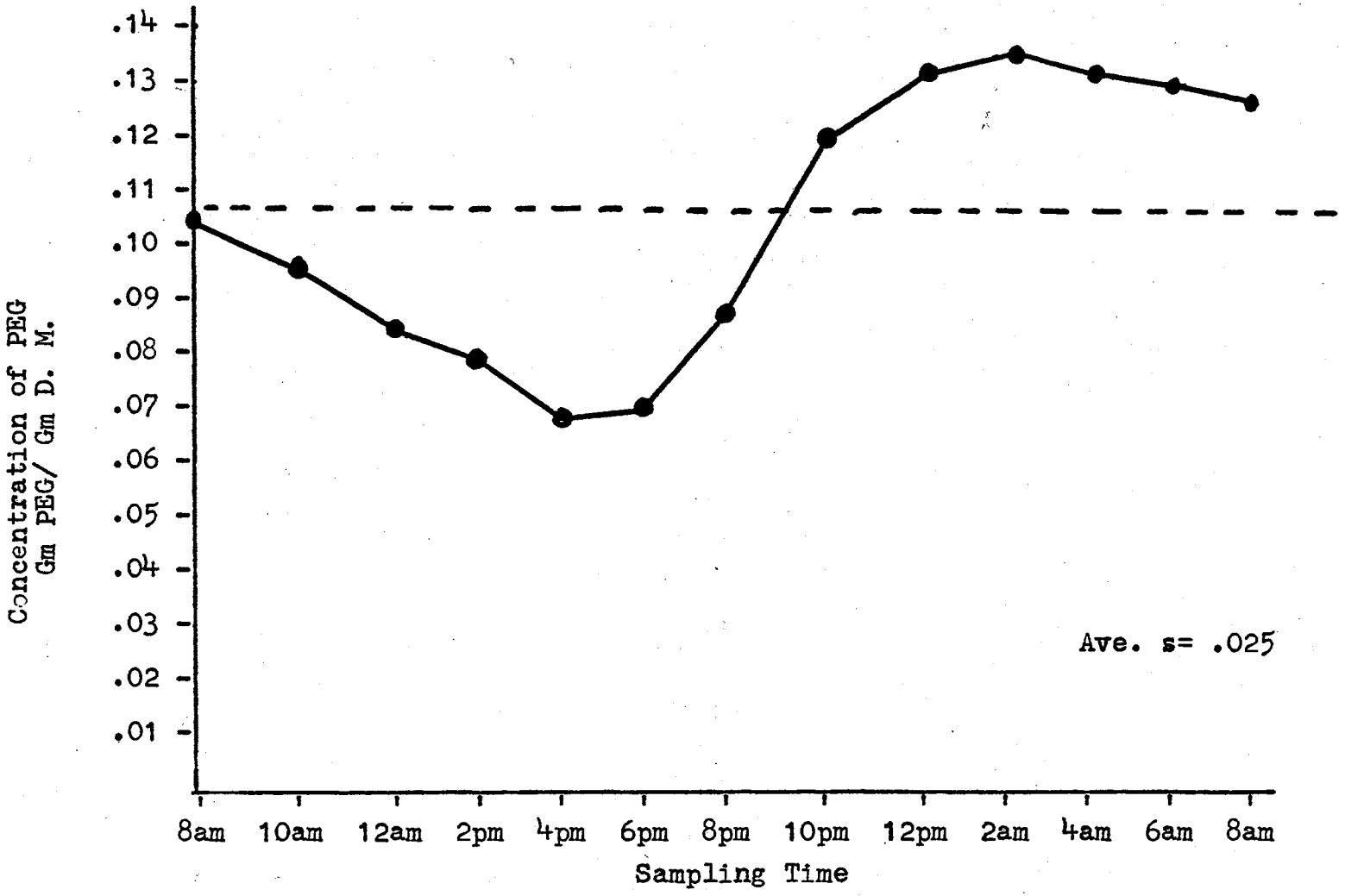

Figure 7. Diurnal Excretion Curve for 200 Gm. PEG Given at 8:00 A.M.

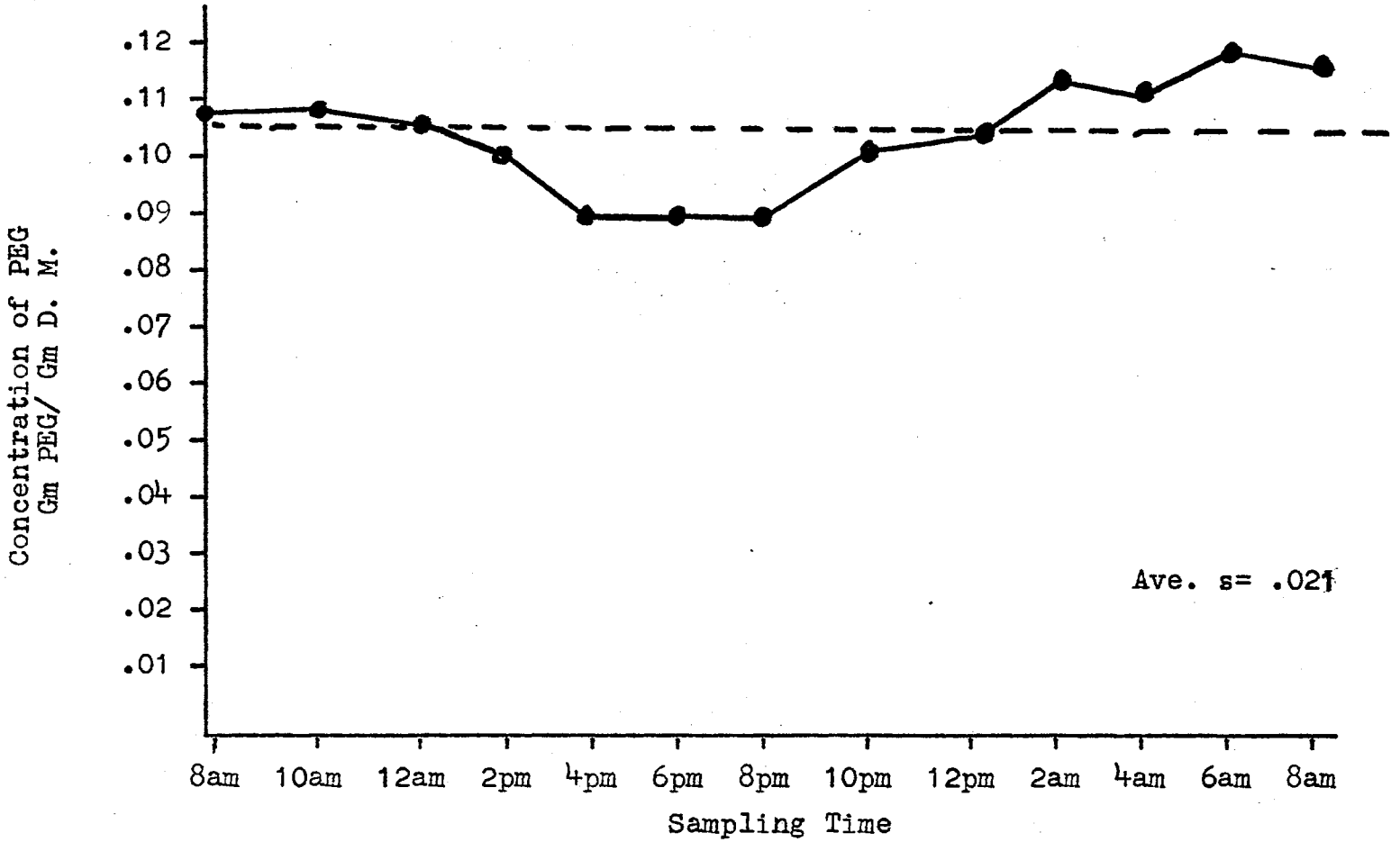

Figure 8. Diurnal Excretion Curve for 100 Gm. PEG Given at 8:00 A.M. and at 4:30 P.M.

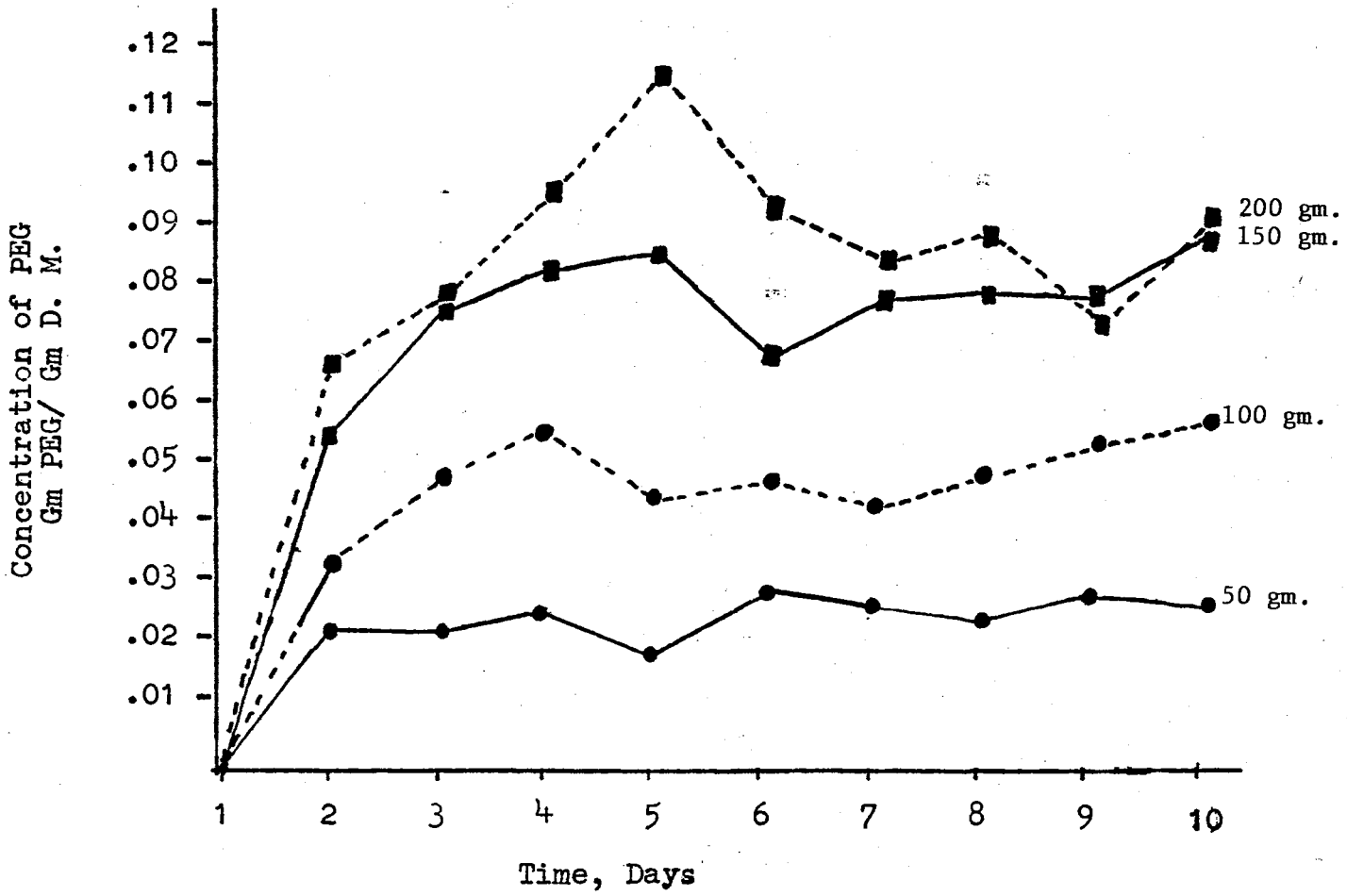

Figure 9. Time Required for PEG to Reach Maximum Concentration in the Feces When Administered Once Daily (One Animal Per Treatment)

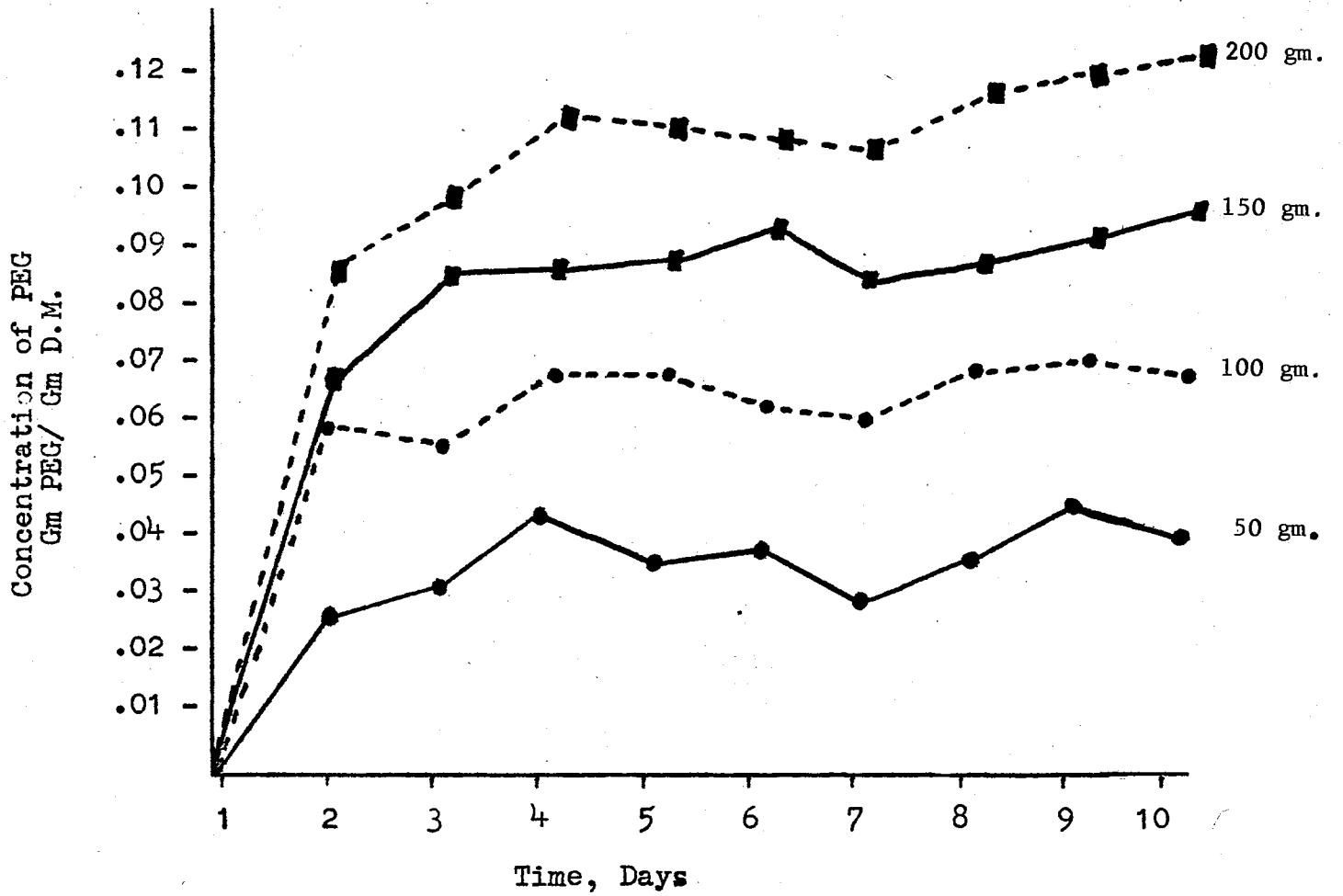

Figure 10. Time Required for PEG to Reach Maximum Concentration in the Feces When Administered Twice Daily in Split Doses (One Animal Per Treatment)

VITA

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David Edmond Hopson

Candidate for the Degree of

Master of Science

Thesis: THE DETERMINATION OF INTAKE AND DIGESTIBILITY OF HARVESTED AND GRAZED FORAGE BY THE USE OF INDICATORS

Major Field: Animal Science

Biographical:

Personal Data: Born at Dallas, Texas, January 13, 1943, the son of Ben R. and Dorothy Hopson.

Education: Graduated from high school in DeQueen, Arkansas, in 1961; received the Bachelor of Science degree from the University of Arkansas, Fayetteville, Arkansas, with a major in Animal Science in January, 1966.

Professional Experience: Raised and worked on a livestock farm in southern Arkansas; worked on research farm at the University of Arkansas, 1964-1966; Research and Teaching Assistant in the Department of Animal Science, Oklahoma State University, 1969-1970.

Professional Organizations: Member of Alpha Gamma Rho and Alpha Zeta fraternities.