AN EVALUATION OF POLYETHYLENE AS AN INDEX FOR DIGESTION STUDIES WITH CATTLE

By

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Bachelor of Science

Texas Technological College

Lubbock, Texas

1964

Submitted to the faculty of the Graduate School of
the Oklahoma State University
in partial fulfillment of the requirements
for the degree of
MASTER OF SCIENCE
May, 1966

AN EVALUATION OF POLYETHYLENE AS AN INDEX

FOR DIGESTION STUDIES WITH CATTLE

Thesis Approved:

Thesis Adviser

Dean of the Graduate School

ACKNOWLEDGMENTS

The author wishes to thank Dr. S. A. Ewing, Professor of Animal Science, for his encouragement, counsel and guidance during the course of this study and in the preparation of this thesis.

He is also indebted to Dr. J. V. Whiteman, Professor of Animal Science, for his assistance in the analyses of the data.

Further acknowledgment is due Dr. C. W. Nichols, Extension Livestock Specialist, for his assistance in improving the manuscript.

Appreciation is also extended to colleagues in the Graduate School at Oklahoma State University for their encouragement and assistance.

Grateful acknowledgment is also extended to the E. I. DuPont De Nemours and Co. (Inc.), Wilmington, Delaware, for providing the polyethylene fluff used in this study.

Special acknowledgment is given to the author's parents for their continuing assistance and encouragement during the course of this study.

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INTRODUCTION

The first experiments to determine digestibility of feeds were conducted at the Weende Experiment Station in Goettingen, Germany, by Henneberg and Stohman in 1864 (Schneider et al., 1955). These trials involved the total collection method. Since that time researchers have sought methods which would reduce the time, facilities and confining animal environment characteristic of the total collection technique.

Indicator methods have provided one approach for determining digestibility without requiring the quantitative measurement of feed intake or fecal output. The index materials evaluated for this purpose have been both compounds that occur naturally in feedstuffs and those that may be added to the diet.

To qualify as an index material a substance must be indigestible, unabsorbable and non-toxic. It should pass through the digestive tract in a uniform mixture with the other ingesta and should not influence the digestibility of the other ingesta. In addition such materials should have no pharmacological action on the digestive tract. Finally, the reference substance should be easily and accurately determined by chemical procedures in both feed and fecal samples.

This study was conducted to evaluate the feasibility of using alathon, a polyethylene material in fluff form, as a reference or index material for digestion studies with cattle.

LITERATURE REVIEW

Reference Materials

Silica

According to Schneider et al. (1955) Wildt, a German worker, used silica as a reference material in digestion studies as early as 1874. Knott et al. (1936) found that silica was unreliable as an index material due to natural feed and fecal contamination. Gallup et al. (1945) reported silica recoveries of 136.3 percent on pasture. It was concluded that the animals consumed silica from soil. When the animals were maintained on concrete floors silica recoveries of 103 percent were reported.

Gallup and Kuhlman (1936) observed that 15 percent of the ingested silica was metabolized. It was apparent that silica distribution was not homogeneous in the mung-bean silage fed, this increased the variability of results. Druce and Willcox (1949) also reported that the amount of silica recovered in the feces was highly variable. From these reports it is generally concluded that silica does not meet the important criteria needed for reference materials in digestion studies.

Ferric Oxide

Bergeim (1926) used ferric oxide as a reference material in a study with albino rats and reported satisfactory results. Based on the assumption that recovery of added iron was not always complete, Heller

et al. (1928) suggested the naturally occurring iron in feeds as a reference material. These workers reported that iron is not eliminated at a sufficiently uniform rate to serve as a reference material.

Knott et al. (1936) found considerable diurnal variation in iron excretion in studies with ruminants. Hamilton et al. (1927-28) reported that ferric oxide recovery was of doubtful value for replacing conventional methods of determining digestibility with cattle or sheep.

Moore and Winter (1934) in a rate of passage study utilizing the ferric oxide technique reported recovery values of only 82.4 to 88.7 percent of the amount of ferric oxide ingested.

Fecal Nitrogen

Gallup and Briggs (1948) evaluated the use of fecal nitrogen as a reference material. The results indicated that there was a close relationship between protein content and nutrient digestibility of hays. Soni et al. (1954) found a high degree of similarity in digestion coefficients between the fecal nitrogen and chromogen techniques. Forbes (1949) found no significant relation between fecal nitrogen and forage protein content and suggested that the use of lignin as a reference substance was a better approach.

Lignin

Lignin is a naturally occurring plant substance of unknown chemical structure. According to Fruton and Simmonds (1961) p-hydroxyphenylpropanes derived from coniferyl alcohol or some closely related compound are fundamental repeating units of lignin. Mertz (1959) states that recent studies suggest that the aromatic amino acids, especially phenylalanine, serve as precursors of lignin in the plant. As the

demands for phenylalanine by the plant decrease, this amino acid is converted to compounds that serve as monomer units in lignin formation.

Ellis et al. (1946), Swift et al. (1947) and Kane et al. (1950) report no digestion of lignin when fed to ruminants. Digestion coefficients obtained by the lignin technique were in close agreement with those derived from the total collection method.

Johnson et al. (1964) reported that about ten percent of the lignin ingested by ruminants was digested, however, when the values were adjusted for this level of digestion the results were comparable to findings obtained by the total collection procedure. In conclusion, it was stated that lignin content, if properly analyzed or adjusted, should serve as an accurate index material. Crampton and Maynard (1938) reported ranges in fecal lignin recovery of 93.4 to 103.7 percent.

Hale et al. (1947) reported rather high digestion coefficients for lignin. In one case 32.7 percent of the fecal lignin was digested. It was concluded that this digestion was taking place in the intestine since no ruminal lignin digestion occurred. Ely et al. (1951) in studies with lactating dairy cows found lignin to be as high as 14 percent digestible. Elam et al. (1962) reported average lignin recoveries of only 90.2 percent.

Kane et al. (1951) and Pigden and Stone (1952) reported species differences in lignin recoveries. Lignin in alfalfa was found to be reliable as an indicator of digestibility while that of orchard grass was not found to be as reliable.

Pazur and De Long (1948) reported that lignin units present in the earlier stages of growth of the clover plant were more readily metabolized by ruminants than those present in more mature clover. Ely

et al. (1953) stated that the stage of maturity of the plant involved can influence the amount of lignin recovered.

Druce and Willcox (1949) reported that results obtained by the two methods of lignin analysis are not comparable. Forbes et al. (1946) stated that there is a need for a method of lignin analysis which will give consistent results.

From the variable results reported and the problems involved in accurate lignin determination, this naturally present reference material is of doubtful value.

Indigestible Protein

Forbes (1950) conducted studies utilizing indigestible protein as a reference material. It was assumed that forage protein level had an influence on the amount of fecal protein per unit of dry matter intake. Results utilizing this technique are very inconsistent; therefore, it is of doubtful value as a pasture forage indicator.

Methoxyl Radical

Richards and Reid (1952) proposed the methoxyl radical as a reference material. This report indicated that the methoxyl content of lignin and the percentage of total methoxyl increases with plant maturity. A considerable portion of the methoxyl consumed disappeared from the plant material as it passed through the digestive tract. Kane et al. (1951) found that approximately 50 percent of the methoxyl content of an orchard grass ration was digested.

Titanic Oxide

Lloyd et al. (1955) compared titanic oxide and chromic oxide as index materials in a rat diet. After a six day preliminary feeding period the length of time necessary for the maximum constant fecal excretion of titanic oxide could not be determined. The reported recovery values of titanic oxide from feces were lower (92 percent) than those of chromic oxide (99.8 percent). As a result, it was concluded that titanic oxide was inadequate as an index material.

Dyes

Corbin and Forbes (1951) reported using anthraquinone violet as a reference material with lambs. The rate of fecal recovery ran from 96.4 to 106.0 percent with an average of 100.5 ± 2.8 percent. Shellenberger and Kesler (1961) in studies with cattle used a crystal violet strain as a marker. The level of dry matter intake was significantly correlated with the rate of passage of ingesta.

Polyethylene Glycol

According to Downes and McDonald (1964) polyethylene glycol was used as a soluble rumen marker in 1953 by Sperber, Hyden and Ekman. Corbett et al. (1956) reported that polyethylene glycol may be a satisfactory marker if it is fed to give no less than 250 mg. per 100 g. of feces. Corbett et al. (1958), working with dairy cows, reported more variability in the excretion of polyethylene glycol than for chromic oxide. Huston and Ellis (1965) stated that results obtained utilizing polyethylene glycol did not differ significantly from those obtained utilizing chromic oxide with sheep. They reported a coefficient of

variation of 26 percent for polyethylene glycol recovery and one of 15.3 percent for chromic oxide recovery. Hyden (1956) and Sutton et al. (1962) stated that when polyethylene glycol was used as a rumen marker in studies of short duration that it was a valuable, accurate reference material. Downes and McDonald (1964) concluded that the most serious limitation to the use of polyethylene glycol as a reference material for determining digestibility is the lack of an accurate method of analysis. Christie and Lassiter (1958) also reported polyethylene glycol to be an unsatisfactory indicator of digestibility. The principal use of polyethylene glycol has been as a measure of rumen volume by dilution technique.

Cerium^{144}

Cerium^{144} is a naturally occurring member of the lanthanide or inner transition series of elements according to Wood and Keenan (1957). Huston and Ellis (1965) compared cerium^{144}, chromic oxide and polyethylene glycol as index materials. Similar excretion patterns were reported for the three substances. A coefficient of variation of 6.4 percent was reported for cerium^{144} between four hour collections over an 80 hour collection period after an eight day preliminary period. The results indicated that cerium^{144} is more reliable than chromic oxide or polyethylene glycol for estimating digestibility and rate of passage of a ration due to its binding properties which result in a lower diurnal variation. It was concluded that the properties of elements of the rare earth group indicate that they will adsorb onto and remain bound to dry matter.

Chromium - 51 Complex

Downes and McDonald (1964) reported the use of the chromium - 51 complex of ethylenediamine tetraacetic acid as a reference material. A slight absorption and subsequent excretion of the chromium - 51 complex was encountered in the urine of experimental animals. The results obtained by this method were similar to those obtained by the polyethylene glycol technique. On the basis of this report it would appear that the chromium - 51 complex of ethylenediamine tetraacetic acid can be used as a satisfactory soluble rumen marker.

Miscellaneous Materials

Kane et al. (1953) in a review of the literature stated that radioactive isotopes as well as barium sulphate have been used as index materials. Welch (1965) using the nylon bag technique reported a dry matter digestion coefficient of 38 percent for sisal fibers and no digestibility of wood shavings.

Chromogen

Chromogens are a group of naturally occurring plant pigments whose exact composition is unknown. Reid et al. (1950) reported work which indicated that chromogens could be used as an index material. Using 85 percent acetone extracts of feces from animals fed various forages an average rate of recovery of 100.5 percent of the chromogenic substances was reported.

Reid et al. (1952) established mathematical relationships between the chromogen-dry matter ratio of feces voided and that of the forage actually consumed. These relationships were judged to be sufficiently

accurate to eliminate the need for manual sampling of forage. It was presumed that the chromogen concentration of the forage could be predicated from a knowledge of the fecal chromogen concentration.

Kane et al. (1951, 1953) stated that the determination of digestibility values using plant pigments were similar to values obtained by the total collection procedure. Eng (1962) reported higher digestion coefficients from the chromogen technique than from the total collection method. Very little diurnal variation was noted in the fecal chromogen concentration.

Soni et al. (1954) and Hardison et al. (1957) reported a definite diurnal variation in fecal chromogen concentration. The apparent digestibility of different portions of the plant were different as calculated by the chromogen technique. It was reported that the top portions of the alfalfa plant were more uniformly excreted than were the lower portions.

Squibb et al. (1958), working with tropical grasses, reported that the chromogen technique was suitable for Kikuyu grass but in trials with Ramie grass incomplete chromogen recovery led to erroneous results. It was concluded that such results would preclude the use of the chromogen method for determination of digestion coefficients for all species of forages.

Cook and Harris (1950) reported that animals fed plants high in ether extract have an extremely high concentration of chromogens in the urine. It is believed that this phenomenon could influence digestion coefficients determined by the chromogen method.

Hamilton et al. (1955) in a comparison of the chromogen ratio technique and the conventional method of determining digestibility observed erratic results from chromogens. It was concluded that the

chromogen ratio technique was not suitable as a measure of digestibility. Thus it would appear that further work must be done before the chromogen technique can be recommended as an accurate method.

Chromic Oxide

Chromic oxide is the most widely used index material. Edin (1918) was the first to use this compound as an index material. Since this early work this material has been used as a reference material in studies with many species of animals.

Dansky and Hill (1952) in a study with chickens, reported that chromic oxide gave more accurate results than did the total collection method. In studies with rabbits, Huang et al. (1954) reported satisfactory digestibility values using chromic oxide. Irwin and Crampton (1951) working with human subjects reported that chromic oxide was voided in a uniform mixture with the feces and that reliable coefficients of digestibility could be determined in this way.

In studies with rats, Schurch et al. (1950) and Lloyd et al. (1955) reported that there was no significant difference in results between the chromic oxide technique and the total collection method. Lloyd and McCay (1954) stated that chromic oxide has also been used successfully with horses, foxes and mink. These workers reported identical digestion coefficient means in comparing chromic oxide and the total collection technique in studies with dogs.

There is general agreement among workers that chromic oxide will give reliable results when used in digestion studies with monogastric subjects. Clawson et al. (1955) reported digestion coefficients derived from chromic oxide that were in close agreement with those obtained by the total collection technique for swine. Horvath et al.

(1958), in studies with swine, reported that there was a difference in the concentration of fecal chromic oxide between morning and afternoon collections. It was postulated that the variation in fecal concentration could be due to differences in digestion time in the lower tract and/or differences in the rate of passage of various feed fractions through the stomach.

There has been some disagreement among researchers as to the value of chromic oxide as a reference material in studies with ruminants. Kane et al. (1950, 1951, 1953) reported that digestibility values derived by the chromic oxide technique compare favorably to the total collection method. Archibald (1958), working with dairy cows, recommended the chromic oxide procedure due to both uniformity of results and ease of determination.

Hardison and Reid (1953), Putnam et al. (1958), Linnereud and Donker (1961) and Clanton (1962) reported considerable variation in the fecal chromic oxide concentration at any sampling time. Kane et al. (1952) reported that fecal chromic oxide concentrations were highest at 9:00 a.m. and lowest at 9:00 p.m. Smith and Reid (1955) reported a low fecal chromic oxide concentration of 65 percent at 2:00 p.m. and a high of 141 percent at 12:00 p.m. for the grazing cow. For steers a low of 52 percent at 12:00 a.m. and a high of 183 percent at 7:00 p.m. was reported.

Davis et al. (1958) in studies with lactating dairy cows reported that the peak level of fecal chromic oxide concentration occurred from 12:00 a.m. to 6:00 p.m. and the lowest level was from 2:00 a.m. to 8:00 a.m. Elam et al. (1958) reported extremes in diurnal variation of 74 percent to 135 percent. Corbett et al. (1958) stated that fecal chromic oxide concentration varied at the rate of ± 10 percent over a 24 hour

period. Huston and Ellis (1965) reported a coefficient of variation of 15.3 percent among four hour fecal collections.

The mode of chromic oxide administration may influence fecal concentration with time. Corbett et al. (1956) reported that chromic oxide capsules may pass through the esophageal groove into the abomasum in sheep, or be regurgitated.

Brissom and Pigden (1957, 1958) reported using a chromic oxide impregnated plaster of paris pellet as a means of index administration. Diurnal variation was extremely low when the pellets were used. Regurgitation and passage of the pellet from the rumen was reported for sheep but not for cattle. It was postulated that one reason for the low variability in fecal chromic oxide concentration was due to a sustained chromic oxide release since there were several pellets in the rumen at all times.

Pigden et al. (1964) used an extruded sustained-release pellet (ESRP) and a pressed sustained-release pellet (PSRP). More diurnal variation was reported for the pressed sustained-release pellet than for the extruded sustained-release pellet. However, a higher proportion of the extruded sustained-release pellets were regurgitated.

Eng (1962) reported a much lower diurnal variation (84.03 to 114.5 percent) for a sustained-release pellet (SRP) than for capsule administered chromic oxide (76.88 to 126.12 percent). Only about 50 percent of the chromic oxide from the sustained-release pellet was recovered. Troelsen (1961) reported marked differences in digestion coefficients obtained with the sustained-release pellet and those obtained by the total collection procedure. A pellet loss due to regurgitation and passage into the abomasum was observed.

Crampton and Lloyd (1951) reported that mixing chromic oxide with the ground concentrate portion of the ration provides a satisfactory method of admistering chromic oxide to sheep. When chromic oxide was mixed in an unground all-roughage ration it tended to be partially retained in the digestive tract and gave unreliable digestibility estimates.

Bradley et al. (1958) and Elam et al. (1960) reported that mixing chromic oxide in a pelleted ration greatly reduced variability of results. They concluded that it can be used as a simple method of determining digestibility.

Corbett et al. (1960) used a chromic oxide impregnated paper as a reference material. Results indicated that the use of the shredded paper gave better results than whole paper or gelatin capsules.

Troelsen (1963) fed a pellet made from chromic oxide impregnated paper and reported no loss from regurgitation or passage into the abomasum.

Campbell (1964) and Johnson et al. (1964) reported a smaller range in fecal chromic oxide recovery when a chromic oxide impregnated paper was used than when chromic oxide was administered in gelatin capsules.

Hardison and Reid (1953) reported that the mean rates of chromic oxide recovery from fecal grab samples taken at 6:00 a.m. and 4:00 p.m. were 71.8 percent and 129.3 percent. When these samples were wet-bulked the average chromic oxide recovery was 99.95 percent of the amount ingested. Smith and Reid (1955) stated that the rate of chromic oxide excretion was near the 24 hour average at 6:00 a.m. and 4:00 p.m. A mean rate of recovery of 100.58 ± .87 percent was reported.

Putnam et al. (1958), Hardison et al. (1959) and Eng (1962) reported that samples taken every 12 hours and combined gave the most accurate

results. It was observed that the amount of chromic oxide fed influenced the results. It was concluded by these workers that the indiscriminant use of chromic oxide in nutritional studies could result in considerable error.

Bradley et al. (1958) stated that the best time to sample feces was between 6:00 and 10:00 a.m. when chromic oxide was administered in gelatin capsules and between 8:00 and 10:00 a.m. when it was fed as a component of a completely pelleted ration.

Pigden et al. (1964) stated that there was less variation in fecal chromic oxide concentration when samples were taken at 7:00 p.m. from aminals given chromic oxide pellets.

Elam et al. (1959) stated that the magnitude of variation of fecal chromic oxide concentration would preclude indiscriminate sampling with regard to time.

Balch et al. (1957) stated that chromic oxide administered before a single daily meal caused a more even excretion rate than when administered after the meal. In pasture studies it was reported that better results were obtained when chromic oxide was given at the beginning of the daily grazing period.

Brissom et al. (1957) reported that animals given chromic oxide six times a day had a constant chromic oxide excretion rate. This led to the conclusion that samples taken at any time of the day would give equally valid results. Davis et al. (1958) and Linnereud and Donker (1961) stated that cows given chromic oxide twice daily exhibited about one-half the range in fecal chromic oxide concentration as did cows receiving chromic oxide only once a day.

Smith and Reid (1955) suggest that the time and mode of chromic oxide administration should be determined by the nature of the

experiment and the convenience of the operator. They concluded that preliminary work to establish excretion patterns with a particular feeding regime may be extremely important.

Kameoka et al. (1956) reported that the time of feed ingestion influenced the excretion rate. The movement of chromic oxide through the digestive tract was not influenced by the type of ration fed. It was concluded that chromic oxide passed through the digestive tract at a rate which may be independent of that characteristic of other feed particles.

Putman (1962) in a survey of 101 laboratories in the United States and Canada reported that most laboratories were satisfied with the chromic oxide technique. However, several of these workers had reservations concerning its use due to variations among animals, low recoveries and variable excretion patterns.

Inert Plastics

Recently the use of inert plastics as index materials has been proposed. Campling and Freer (1962) used polystyrene in a study of ruminal mean retention time. It was concluded that polystyrene particles were unlikely to provide an alternative method of estimating the mean retention time of food in the alimentary tract of the cow.

Chandler et al. (1964a, 1964b) working with young dairy calves, have in both cases reported values quite similar to those obtained by the total collection technique when polyethylene was used as an index material to study digestibility. Further work by this group (Chandler et al., 1966) confirmed their previous findings. More variation was noted in the total collection method than in the polyethylene technique. The lowest fecal concentration observed occurred at 2:00 a.m. and the

highest concentration was observed at 2:00 p.m. They concluded that the number of animals and sampling times employed are thought to be important factors in predicting mean polyethylene fecal concentration.

Welch (1965) utilizing the nylon bag technique observed no dry matter digestibility of polypropylene which indicates that some materials of this type may qualify as totally inert substances.

EXPERIMENTS

A series of experiments was conducted to evaluate the feasibility of using alathon,[1] a polyethylene material in fluff form, as a reference material for digestion studies with cattle. It has been established that polyethylene is not affected by digestive enzymes, intestinal juices or mechanical processes within the digestive tract. It is also apparent that this material may hold promise as an index material due to it bulky nature and low specific gravity (0.91) which are similar to the characteristics of many natural feedstuff particles. Since this material possesses these important characteristics it was desirable to develop and evaluate the accuracy with which polyethylene could be quantitatively determined in feed and fecal samples by a gravimetric technique. Following this evaluation, tests were designed to evaluate the material as a reference compound in diets for cattle by studying such factors as excretion patterns, uniformity of concentration in intestinal ingesta and fecal excreta. In addition, coefficients of digestibility determined by this method and by the total fecal collection procedure were compared. The procedures used and the results obtained are discussed below.

Experiment I

Procedure

This experiment was conducted to refine and evaluate a gravimetric technique for quantitative determination of polyethylene in feed and fecal samples.

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[1] Alathon, E. I. Du Pont de Nemours and Co., Inc., Wilmington, Delaware.

The analytical procedure developed for evaluation is explained in detail below:

1. A sample of feed or fecal material containing alathon was prepared for analysis by drying at 103° C. to a constant weight.
2. The sample was then weighed and transferred into a 400 ml. pyrex beaker.
3. Approximately 70 ml. of concentrated nitric acid was added to the beaker for digestion. Since the quantity of nitric acid is not critical a sufficient quantity of acid was used to prevent the digestion medium from becoming thick.
4. The sample was then digested for at least 90 minutes at approximately 80° C. The digestion mixture was swirled periodically and the sides of the beaker washed with distilled water from a fine stream wash bottle as needed to keep polyethylene and feed particles from adhering to the sides of the beaker.
5. When the digestion period was complete the contents were transferred into a 250 ml. separatory funnel. The acid and polyethylene were allowed to separate by floatation.
6. The acid layer was then slowly withdrawn from the bottom of the separatory funnel and discarded.
7. The remaining polyethylene was washed at least twice by adding distilled water to the separatory funnel, shaking and withdrawing the water in the same manner used for removing the acid.
8. The cleaned polyethylene was then flooded with acetone from a fine stream wash bottle and transferred from the separatory funnel into a 250 ml. beaker. Care was taken to insure that all of the polyethylene was washed out.

9. The mixture of polyethylene and acetone was then filtered into dried, weighed Gooch crucibles. An asbestos mat was used in the crucibles as a suitable filter.

10. The polyethylene was then dried at 103° C. and weighed.

In order to evaluate the accuracy of the above analytical procedure a series of experiments was conducted to determine the recovery of known amounts of polyethylene when added to various feed materials and to feces collected from steers fed typical finishing rations.

After known quantities of polyethylene were added to oven-dried feed and fecal samples the mixtures were analyzed by the technique described above. In addition, the recovery of known quantities of polyethylene exclusive of feed and fecal materials was determined by the technique. Samples of a complete ration exclusive of polyethylene were also subjected to the analysis to determine if significant quantities of naturally occurring feed compounds would be reflected as polyethylene by the analysis.

Results and Discussion

The results of these tests are shown in Table I. The data are expressed as mean percentages of polyethylene recovered along with the associated standard deviations and standard errors.

The average percentage recovery of polyethylene from all samples containing the material was 99.89 percent. When pure polyethylene was subjected to the analysis a recovery of 98.94 percent was noted. The main reason for this value being 1.06 percent less than unity may be due to losses incurred during transfer of the material during the analytical procedure. In the evaluation to determine the quantity of naturally occurring feed constituents that could be reflected as

TABLE I

PERCENTAGE RECOVERY OF KNOWN AMOUNTS OF POLYETHYLENE

Material	Number of Determinations	Mean Recovery Percentage	Standard Deviation	Standard Error of the Mean
Polyethylene	19	98.94	2.6938	.6179
Complete finishing ration without polyethylene	13	0.07	.0583	.0162
Cottonseed hulls and polyethylene	11	100.15	.4613	.1391
Complete finishing ration and polyethylene	34	99.56	1.9065	.3270
Feces and polyethylene	23	99.97	5.1200	1.0676

polyethylene by the analysis when no polyethylene was present, it was apparent that this source of error was negligible. The quantities of naturally occurring feed constituents that remained after digestion and separation was 0.07 percent.

It is of interest to note that when the three most widely divergent recoveries of polyethylene added to fecal material were discarded that the standard deviation dropped from 5.12 percent to 3.13 percent. In the case of samples of feces containing polyethylene, apparently the main factor likely to contribute to error in the analysis is the accuracy and competence of the individual conducting the analysis.

The results would indicate that the analytical procedure used is sufficiently accurate to permit the use of polyethylene as a reference material in digestion studies with cattle. This conclusion is based on both percentage polyethylene recovery values and standard errors observed.

Experiment II

Procedure

Phase 1

The first phase of this experiment involved two trials designed to study the diurnal variation in concentration of polyethylene in feces from steers fed a ration containing a known percentage of the reference material.

Trial 1. In the first trial five Hereford steers averaging approximately 458 kilograms were used as experimental animals. This group of steers was given access to a self-feeder which provided a complete finishing ration containing 13 percent polyethylene. The composition of the ration used is shown in Table II. At the conclusion of a 132-day feeding period fecal grab samples were collected from each steer every four hours starting at 3 a.m. for portions of three days in October, 1964. At the conclusion of the first 24-hour collection period it was noted that the consistency of the feces was abnormal, thus further collections were suspended for a period of 20 hours after which time collections were resumed on schedule.

Immediately after collection the samples were placed in labeled plastic bags, frozen and stored for future analysis.

Trial 2. A second trial was conducted in a similar manner in June, 1965. This trial involved two groups of Hereford steers averaging approximately 458 kilograms which were fed rations 2 and 3, shown in Table II. Both groups of steers were self-fed the assigned rations containing 13 percent polyethylene for 115 days. At the conclusion of

TABLE II

PERCENTAGE COMPOSITION OF RATIONS

Ingredients	Ration Number		
	1	2	3
Steam rolled milo	69.17	75.64	70.16
Cottonseed meal (41 % solvent)	4.35	2.61	3.48
Alfalfa meal (19 % protein)	4.35	4.35	4.35
Molasses	2.61	2.26	2.61
Urea (262)	1.30	.70	.96
Stabilized animal tallow	4.35	----	4.35
Salt	.44	.44	.44
Calcium carbonate	.35	.57	.57
Vit. A premix	.03	.03	.03
Trace mineral premix	.02	.02	.02
Polyethylene (alathon)	13.03	13.03	13.03

this feeding period fecal grab samples were collected at 6 a.m. and 4 p.m. for two days from each of five steers receiving ration 2 and from each of six steers receiving ration 3.

Immediately after the fecal grab samples were collected they were placed in labeled plastic bags, frozen and stored for future analysis.

Upon completion of the collection period the samples obtained in each trial were analyzed by the procedure outlined in Experiment I.

Phase 2

A second phase of this experiment involved a trial designed to study the concentration of polyethylene in various digestive tract compartments of steers fed ration 1. The composition of the ration is shown in Table II. Four Hereford steers averaging approximately 458 kilograms were slaughtered at the Oklahoma State University Meat Laboratory in October 1964, after 132 days on full-feed. Samples of ingesta from the rumen, abomasum, duodenum and large intestine were taken immediately post-slaughter. These steers were selected from the same group of steers involved in the first trial of phase one of this experiment.

The ingesta samples collected were placed in labeled plastic bags, frozen and stored for future analysis by the method outlined in Experiment I.

Results and Discussion

Phase 1

Trial 1. The mean percentage concentration of fecal polyethylene for each collection time during trial 1 of the first phase of this experiment is reported in Table III. These values are pooled for all

TABLE III

PERCENTAGE MEAN POLYETHYLENE CONCENTRATION OF FECAL SAMPLES FROM STEERS FED RATION 1, TRIAL 1, EXPERIMENT II

Time	Steer 5	11	38	39	40	Mean
Day 1						
3 a.m.	38.0	34.0	34.9	44.0	45.3	39.2
7 a.m.	38.5	43.9	37.9	40.8	41.7	40.6
11 a.m.	35.6	39.9	38.3	39.5	40.1	38.7
3 p.m.	32.2	48.0	33.0	36.4	36.2	37.2
7 p.m.	43.3	48.3	39.3	32.8	41.7	41.1
11 p.m.	40.2	19.9	40.1	45.5	38.3	36.8
Day 2						
7 p.m.	38.2	39.9	40.6	33.5	47.1	39.9
11 p.m.	47.1	46.5	44.1	27.7	49.3	42.9
Day 3						
3 a.m.	37.9	43.8	43.6	40.0	48.5	42.8
7 a.m.	39.9	50.7	38.2	35.6	46.8	42.2
11 a.m.	37.4	40.1	39.2	35.4	39.4	38.3
3 p.m.	41.1	49.4	40.8	42.1	35.6	41.8
Mean	39.1	42.0	39.2	37.8	42.5	
Overall mean						40.1

steers and plotted against time in Figure 1. The overall mean is superimposed on the graph to illustrate how the concentration at each collection time differed from the average for all times.

The variance associated with duplicate determinations of polyethylene concentration on each sample and the variance assoicated with different samples was determined by analysis of variance for the steers fed ration 1, as shown in Appendix Table X. The average variance observed between duplicate analyses of the same sample was 10.84. The standard deviation between these duplicate analyses was 3.29 percentage units. When the average variance observed between duplicate analyses of the same samples was expressed as a percentage of the total variance the portion attributable to differences between duplicate analyses was only 0.65 percent. This includes any error inherent in the procedure, subsampling error and error comitted by the person performing the procedure. That portion of the total variance due to differences between samples was 2.33 percent.

Since these data were disproportionate in some cases, the method of unweighted means described by Snedecor (1956) was used to test for differences between times and between steers. This analysis of variance is reported in Appendix Table XI. A significant interaction ($P < .01$) was observed between steers and time, but neither the variance associated with steers nor time was found to be significant.

Trial 2. The mean percentage concentration of fecal polyethylene for each steer fed rations 2 and 3 for each collection time during trial 2 of the first phase of this experiment is presented in Table IV. These values are pooled and the mean value for all steers on each ration is plotted against time in Figure 1. In both cases the overall mean for

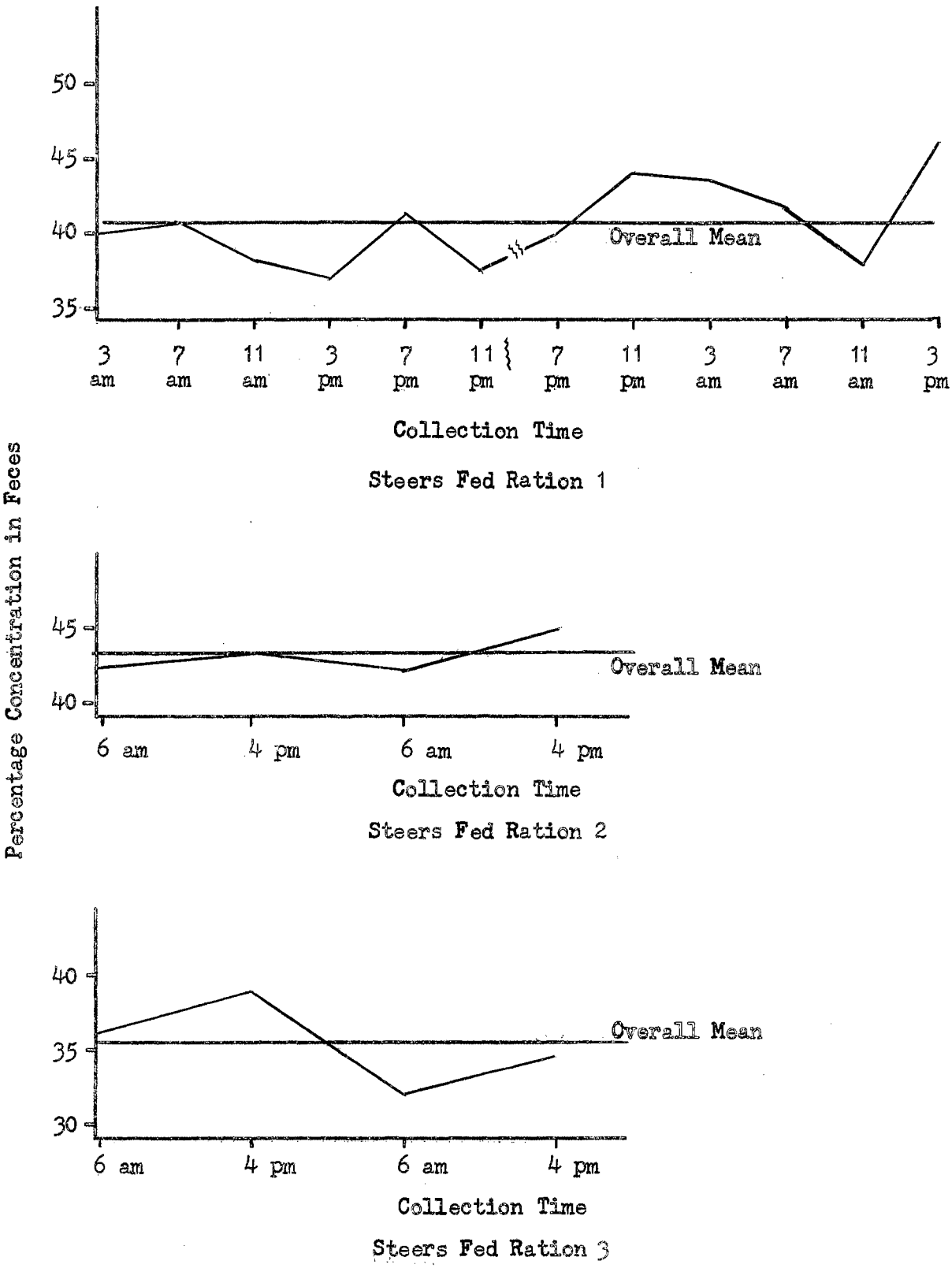

Figure 1. Percentage Fecal Polyethylene Concentration Plotted Against Time for Steers Fed Rations 1, 2 and 3

TABLE IV

PERCENTAGE MEAN POLYETHYLENE CONCENTRATION OF FECAL SAMPLES FROM STEERS FED RATIONS 2 AND 3, TRIAL 2, EXPERIMENT II

Time	Steer 6	7	23	26	36	Mean
			Ration 2			
6 a.m.	44.7	41.2	38.1	49.0	40.2	42.6
4 p.m.	44.8	40.9	40.8	42.4	48.2	43.4
6 a.m.	47.0	34.1	34.9	46.4	49.3	42.3
4 p.m.	40.4	42.1	43.3	44.5	55.0	45.0
Mean	44.2	39.6	39.3	45.6	48.1	
Overall mean						43.3

	Steer 9	15	19	24	29	39	Mean
			Ration 3				
6 a.m.	34.5	24.9	45.2	45.1	32.5	32.8	35.8
4 p.m.	29.7	37.0	42.2	26.3	31.7	25.4	32.0
6 a.m.	25.2	37.9	29.5	36.4	36.1	29.5	32.4
4 p.m.	39.4	38.7	22.4	39.3	35.4	32.9	34.7
Mean	32.2	34.6	34.8	36.8	33.9	30.1	
Overall mean							33.7

that ration is superimposed on each graph as an aid in depicting the deviation from the mean at any time.

The variance associated with duplicate determinations of polyethylene concentration on each sample and the variance associated with different samples was determined by analysis of variance, for the steers fed ration 2, as shown in Appendix Table XII. The average variance observed between duplicate analyses of the same sample was 2.90. The standard deviation between these duplicate analyses was 1.70 percentage units. When the average variance observed between duplicate analyses of the same samples was expressed as a percentage of the total variance the portion attributable to differences between duplicate analyses was only 0.15 percent while that amount of the total variance due to differences between samples was 0.36 percent.

Since these data were proportionate the analysis for subsampling was used to test for differences between times and between steers. This analysis of variance is reported in Appendix Table XIII. A significant interaction ($P < .01$) was found between steers and time, in addition the variance due to differences between steers was found to be significant ($P < .01$). However, the variance due to differences between times was not significant.

The variance associated with duplicate determinations of polyethylene concentration on each sample and the variance associated with different samples was determined by analysis of variance, for the steers fed ration 3, as shown in Appendix Table XIV. The average variance observed between duplicate analyses of the same sample was 41.11. The standard deviation between these duplicate analyses was 6.41 percentage units. When the average variance observed between duplicate analyses

of the same samples was expressed as a percentage of the total variance the portion attributable to differences between duplicate analyses was 3.49 percent while that amount of the total variance due to differences between samples was 3.32 percent.

Since these data were proportionate the analysis for subsampling was used to test for differences between times and between steers. This analysis of variance is reported in Appendix Table XV. A significant interaction ($P<.01$) was encountered between steers and time; however, neither the variance attributable to differences between steers nor times was significant.

The results of the first phase of this experiment would indicate that there is no significant diurnal variation in percentage fecal polyethylene concentration among any of the times studied. There does appear to be a significant effect due to differences between steers but this could be expected in lieu of the great variability exhibited by biological materials. Ample numbers of experimental animals should be used to allow for this variability. It is thought that the interaction between steers and times was caused by the fact that excretion patterns between steers were different.

Phase 2

The mean percentage concentration of polyethylene in the four digestive tract compartments studied is presented in Table V. Since these data were proportionate the analysis for subsampling was used to test for differences between compartments and between steers. This analysis of variance is reported in Appendix Table XVI. A significant interaction ($P<.01$) was found between steers and compartments. In addition the variance due to differences between compartments was found to be

TABLE V

PERCENTAGE MEAN POLYETHYLENE CONCENTRATION
OF DIGESTIVE TRACT CONTENTS

Compartment	Steer				Mean
	21	27	43	46	
Abomasum	19.3	22.8	47.8	34.6	31.1
Rumen	54.5	27.9	39.2	42.2	41.0
Large intestine	43.8	32.4	43.7	38.9	39.7
Duodenum	8.9	12.8	11.7	3.5	9.2
Mean	31.6	24.0	35.6	29.8	
Overall mean					30.3

significant ($P < .01$); however, the variance attributable to differences between steers was not significant. The coefficients of variation, means and standard deviations for each compartment are given in Table VI. Based on these results it would appear that the duodenal ingesta was the most variable with respect to percentage polyethylene concentration, followed by ingesta taken from the abomasum, rumen and large intestine in decreasing order of variability.

The results of the second phase of this experiment would indicate that there was a significant variation in the concentration of polyethylene in the various compartments of the digestive tracts of the cattle used in this trial. Due to the great variation in percentage concentration of polyethylene in the ingesta collected from different compartments of the tract, it would appear to raise some doubt as to the value of this technique for rate of disappearance studies. Since the numbers

TABLE VI

CONCENTRATION OF POLYETHYLENE BY COMPARTMENT

Compartment	Mean Percentage	Standard Deviation	Coefficient of Variation
Duodenum	9.23	5.80	62.88
Abomasum	31.10	11.75	37.78
Rumen	40.95	9.95	24.29
Large Intestine	39.71	5.08	12.79

involved in this study were small, additional studies would be desirable to more clearly evaluate concentration uniformity among the different digestive tract compartments.

Experiment III

Procedure

This experiment was designed to study the length of preliminary feeding period required in a digestion study by determining the time required for a fecal polyethylene concentration curve to reach its peak. Following this preliminary period of study, a total fecal collection period was conducted to study the variability in polyethylene excretion patterns and to compare digestion coefficients obtained by the polyethylene technique with those obtained by the total collection method.

Eight Hereford steers averaging 315 kilograms in weight were used as experimental animals. These steers had been used previously in a series of digestion trials by Brown (1966). Following a 14-day rest

period the animals were placed in metabolism stalls described by Nelson et al. (1954).

The steers were fed seven kilograms of feed per day in two equal portions at 6:00 a.m. and 6:00 p.m. The composition of the ration is given in Table VII. Representative feed samples were taken and ground in a Wiley mill for proximate analysis by the method of A.O.A.C. (1960). The proximate composition of the feed is presented in Table VIII. Polyethylene was added, as the reference material, at the rate of five percent of the ration.

Fecal grab samples were taken 24 hours after the initiation of polyethylene administration and every six hours thereafter for the duration of the trial. Immediately after collection, the samples were placed in labeled plastic bags, frozen and stored for future analysis.

After eight and one-half days the preliminary period was terminated and total fecal collections were made for the subsequent seven days. At the conclusion of each 24-hour period during the total collection phase of this experiment the feces were mixed thoroughly with an electric mixer and a five percent aliquot was retained. The samples from each animal were then wet-bulked and dried in a forced air oven at 70° C. At the conclusion of the trial each composite fecal sample was ground in a Wiley mill and representative samples taken for proximate analysis by the method of A.O.A.C. (1960).

At the conclusion of the trial the fecal grab samples and samples obtained from the composite sample were analyzed by the analytical procedure described in Experiment I.

TABLE VII

COMPOSITION OF RATION 4

Ingredient	Percentage
Steam rolled milo	65.845
Cottonseed hulls	20.000
Cottonseed meal (41 % solvent)	4.000
Dehydrated alfalfa meal (19 % protein)	5.000
Molasses	3.000
Urea (262)	1.000
Salt	.500
Calcium carbonate	.600
Vit. A premix	.030
Trace mineral premix	.025
Added Per Kilogram	
Polyethylene fluff (alathon)	50 g.

TABLE VIII

PROXIMATE ANALYSIS OF RATION 4

Dry Matter %	Ash %	Crude Protein %	Fat %	Crude Fiber %	N.F.E. %
91.55	4.14	11.99	3.10	14.25	58.08

Results and Discussion

The length of time required for a fecal polyethylene concentration curve to peak was four days. This is illustrated in Figure 2. The length of time required for polyethylene concentration to peak was considerably less than the seven day preliminary period requirement reported by Chandler et al. (1966).

Dry matter digestion coefficients obtained during the seven day collection period by the total collection technique and those obtained during this period by the polyethylene technique are shown in Table IX. Mean dry matter digestibility values determined by the two methods were 68.62 percent and 68.95 percent, respectively, for the polyethylene and total collection techniques. Using the paired "t" test no significant difference was found between dry matter digestion coefficients obtained by the two methods. The correlation coefficient between dry matter digestibility values determined by the two methods was 0.92. The standard error of the digestion coefficients determined by polyethylene was 1.28 percentage units while that of the digestion coefficients determined by total collection was 0.67.

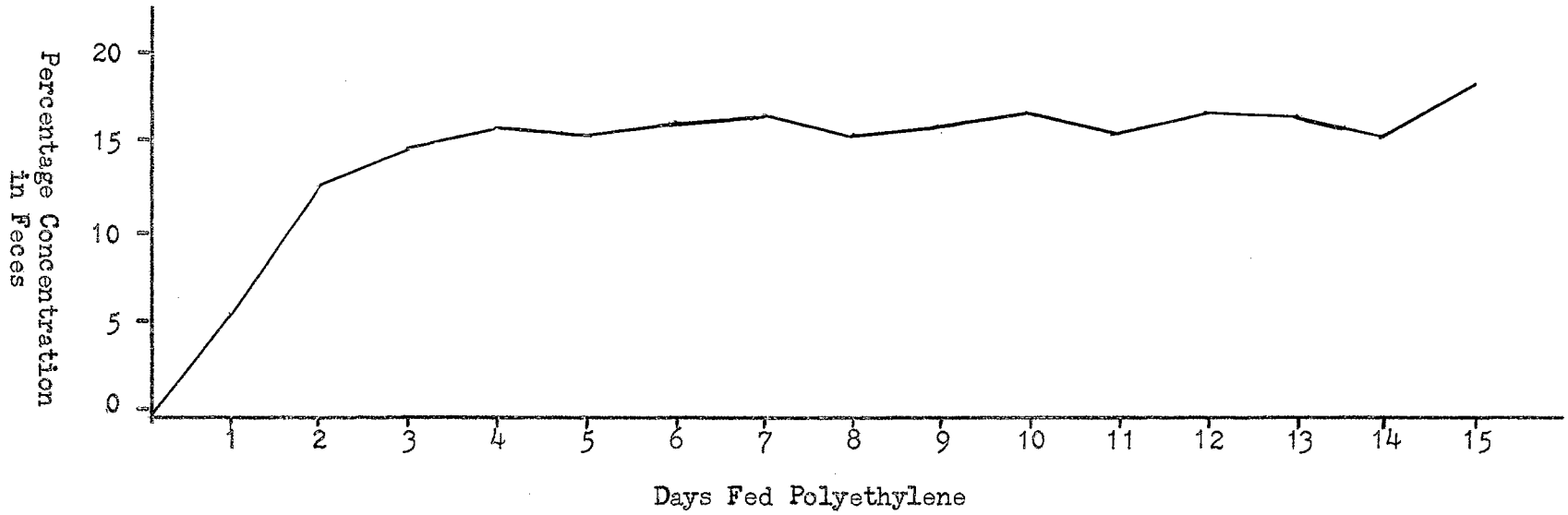

Figure 2. Percentage Fecal Polyethylene Concentration Plotted Against Time for Steers Fed Ration 4.

TABLE IX

DRY MATTER DIGESTION COEFFICIENTS CALCULATED FROM THE POLYETHYLENE TECHNIQUE AND FROM THE TOTAL COLLECTION METHOD

Day	Polyethylene	Total Collection
1	71.21	69.46
2	67.93	68.29
3	70.18	68.75
4	72.14	71.48
5	72.63	71.87
6	62.03	66.43
7	65.66	67.51
$\bar{x}$	68.62	68.95

Due to an oversight records of fecal moisture content were not obtained. To compensate for this the moisture content of fecal samples obtained from similar steers receiving ration 4 minus polyethylene was employed. The average of the values obtained in this manner was then adjusted by the following method to include polyethylene:

$$X - .1606X = \text{dry weight}$$

$$.8394X = \text{dry weight}$$

$$\frac{\text{Dry weight}}{.8394} = \text{adjusted dry weight}$$

$$\left[\text{Adjusted dry weight} - (\text{adjusted dry weight} + \text{water})\right] \times 100 =$$

$$\text{percentage adjusted dry matter}$$

where:

.1606 = mean percentage fecal polyethylene concentration

X = adjusted dry weight.

A value of 66.65 percent moisture on a polyethylene adjusted basis was obtained when this procedure was utilized. It was felt that this method might have underestimated the true amount of moisture in the feces; however, all digestion coefficients reported for the total collection technique were based on this value. Using the moisture content described above it was calculated that 96.63 percent of the polyethylene fed was recovered in the feces.

The pooled mean percentage concentration of fecal polyethylene for all steers is shown plotted against time in Figure 3. The overall mean is superimposed on the graph to illustrate how the concentration at each collection time differed from the average for all times.

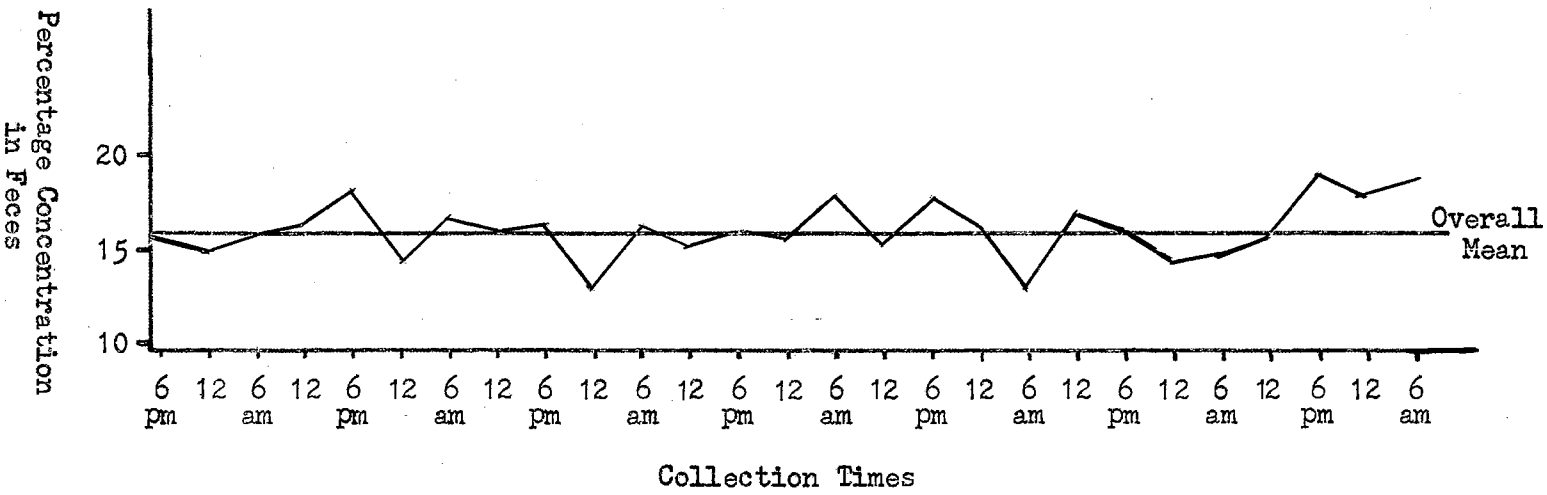

Figure 3. Percentage Fecal Polyethylene Concentration of Steers Fed Ration 4 at Individual Collection Times During the Seven-Day Collection Period

The mean fecal polyethylene concentration of all grab samples obtained during the seven day collection period was 16.06 percent which is in extremely close agreement with the 15.96 percent mean of the composite samples analyzed by the polyethylene technique.

The variance associated with duplicate determinations of polyethylene concentration on each sample collected during the preliminary period and the variance associated with different samples collected during the collection period was determined by analysis of variance as shown in Appendix Table XVII. The average variance observed between duplicate analyses of the same sample was 1.19. The standard deviation between these duplicate analyses was 1.09 percentage units. When the average variance observed between duplicate analyses of the same samples was expressed as a percentage of the total variance the portion attributable to differences between duplicate analyses was only 0.55 percent while that amount of the total variance due to differences between samples was 13.65 percent.

The variance associated with duplicate determinations of polyethylene concentration on each sample collected during the collection period and the variance associated with different samples collected during the collection period was determined by analysis of variance as shown in Appendix Table XVIII. The average variance observed between duplicate analyses of the same sample was 3.38. The standard deviation between these duplicate analyses was 1.84 percentage units. When the average variance observed between duplicate analyses of the same samples was expressed as a percentage of the total variance the portion attributable to differences between duplicate analyses was only 1.25 percent while that amount of the total variance due to differences between samples was 7.32 percent.

Since these data were disproportionate in some cases, the method of unweighted means described by Snedecor (1956) was used to test for differences between times and between steers. This analysis of variance is reported in Appendix Table XIX. A significant interaction ($P < .01$) was found between steers and time, in addition, the variance due to differences between steers was found to be significant ($P < .01$). However, the variance due to differences between times was not found to be significant.

The results would indicate that the fecal excretion of polyethylene peaks at approximately four days after initial administration in the type of ration used in this study. This may eliminate the need for lengthy preliminary feeding periods in digestion trials conducted by this method when the type of ration is similar to that used in this study.

Since variation in polyethylene concentrations at the different collection times was not significant it would appear that there is no significant diurnal variation in polyethylene excretion by steers. Thus digestion coefficients calculated from fecal samples taken at any time could be considered as valid, especially since digestion coefficients calculated from the polyethylene technique were so highly correlated with those from the total collection method. All of these results would lead to the conclusion that polyethylene is a reliable index material for digestion studies with cattle.

SUMMARY

Alathon, a polyethylene material in fluff form, was evaluated as a reference material for digestion studies with cattle. In Experiment I the accuracy with which polyethylene could be quantitatively determined in feed or fecal samples by a gravimetric technique was evaluated. In Experiment II excretion patterns and the uniformity of concentration of polyethylene in digestive tract ingesta and fecal excreta were studied. In Experiment III the length of time necessary for a fecal polyethylene concentration curve to peak was plotted and coefficients of digestibility determined by the polyethylene reference technique and by the total fecal collection procedure were compared.

The average percentage recovery of polyethylene from 68 samples containing known quantities of the material was 99.89 percent. When samples containing no polyethylene were analyzed by this technique the average quantity of naturally occurring feed constituents that were isolated after digestion and separation was 0.07 percent.

No significant diurnal variation was found in the excretion rate of polyethylene but the concentration of polyethylene in fecal excreta tended to vary between animals. This was thought to be due to biological variability among animals. A significant interaction was observed between steers and collection times which was possibly due to differences in the excretion patterns of the steers. The concentration of polyethylene in digestive tract ingesta was not found to be uniform in different compartments of the digestive tract.

A preliminary period of four days was found to be necessary for a fecal polyethylene concentration curve to peak. A correlation of 0.92 was found between dry matter digestibility coefficients determined by the polyethylene reference technique and by the total fecal collection procedure. Mean dry matter digestibility values for a typical finishing ration as determined by the polyethylene technique and the total collection method were 68.62 percent and 68.95 percent, respectively.

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A P P E N D I X

TABLE X

ANALYSIS OF VARIANCE FOR VARIANCE DUE TO DETERMINATION FOR STEERS FED RATION 1

Source	df	Mean Square
Total	97	
Between samples	48	39.077
Within samples (error)	49	10.840

TABLE XI

ANALYSIS OF VARIANCE FOR UNWEIGHTED MEANS FOR STEERS FED RATION 1

Source	df	Mean Square
Total	108	
Steers	4	50.220
Time	11	22.439
Interaction	44	52.010**
Determinations	49	10.840

**P <.01.

TABLE XII

ANALYSIS OF VARIANCE FOR VARIANCE DUE TO DETERMINATION FOR STEERS FED RATION 2

Source	df	Mean Square
Total	39	
Between samples	14	68.053
Within samples (error)	15	2.903

TABLE XIII

ANALYSIS OF VARIANCE FOR SUBSAMPLING FOR STEERS FED RATION 2

Source	df	Mean Square
Total	39	
Steers	4	119.173**
Time	3	15.210
Interaction	12	35.871**
Error (within)	20	2.176

**P < .01.

TABLE XIV

ANALYSIS OF VARIANCE FOR VARIANCE DUE TO DETERMINATION FOR STEERS FED RATION 3

Source	df	Mean Square
Total	47	
Between samples	23	39.097
Within samples (error)	24	41.106

TABLE XV

ANALYSIS OF VARIANCE FOR SUBSAMPLING FOR STEERS FED RATION 3

Source	df	Mean Square
Total	47	
Steers	5	42.258
Time	3	39.177
Interaction	15	101.361**
Error (within)	24	1.523

**P < .01.

TABLE XVI

ANALYSIS OF VARIANCE FOR SUBSAMPLING
FOR DIGESTIVE TRACT INGESTA

Source	df	Mean Square
Total	47	
Steer	3	275.007
Compartment	3	2586.893**
Interaction	9	238.046**
Error (within)	32	9.167

**P < .01.

TABLE XVII

ANALYSIS OF VARIANCE FOR VARIANCE DUE TO DETERMINATION
DURING THE PRELIMINARY PERIOD FOR
STEERS FED RATION 4

Source	df	Mean Square
Total	447	
Between samples	223	28.6609
Within samples (error)	224	1.1856

TABLE XVIII

ANALYSIS OF VARIANCE FOR VARIANCE DUE TO DETERMINATION DURING COLLECTION PERIOD FOR STEERS FED RATION 4

Source	df	Mean Square
Total	415	
Between samples	207	19.7595
Within samples (error)	208	3.3802

TABLE XIX

ANALYSIS OF VARIANCE FOR UNWEIGHTED MEANS FOR STEERS FED RATION 4

Source	df	Mean Square
Total	423	
Steers	7	85.736
Time	26	15.164
Interaction	182	11.343**
Determinations	208	3.380

**P< .01.

VITA

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