POLYETHYLENE GLYCOL AS AN INDICATOR OF DIGESTIBILITY IN DAIRY COWS

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By

LLOYD E. CHRISTIE

AN ABSTRACT

Submitted to the College of Agriculture of Michigan State University of Agriculture and Applied Science in partial fulfillment of the requirements for the degree of

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Approved C A Lassiter

ABSTRACT LLOYD E. CHRISTIE

Eight mature dairy cows on hay-grain rations were used to evaluate the use of polyethylene glycol (PEG) as an inert reference substance in ruminant digestion studies.

Molecular weights of 4,000, 6,000, 9,000, and 20,000 were tested, using a turbidimetric procedure to analyze the feces for PEG. Fifteen gm. of PEG was administered daily to each cow.

Recoveries of PEG after passage through the digestive tract were: PEG-4,000, 77.7% (71.4-82.9%); PEG-6,000, 60.7% (55.5-66.0%); PEG-9,000, 58.7%; and PEG-20,000, 50.1%.

Excretion curves representing 24-hour periods revealed a definite excretion pattern with lowest concentrations of PEG at 4:00 p.m. and highest concentrations at 11:00 p.m.

Average recovery of PEG added to dried feces was 103.7%. When PEG was added to fresh feces, 78.7% of PEG-4,000 and 66.8% of PEG-9,000 was recovered. Essentially a linear relationship was found between weights of dried feces added to aqueous PEG solutions and weights of PEG removed from the solutions.

The most plausible explanation for the low recoveries observed, in view of the data presented, would seem to be adsorption of the PEG on the feces.

Under the conditions of the present experiment, PEG was not found to be a satisfactory indicator of digestibility.

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INTRODUCTION

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

Indicators may be used in large, group-feeding experiments since no special feeding or collecting equipment is normally required. Indirect methods therefore may be less expensive than direct methods of determining digestibility.

In the conventional approach of estimating digestibility; % apparent digestibility = $100 - 100 \left(\frac{\% \text{ nutrient in feces}}{\% \text{ nutrient in feed}} \times \frac{\text{feces weight}}{\text{feed weight}} \right)$

The digestibility formula, when using indicators becomes; % apparent digestibility = $100 - 100 \left(\frac{\% \text{ nutrient in feces}}{\% \text{ nutrient in feed}} \times \frac{\% \text{ indicator in feed}}{\% \text{ indicator in feces}} \right)$

Digestibility indicators may be divided into two categories; "external" and "internal" indicators. External indicators are those not normally present in the feedstuff, while internal indicators are natural feed constituents.

To give a valid estimation of digestibility an external indicator substance must (1) be quantitatively recovered in the feces (not produced, absorbed, broken down, or adsorbed in the digestive tract); (2) be non-toxic to the animal and the microorganisms of the digestive tract;

(3) have no effect on the digestibility of the ration; (4) pass through the digestive tract in a manner identical to the nutrient or nutrients being studied; (5) be readily analyzed by physical or chemical methods; and (6) have a distinct excretion pattern over the 24-hour day.

It is desirable that the external indicator substance (1) be inexpensive, and (2) reach an equilibrium rapidly in the digestive tract.

Since no material has thus far been shown to satisfy all these requirements, the present experiment was conducted to further evaluate polyethylene glycol as an indicator substance.

REVIEW OF LITERATURE

The earliest known balance trials were conducted with a milk cow in 1839 by Boussingault.

Digestibility trials were conducted in Germany by Henneberg and Stohman in 1864, according to Schneider et al. (1955). Just 10 years later, the first indicator of digestibility was used.

Silicon. Wildt (1874) made use of silicon to indirectly study the digestibility of hay and straw by sheep and obtained 86.1-91.6% recovery of the indicator. Close approximations of digestibilities were observed by Skulmowski et al. (1943) with sheep and horses, and Druce and Willcox (1949) with rabbits, using silicon ratios. Gallup (1929) obtained digestibilities of protein by rats using the silicon ratio method which varied by only 0.01-0.50% from those obtained by the conventional method.

Knott et al. (1936) concluded that the silicon ratio technique was unreliable due to contamination of the feed with soil. Gallup and Kuhlman (1936) observed wide variations in silica content of silage and recovered only 85% after passage through the digestive tract of cows. Mineral balance studies by Forbes et al. (1916) indicated the existence of extensive metabolism of silicon by the cow. Gallup et al. (1945) recovered 105.6-111.6% of silica fed to sheep. Gallup and Kuhlman (1936) observed variations in feces concentration of silicon during the day with cows.

Iron Oxide. Bergeim (1926) proposed the use of iron oxide as an

indigestible reference material. Hamilton et al. (1927) found close agreement between the results of iron oxide ratios and the conventional digestibility trial technique with steers. Favorable results were also reported by Gallup (1929) with rats. Low digestibility estimates were reported using iron oxide ratios by Gallup (1928) and Heller et al. (1928) with rats, Moore and Winter (1934) with cows, and Druce and Willcox (1949) with rabbits.

The use of naturally occurring iron in the feedstuff as an indicator was suggested by Heller et al. (1928). Knott et al. (1936) pointed out that iron occurred in feeds in such small amounts that determination methods were inadequate. Hale et al. (1940), working with cows, concluded that the iron ratio technique was unreliable due to accumulation of iron in the rumen in some instances and passage from the rumen ahead of the ingesta at other times.

Titanium Dioxide. Fournier et al. (1954) reported that titanium dioxide passed through the digestive tract of the rat at a rate similar to that of phosporous and was useful in absorption studies of that element. Lloyd et al. (1955) reported 99.8% recovery of titanium dioxide over a 7-day period with rats.

Barium Sulfate. Brown (1954) found great variations in the concentration of barium sulfate from day-to-day in the feces of cows. Muller (1953) encountered difficulties in chemical determination of this indicator from the feces of chickens. Radioactive barium sulfate was tested by Van Der Kley (1956), who reported large variations in excretion rate and large day-to-day variations with steers.

Dyes. Anthraquinone violet was found to be rapidly absorbed from doubly-ligated rumens of open-abdomen calf preparations and rumen-fistula calves by Flatt et al. (1956). An average recovery of 72.4% was found after 1 hour. Corbin and Forbes (1951) obtained an average recovery of 100.5% of this dye and observed a variation in feces concentration during the day with sheep.

Monastral blue (copper phthalocyanin) was tested by Coup and Lancaster (1952) by addition to cow feces. The average recovery was 99.6%. Lambourne (1957a) obtained 101-109% recovery of a single dose of this dye in 72 hours using sheep. Slightly lower recoveries were observed when administered in a hard gelatin mixture than in powder form. Higher recoveries of this dye were found with morning than evening dosage.

"Oil Red O" was only 81.6% recovered from lambs by Corbin and Forbes (1951).

Chromic Oxide. The most widely used external indicator of digestibility is chromic oxide (chromium sesquioxide). Edin (1918) recovered chromic oxide almost completely when fed in the ration impregnated in blotting paper. Edin (1926) reported that a 2-day collection period using chromic oxide ratios gave as accurate digestion coefficients as a standard 7-day trial.

Acceptable recoveries and digestion coefficients using chromic oxide ratios were obtained by Kane et al. (1949, 1953a, 1953b, 1957), Crampton and Lloyd (1951), Smith and Reid (1955), and Putnam et al. (1957) working with cows; Hardison and Reid (1953) and Brannon et al. (1954) with steers; and Armstrong and Preston (1954) with calves. The chromic oxide

technique was considered valid by Andersen (1934), Skulmowski (1943), Raymond and Minson (1955), Woolfolk et al. (1955), and Pigden and Brisson (1956) using sheep.

Satisfactory recoveries of chromic oxide were also reported by Skulmowski (1951) with horses; Schurch (1952) and Schurch et al. (1952) with swine; Chands et al. (1951) with goats; Lloyd and McCay (1954) with dogs; Eriksson (1953) and Yoshida and Morimoto (1957) with hens; Huang (1954) with rabbits; Schurch et al. (1950) and Schurch (1952) with rats; and Kreula (1947), Irwin and Crampton (1950), Virtanan (1950), and Schurch (1952) with humans. Edin (1954) reported favorable results with chromic oxide using fox and mink.

Chromic oxide was used successfully in conjunction with chromogens in estimating pasture digestibility and dry matter intake by Kane et al. (1953a, 1953b) with cows and Brannon et al. (1954) with steers. Kane et al. (1957) reported satisfactory results using radioactive methods of analyzing feces for chromic oxide.

Even though Lancaster and Coup (1953) recovered 100-102% of the chromic oxide fed, they found a coefficient of variation over a 5-day period of 10% with cows. Linkous et al. (1954) noted large between-cow variations. Murdock et al. (1957) noted low recoveries of chromic oxide with heifers which were in estrus. Low recoveries were found by Crampton and Lloyd (1951) when chromic oxide was administered to cows in oats-molasses pellets. Hardison et al. (1953) recovered 106% of the indicator fed over a 7-day period with steers. Lassiter and Davis (1954) failed to obtain satisfactory results in one of three trials. Also working with steers, Oldfield et al. (1956) found 93.2% recovery of the

indicator. Woolfolk et al. (1950) found the chromic oxide ratio technique to be inaccurate due to variable recovery from the feces of calves.

Pigden (1953) failed to obtain complete recovery of chromic oxide from sheep. Schurch (1952) found that results were not satisfactory with sheep unless large amounts of concentrates were fed. Lambourne (1957a) recovered 83-104% of single doses of chromic oxide from sheep in 72 hours. Only 67-90% of single doses of chromic oxide given to sheep in a hard gelatin mixture was recovered. Lambourne (1957b) was able to estimate fecal output of sheep over a 10-day period within only 8% by the chromic oxide technique. Barnicoat (1945) recovered 75-95% of the chromic oxide fed to lambs, wethers, calves, and pigs. Schurch (1952) observed higher recovery of chromic oxide with sheep when larger doses were given. Lambourne (1957a) obtained a higher recovery from a morning dose than from one given in the evening to sheep.

An average recovery of 90% of single doses of chromic oxide fed to horses was found by Olsson (1950). Kameoka (1956) observed 10% day-to-day variations in chromic oxide excretion with goats. Olsson et al. (1949), Dansky and Hill (1952), and Mueller (1956) each obtained approximately 95% recovery of chromic oxide with chickens. Muller (1953) obtained only 88% recovery of this indicator with chickens. Mueller (1956) found higher chromic oxide recoveries with chickens fed a more finely ground ration.

Definite diurnal patterns of chromic oxide excretion have been reported by many workers, including Kane et al. (1950, 1952), Hardison et al. (1953), Reid (1952), Lancaster and Coup (1953), Oldfield et al. (1956), Murdock et al. (1957), and Putnam et al. (1957) with cattle;

and Muller (1953) and Mueller (1956) with chickens.

A diurnal variation necessitates care in establishing a grab-sampling regime. The most valid sampling times have not been established, however, since the various workers do not agree as to the time of day of highest and lowest feces concentrations of chromic oxide.

Andersen and Winther (1934) and Woolfolk et al. (1955) failed to find a definite pattern of chromic oxide excretion during the day with sheep. Raymond and Minson (1955) observed similar results with sheep and pointed out possible errors in sampling brought about by patterns of chromic oxide excretion due to grazing habits of animals on pasture. Barnicoat (1945) using sheep, calves, and pigs, and Clawson et al. (1955) using pigs reported results more nearly approximating the standard collection method when more samples were taken during the day than the usual two. Satisfactory results with a random-sampling program were obtained by Huang (1954) with rabbits and Schurch et al. (1950) with rats. Time of day of chromic oxide administration was found to have no effect upon excretion curves of cows by Smith and Reid (1955) or Hardison (1956)

Working with cows, Hardison (1956) noted a more even excretion of chromic oxide with twice-daily than with once-daily dosing. Irwin and Crampton (1950) found the same effect of dosing frequency with humans. Kameoka (1956) working with goats, found that more frequent dosing shifted the excretion curve and gave a more definite peak excretion. Pigden and Brisson (1956) found no diurnal excretion pattern with sheep dosed six times daily. Linkous et al. (1954) observed that the intervals between highest concentrations of chromic oxide in feces of cows during a 24-hour period corresponded to the intervals between dosing times.

Kameoka (1956) found less range of feces concentration during the day when larger amounts of chromic oxide were administered to goats. No effect of age of cattle upon the excretion curves was found by Hardison (1956).

Bloom et al. (1957) found little effect of level of hay and grain feeding on chromic oxide excretion curves of cows. Makaffey et al. (1954) reported greater ranges of chromic oxide concentrations in the feces of steers during the day with more frequent feeding. Little effect of hay:grain ratio on chromic oxide excretion curves of cows was found by Bloom et al. (1957). According to Barnicoat (1945) the fibrous nature or fineness of the feed did not alter excretion curves of sheep, calves, or pigs. Crampton and Lloyd (1951) found large variations in daily levels of chromic oxide excreted when administered to cows in oats-molasses pellets.

Makaffey et al. (1954) reported a smaller range of fecal concentrations when chromic oxide was administered to steers in pure form or dried with collodion than in gelatin suspension or baked flour paste with grain. Raymond and Minson (1955) were unable to reduce diurnal variation by the administration of chromic oxide in a bentonite solution to cows. A greater diurnal variation was observed when using gelatin capsules than when administering chromic oxide in the feed, according to Reid (1952). Barnicoat (1945), however, failed to find this difference with sheep, calves, and pigs.

Radiography studies by Corbett and Benzie (1953) showed the passage of chromic oxide in gelatin capsules directly into the omasum or abomasum of sheep in some cases. Similar observations were reported by

Balch et al. (1957) who found that as much as 60% of the chromic oxide administered to rumen-fistula steers in gelatin capsules passed to the omasum in 30-60 minutes. Lambourne (1957) observed fairly even mixing of chromic oxide in the rumen before passage to the remainder of the digestive tract of sheep.

Lignin. The use of the lignin portion of the plant as an indicator of digestibility was proposed by Ellis et al. (1946). Using a 72% sulfuric acid determination method, they found the lignin ratio technique to compare closely to conventional methods of estimating digestibility in cows, sheep, and rabbits. Bondi and Meyer (1943) found lignin digestibilities of 35.1-64.0% in green forages containing 15.1-22.8% lignin. Ely et al. (1953) observed 86.0-96.2% recovery of lignin from cows fed hay of varying degrees of maturity.

Methoxyl Groups. Richards and Reid (1952) suggested the use of the methoxyl groups of lignin as a digestibility indicator. Ely et al. (1953) found 20.3-31.6% digestibility of methoxyl groups of hay using sheep.

Normal Acid Fiber. "Normal acid fiber" ratios (15% sulfuric-acid method) were reported by Raymond et al. (1955) to closely approximate digestibilities found in the conventional manner with fresh forage fed indoors to cows.

Fecal Nitrogen. The total fecal nitrogen excretion of steers was reported by Gallup and Briggs (1948) to be related to dry matter intake in such a manner that either being known, the other could be closely

approximated with an appropriate factor. Forbes (1949) found no apparent constancy between fecal nitrogen excretion and dry matter intake of steers.

Plant Pigments. Reid et al. (1950) developed the "chromogen" ratio technique using steers, calves, and wethers. Spectral examination of 85% acetone extracts of feeds and feces at 406 mμ revealed 100.5% recovery of certain plant pigments in 36 trials.

Irvin et al. (1953) found pigment nitrogens or porphyrins to be satisfactory as digestibility indicators in chromatographic and spectrophotometric studies with alcohol extracts of forages and feces.

Kane and Jacobson (1954) proposed the use of pheophytin ratios for the determination of digestibility. No significant differences between the chromogen and pheophytin ratios and conventional methods of determining digestibility were found by Grainger and Woolfolk (1954) with lambs.

Polyethylene Glycol (PEG). The way was opened for the use of a different sort of inert reference substance when Shaffer and Critchfield (1947a) developed a gravimetric and a colorimetric method for the determination of solid polyethylene glycols in blood and urine. In terminal experiments with rats, Shaffer and Critchfield (1947b) found approximately 2% intestinal absorption of PEG-1,000 (PEG of molecular weight 1,000) and PEG-1,540 in 5 hours, but no absorption of PEG-4,000 or PEG-6,000. With human subjects, no significant absorption of PEG-4,000 was detected, but an absorption of 8% of PEG-1,000 was estimated. All molecular weights of PEG from 1,000 to 6,000 were found to be readily

excreted by the kidney through glomerular filtration.

Hyden (1955b) obtained recoveries of single drench doses of PEG-4,000 (3,500-4,200) from a rumen-fistula cow of 92.3% and an abomasal-fistula cow of 95.9%. In sheep, the recovery was 92.1%, hens 97%, and man 96.6%. The low recovery of 79.2% with the rabbit was attributed to delayed passage through the cecum. Almost perfect recovery was obtained when PEG was added to the excreta of these subjects, and in terminal experiments with a cow and goats in which the total digestive tract contents were analyzed. The Shaffer and Critchfield (1947a) method of determination was used.

Recoveries of 40-70% of the PEG-4,000 fed to cows in grass-meal cubes over 5-day periods were reported by Corbett et al. (1956). Using PEG-4,000 (3,000-3,700) Sperber et al. (1953) concluded that there was no significant precipitation, uptake, or adsorption of the indicator by rumen contents, on the basis of comparisons between centrifuged and non-centrifuged samples. Corbett et al. (1956), however, conducted in vitro trials which suggested adsorption.

No microbial breakdown or toxicity to rumen microorganisms in vitro in 24 hours was found by Sperber et al. (1953) using glucose utilization as an indication of microbial actibity. These findings are in agreement with those of Corbett et al. (1956). No destruction of PEG-4,000 was found over short periods of time in stored samples by Hyden (1955b). However, recovery results suggested destruction in the digestive tract over longer periods of time. Doses up to 500 gm. of PEG-4,000 per day produced no signs of toxicity in cows according to Sperber et al. (1953). No passage of PEG-4,000 through the rumen wall was believed to take

place by these workers or by Corbett *et al.* (1956) as none was detected in the urine.

Since the methods of Shaffer and Critchfield (1947a) were found to be time consuming and impractical for routine determinations of PEG, Hyden (1955a) introduced a simplified turbidimetric procedure for the determination of PEG in biological compounds. The method checked favorably with known concentrations of PEG in rumen contents and feces, and with the Shaffer and Critchfield (1974a) gravimetric method. Corbett *et al.* (1956) also tested the method and found that it checked favorably with the Shaffer and Critchfield (1947a) colorimetric method.

EXPERIMENTAL PROCEDURE

The polyethylene glycols are long-chain, highly polymerized hydrocarbon compounds with the general formula $HO-CH_2(CH_2-O-CH_2)_n-CH_2-OH$. The solid polyethylene glycols are wax-like compounds of varying degrees of hardness, soluble in water, aromatic hydrocarbons, acetone, and ethyl alcohol, and slightly alkaline.

This experiment was initiated to evaluate a wide range of molecular weights of PEG for use as digestibility indicators. The experiment was divided into two trials. PEG-4,000 was tested in Trial I, using four mature, healthy Holstein cows; A-110, K-239, K-139, and A-71. In Trial II, cows K-302 and A-71 were used with PEG-6,000, K-239 with PEG-9,000, and K-139 with PEG-20,000.

The ration fed in Trial I consisted of 20 lb. of alfalfa hay and 6 lb. of a grain mixture. In Trial II, 20 lb. of alfalfa hay was fed with 8 lb. of grain mixture. The rations were fed at 8:00 a.m. and 2:00 p.m. A 14-day preliminary feeding period was allowed in both trials.

In both trials, 15 gm. of PEG was administered orally with a balling gun to each cow daily in two 22 mm. X 75 mm. gelatin capsules, each containing 7.5 gm. of PEG. The capsules were administered at 6:30 a.m. in Trial I and at 8:15 a.m. in Trial II. No loss of capsules due to regurgitation or other causes was detected.

Total collection of feces was made in both trials with mechanical digestion stalls, beginning at the time of the first administration of

PEG. In Trial I, total collections were made for 16 days and Trial II for 11 days. Aliquot samples were obtained from each day's collection.

In Trial I, rectal grab samples (approximately 14 oz.) were taken from cows K-239 and A-71 at 2-hour intervals from 6:00 a.m. of the 11th day of the trial through 6:00 a.m. of the 12th day, and from 6:00 a.m. of the 13th day to 6:00 a.m. of the 14th day of the trial.

Feces samples were stored at 4-5°C. until analyzed, with the exception of the total collection aliquots of the first 5 days of Trial I, which were held at -20°C.

Analyses on all feces samples were completed within 2 weeks following the end of the collection period in Trial I, and on the last day of collection in Trial II. Daily total collection aliquots were analyzed for PEG content to obtain an estimate of the recovery of PEG after passage through the digestive tract. The rectal grab-samples were analyzed to obtain excretion curves for PEG for the 24-hour periods.

Analyses of PEG content of the feces were made by the turbidimetric method of Hyden (1955a). The only modification was the use of approximately 10 gm. samples instead of 1 gm. samples of feces. After macerating in water, the samples were allowed to equilibrate for 15-30 minutes before being diluted to 50 ml. The diluted feces samples were then centrifuged at 2,500 r.p.m. for 30 minutes to obtain a supernatant liquid for precipitation and filtration. A 5 ml. graduated syringe pipette was used to add the trichloroacetic acid-barium chloride solution to develop turbidity. Duplicate determinations were made on all samples. A standard curve was plotted for each group of filtrates analyzed.

Since a slight yellow color was observed in the filtrates, absorption and transmission curves were determined with a Beckman Model DK-2 Spectrophotometer. Maximum absorption was observed at a wave length of 300 mμ and maximum transmission at 750 mμ. Maximum transmission and absorption occurred at the same wave length with or without turbidity being developed in the samples. On the basis of these observations, turbidity was read in a Beckman Model B Spectrophotometer at 750 mμ, using a red photosensative tube with no filter.

Analyses for dry matter were conducted in duplicate on all rectal grab samples for excretion curves. Samples were dried to constant weight in porcelain crucibles at 100°C. in a convection oven.

To determine if PEG could be quantitatively recovered from biological materials under the analytical conditions of this experiment, PEG-4,000 was added to dried feces to give a concentration of 0.39 mg. PEG per gm. and mixed thoroughly in a mechanical mixer. Samples of the dried feces weighing 2 gm. were diluted to 50 ml. and allowed to equilibrate for 1 hour before centrifuging.

In a trial more closely approximating <u>in vivo</u> conditions, PEG-4,000 and PEG-9,000 were added to duplicate 300 gm. samples of slightly diluted fresh feces in Waring blendors to give concentrations of 0.67 mg. PEG per gm. These samples were mixed 3-4 minutes three times daily for 5 days and analyzed for PEG in the usual manner.

Another <u>in vitro</u> trial was conducted to determine if PEG was lost in the presence of fecal material. One set of samples of dried feces of weights 0.5 gm., 1.0 gm., 1.5 gm., 2.0 gm., and 2.5 gm. were placed in flasks containing 50 ml. each of 0.1 mg. per ml. aqueous solution of

PEG-4,000, and a set of duplicates were placed in flasks containing 50 ml. each of 0.1 mg. per ml. aqueous solution of PEG-9,000. The samples were allowed to set for 1 day with occasional shaking. The supernatant liquid was analyzed in the same manner as that from centrifuged, diluted feces samples to detect any change in concentration of the PEG solutions.

RESULTS AND DISCUSSION

Solubilities of the PEG compounds used in this experiment expressed as percent by weight in water were approximately as follows: PEG-4,000, 125%; PEG-6,000, 90%; PEG-9,000, 85%; and PEG-20,000, 55%. The compound PEG-4,000 (3,000-3,700) used by Sperber et al. (1953) was reported to be 60% soluble by weight in water. The use of a water-soluble compound as an indicator would seem to eliminate some of the difficulties encountered with high specific gravity substances due to complications in passage through the digestive tract.

Curves plotted from optical density readings on identical concentrations of PEG-4,000, 6,000, 9,000, and 20,000 all fell on the same line. Thus it was considered valid to use the simplified turbidimetric method of Hyden (1955a) for determination of this range of molecular weights. Hyden (1955a) found very similar curves for PEG-4,000 and PEG-6,000 when comparing effects of trichloroacetic-acid concentrations. However, it was found that molecular weights lower than 4,000 had considerably lower optical densities at a given trichloroacetic-acid concentration.

Hyden (1955a) reported the slope of the calibration curve to be stable. This was true in the present experiment with 0.05-0.10 mg. per ml. aqueous solutions of PEG until the solutions were 4-6 weeks old. At that time, the calibration curve was found to shift upward and take on a greater slope. The high stability of PEG in urine reported by Hyden (1955b) was for a 6-day period only.

The yellow color observed in the filtrates was felt to be due to the low feces:water ratio required to attain levels of PEG in the filtrates which could be detected with accuracy. Since Hyden (1955a, 1955b) used large doses of PEG, this problem was not encountered in their work.

In Vivo Recovery of PEG. The recovery percentages of all molecular weights of PEG tested, as shown in Tables I and II were low and approximately within the 40-70% recovery range reported by Corbett et al. (1956). Recoveries of all molecular weights tested were lower than the 93% (82.9-102.0%) recovery of single, large doses given cows by Hyden (1955a). An inverse relationship was observed between the molecular weight of the PEG compound and the percent recovery. There was no uniform tendency for daily recoveries to increase or decrease as the trial progressed with PEG-4,000 and only between the first and second collection days with the higher molecular weights. Thus it appears that PEG reaches a rapid equilibrium in the digestive tract, however the daily excretions observed were erratic with no constant day-to-day level of PEG excretion being reached.

The highest feces concentration of PEG attained with the 15 gm. daily doses used in this experiment was 0.99 mg. PEG per gm. of feces. Complete recovery was represented by approximately 0.6-0.8 mg. PEG per gm. of feces. Corbett et al. (1956) reported feces concentrations of 0.55-0.80 mg. PEG per gm. of feces with 25 gm. per day administration of PEG to cows. These workers found that increasing feces concentrations of PEG gave increasing, but still incomplete recovery. They suggested a feces concentration of 2.50 mg. PEG per gm. of feces, which would involve

Table I

Percent Recovery of Polyethylene Glycol-4,000
after Passage through the Digestive Tract

	Cow			
	A-110	K-239	K-139	A-71
Day				
1	52.7	62.7	53.1	60.3
2	61.6	57.1	105.5	84.3
3	67.1	89.4	83.9	74.3
4	61.0	114.3	117.3	116.1
5	136.2	80.1	116.9	69.0
6	79.8	80.9	81.5	88.4
7	60.2	93.1	65.1	70.6
8	73.7	96.8	83.3	64.1
9	103.7	92.5	105.9	78.3
10	98.9	69.8	99.3	128.6
11	62.8	69.9	65.4	61.4
12	75.2	85.8	50.4	59.4
13	85.1	96.6	64.5	82.6
14	113.6	62.6	87.3	45.3
15	128.9	54.2	56.9	20.8
16	66.1	61.8	00.0	39.1
Av. Recovery	82.9	79.2	77.3	71.4
Std. Dev.	±25.9	±17.3	±30.1	±26.6

Table II

Percent Recovery of Polyethylene Glycol after Passage through the Digestive Tract

Mol. Wt. PEG	6,000	6,000	9,000	20,000
Cow	K-302	A-71	K-239	K-139
Day				
1	24.8	27.1	00.0	00.0
2	57.2	44.1	48.7	43.9
3	55.5	41.2	48.3	38.1
4	38.2	35.9	31.3	45.4
5	114.6	74.2	65.8	76.8
6	51.9	73.2	67.8	54.2
7	51.2	44.8	53.4	48.6
8	48.2	59.4	42.7	46.8
9	62.7	46.9	73.6	38.1
10	69.4	64.9	69.3	53.9
11	110.9	70.4	85.7	54.9
Av. Recovery*	66.0	55.5	58.7	50.1
Std. Dev.	±26.0	±14.5	±16.5	±11.2

*Excluding 1st day's recovery.

administration of 80-100 gm. of PEG per day. Sperber et al. (1953) gave doses of 100-500 gm. of PEG per day and Hyden (1955b) administered 100-200 gm. of PEG per day to cows. The writer feels that these higher levels of PEG might be impractical for use in prolonged studies.

In Vitro Recovery of PEG. The calculated recovery of PEG added to dried feces (Table III) was assumed to be within the range of experi-

Table III

Recovery of Polyethylene Glycol-4,000 from Dried Feces

Sample	Recovery
	%
1	101.8
2	92.3
3	113.1
4	101.8
5	89.5
6	123.6
Average Recovery = 103.7%	

mental error due to the technical difficulties involved. The flaky nature of the PEG could very likely result in an uneven distribution of the marker leading to sizeable errors when using small dried feces samples. The results indicate that PEG may be recovered quantitatively with the alterations of the determination method imposed in this experiment.

Recoveries of PEG-4,000 and PEG-9,000 mixed with fresh feces (Table IV) were very similar to the recoveries of these compounds after passage through the digestive tract. In contrast, Hyden (1955a, 1955b) reported almost complete recovery of PEG added to the feces of cows and sheep.

Table IV

Recovery of Polyethylene Glycol added to Fresh Feces

Mol. Wt.	Sample	Recovery	Av. Recovery
		%	%
PEG 4,000	1a	74.6	78.7
	1b	73.1	
	2a	85.0	
	2b	82.1	
PEG 9,000	3a	68.7	66.8
	3b	67.2	
	4a	64.2	
	4b	67.2	

As shown in Table V, an essentially linear relationship was found between the weight of dried feces added to aqueous solutions of PEG-4,000 and PEG-9,000 and the weight of PEG removed from the solutions. The removal rate of PEG from the solutions was higher with the higher molecular weight of PEG. This observation is in agreement with the results observed with varying molecular weights in the studies on recovery of PEG after passage through the digestive tract and recovery from fresh feces.

Excretion Curves. The PEG excretion curves (Figure 1), drawn by plotting mg. PEG per gm. of feces dry matter against time, reveal a definite excretion pattern with the average low concentration of PEG at about 4:00 p.m. and the average high concentration at about 11:00 p.m. The high concentrations observed during the night can partially be accounted for by the higher dry matter content of the fecal samples taken during that part of the day.

Table V

Change in Concentration of Polyethylene Glycol Solution with Addition of Dried Feces

Mol. Wt.	Dry Feces Added	PEG/ml.	PEG Lost/gm. Feces Added
	gm.	mg.	mg./ml.
	0	0.104	--
	0.5	0.102	0.004
PEG	1.0	0.099	0.005
4,000	1.5	0.096	0.005
	2.0	0.094	0.005
	2.5	0.085	0.008
	0	0.104	--
	0.5	0.095	0.017
PEG	1.0	0.092	0.012
9,000	1.5	0.088	0.011
	2.0	0.085	0.010
	2.5	0.078	0.011

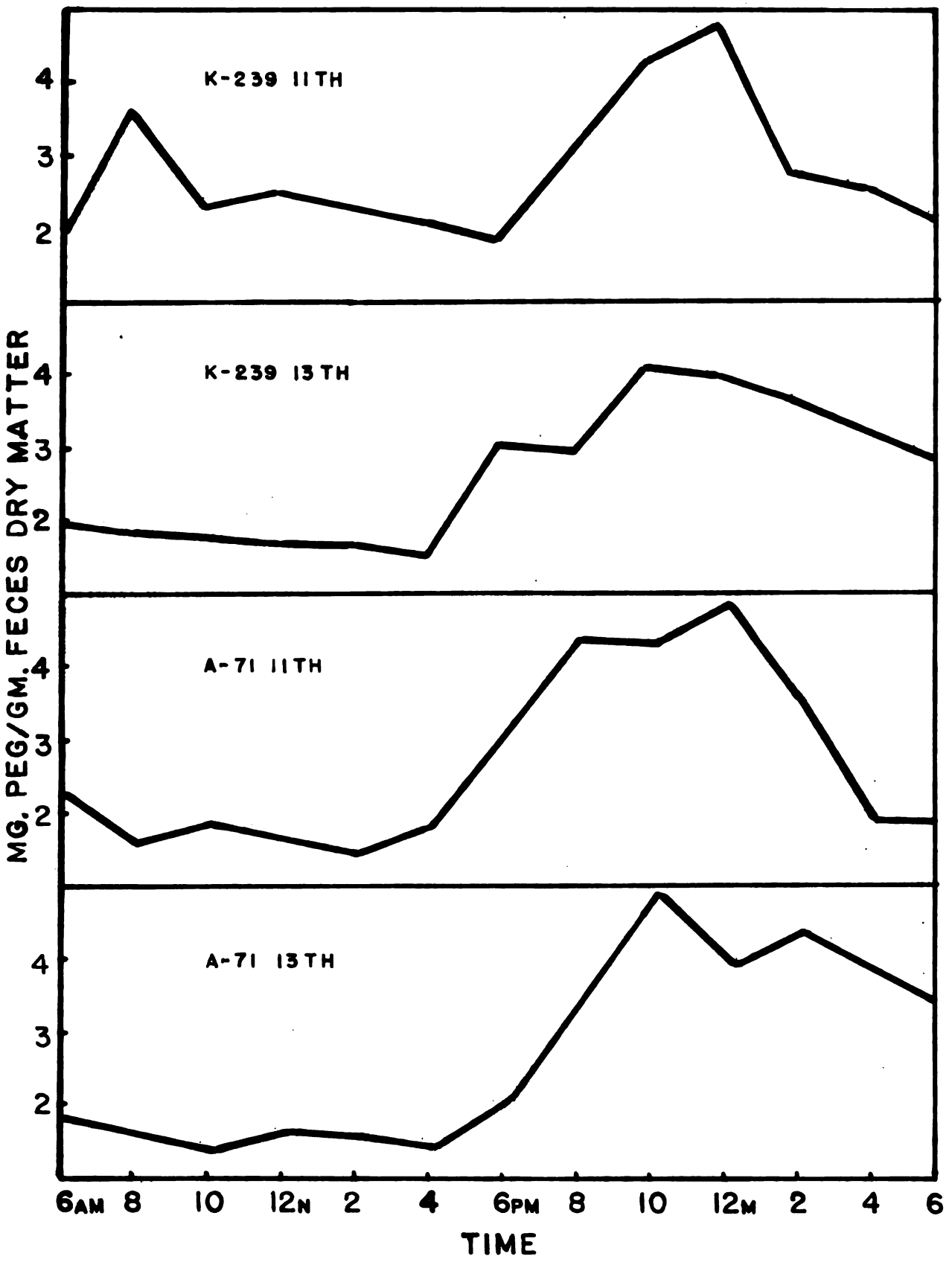

FIG. 1. EXCRETION CURVES OF POLYETHYLENE GLYCOL-4,000

Possible Mode of Loss of PEG. The loss of PEG from the solutions with the addition of dried feces could be due either to adsorption of the PEG on the feces or to removal from the centrifugation supernatant in the precipitation process.

No reliable quantitative method was found to determine if adsorption of PEG on the feces took place. Studies in which centrifuged, diluted feces samples were compared with diluted samples which were allowed to sediment without centrifugation revealed higher apparent concentrations of PEG in the centrifuged samples. However, due to the smaller amount of foreign material remaining in the supernatant liquid of the centrifuged samples, the differences were not considered conclusive. On the basis of similar tests with rumen fluid, Sperber et al. (1953) concluded that there was no significant adsorption, precipitation, or uptake of PEG-4,000 by rumen contents.

Attempts to carry through the sulfate-protein precipitation process with pure aqueous solutions of PEG, to determine if PEG is precipitated in the process, failed due to the incomplete filtration of barium sulfate. The result was a turbid filtrate which could not be used.

If PEG were precipitated by the sulfate-protein precipitating reagents, a constant weight of PEG would be expected to be lost. Since very small concentrations of PEG may be detected (Hyden, 1955b), the loss due to this type of interaction could not account for enough PEG loss to explain the low recoveries observed. The high recoveries of PEG from dried feces are also in opposition to this theory.

Since the loss of PEG from the aqueous solutions to which dried feces were added was a function of the weight of feces added, it would

seem that adsorption on the feces is the most likely mode of loss of PEG. Precipitation of PEG with the proteins or sulfates from the supernatant liquid due to adsorption to these components would allow the above linear relationship to hold, as well as adsorption on other feces constituents which would not allow the PEG to be released to the supernatant liquid for analysis. It may therefore be concluded that the most likely mode of loss of PEG in the digestive tract is through some type of adsorption on the feces.

The use of large doses of PEG, as by Hyden (1955b) and as suggested by Corbett et al. (1956) would make the losses of PEG observed in this experiment less significant in terms of total recovery of the indicator. This could explain the high apparent recoveries of PEG observed by Hyden (1955b). If the large amounts of PEG did not prove to be prohibitive, the practice of administering large doses might solve the major problems involved in the use of PEG as an indicator.

SUMMARY

Eight mature dairy cows on hay-grain rations were used to evaluate the use of polyethylene glycol (PEG) as an inert reference substance in ruminant digestion studies.

Fifteen gm. of PEG was administered daily. Molecular weights of 4,000, 6,000, 9,000, and 20,000 were tested, using the simplified turbidimetric method of Hyden (1955a) to analyze the feces for PEG.

Recoveries of PEG after passage through the digestive tract were: PEG-4,000, 77.7% (71.4-82.9%); PEG-6,000, 60.7% (55.5-66.0%); PEG-9,000, 58.7%; and PEG-20,000, 50.1%.

Excretion curves representing 24-hour periods revealed a definite excretion pattern with lowest concentrations of PEG at 4:00 p.m. and highest concentrations at 11:00 p.m.

Average recovery of PEG added to dried feces was 103.7%. When PEG was added to fresh feces, 78.7% of PEG-4,000 and 66.8% of PEG-9,000 was recovered. Essentially a linear relationship was found between weights of dried feces added to aqueous PEG solutions and weights of PEG removed from the solutions.

The most plausible explanation for the low recoveries observed in view of the data presented, would seem to be adsorption of the PEG on the feces.

Under the conditions of the present experiment, PEG was not found to be a satisfactory indicator of digestibility.

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