THE ARTIFICIAL RUMEN AS A TECHNIQUE FOR EVALUATING DRY MATTER DIGESTIBILITY OF PASTURE FORAGES

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INTRODUCTION

A simple and accurate technique for rapidly evaluating pasture forage quality is seriously lacking. Such a technique would be of great value to the plant breeder and others, in providing a rapid and inexpensive procedure for estimating forage digestibility and quality.

Digestion trials with ruminants provide information regarding the nutritive value of forage in a satisfactory manner, however they are expensive in terms of time, labor, and equipment and require large amounts of forage. Furthermore the investigator is working with a dynamic system, in which the quality of the forage may change considerably during the experimental period.

Recent reports on the use of the artificial rumen as a means of evaluating forage quality have indicated that this method may prove to be a satisfactory and reliable technique.

Therefore the principle purpose of this study was to evaluate the use of the artificial rumen as a technique for determining pasture forage quality.

REVIEW OF LITERATURE

The first studies on the digestibility of the dry matter of forages were made by Reamur (1752), Stevens (1777), and Spallanzani (1784), as cited by Huffman (1958). However, the earliest known metabolism trials were conducted with a milk cow in 1839 by Boussingault. According to Schneider (1955) in 1864 Henneberg and Stohman, pioneer German workers in the nutrition of farm animals, conducted digestibility trials, which have changed but little since their adoption.

Techniques in Forage Digestibility Evaluation

The evaluation of pasture forage, until recently, was primarily accomplished by two methods. One consisted of calculating the nutrients the cow received from an area of pasture forage, on the basis of her milk production and change in body weight. This method did not prove satisfactory because of the many variables involved. The second method consisted of harvesting the forage and feeding it to cows in digestion stalls. This method provided more accurate information on forage intake and digestibility, however the results seldom agreed with those obtained from grazing cows.

McCullough (1952) in his review pointed out that little progress was made in determining pasture forage quality by either of these two methods.

Therefore, the natural constituents and chemical composition of forage plants, indigestible "external" indicators and other techniques adaptable to grazing animals have been studied as a means of evaluating pasture forage quality.

Lignin Ratio Technique

Lignin, a natural constituent of plant material, has been studied as a possible plant indicator of digestibility. Ellis, Matrone and Maynard (1946), using the lignin ratio technique and the standard total fecal collection technique, found the digestibility to be similar in both techniques using cows, sheep, and rabbits. Hale, Duncan, and Huffman (1940) also reported that lignin may be used as an indicator for estimating cellulose digestion in the rumen. Kane (1950) found no significant difference between the lignin and chromic oxide techniques as compared to the conventional collection technique of evaluating digestibility coefficients with dairy cattle. Cook and Harris (1951) showed a variation in dry matter digestibility using the lignin ratio technique. Richards et al. (1958) reported considerable doubt on the predictive value of lignin as an indirect indicator of dry matter digestibility.

Nitrogen Ratio Technique

The calculation of forage digestibility by the forage and fecal nitrogen technique has been reported in a number of studies.

Gallup and Briggs (1948) reported that the total fecal nitrogen excretion of steers was related to dry matter intake and that a constant relationship existed. They reported, that feed consumption of grazing animals could be determined by taking advantage of this relationship to determine dry matter intake and that a close relationship existed between protein content and digestibility of nutrients in the hay. Lancaster (1949) presented a formula for evaluating pasture forage digestibility using fecal nitrogen as an indicator. Soni et al. (1954) reported that the fecal-nitrogen method appears to be as reliable as the chromogen method for determining dry matter digestibility and found no diurnal variation in nitrogen excretions when using Lancaster's formula. Forbes (1949) reported data to show that no constant relationship existed between dry matter intake and the excretion of fecal nitrogen.

Iron Oxide Technique

Iron oxide, as an indicator of the digestibility of feed, has been proposed by Bergeim (1926). Gallup (1929) obtained favorable results using the iron oxide technique with rats.

Heller *et al*. (1928) used the normal iron content of a ration fed to rats as an indicator for determining the digestibility of the ration. Knott (1936) compared the iron ratio technique using the natural iron content of feeds and reported it was not adaptable to ruminants. Hale (1940) in a study with alfalfa hay found the results highly variable and not reliable for use with ruminants. Druce and Willcox (1949) reported unreliable results with this method because of the low recovery of iron oxide.

Equations for Predicting Digestibility

Schneider *et al*. (1951) published equations for estimating the digestion coefficients of nutrients in feeds fed to cattle and sheep and in concentrates fed to swine. They reported that the use of these equations will result in prediction error variances which are 25 to 45 per cent below those incurred if tabled average digestion coefficients are employed. Equations were presented for predicting the digestion coefficients for organic matter, crude protein, crude fiber, nitrogen free extract, ether extract and the content of total digestible nutrients in feed, using their percentages of crude protein, crude fiber, nitrogen-free extract, and ether extract as predicting factors. These equations were reliable for only those feeds for which digestibility data were available. However, Schneider *et al*. presented equations to estimate the digestion coefficients of crude protein, crude fiber, nitrogen-free extract and ether extract and the content of total digestible nutrients in feeds for which few or no digestibility data were available. These equations should be distinguished from those designed for use with feeds for which sufficient digestible data are available as they do not yield as precise estimates.

Plant Pigment Technique

Indigestible pigments, occurring naturally in plants, which are fully recoverable from the feces can be reliable indicators of forage intake and digestibility. Reid *et al*. (1950) first reported the use of the plant pigment technique (chromogen ratio technique) using steers, calves, and wethers. Eighty-five per cent acetone was used as the solvent for extracting the plant pigment or pigments from the feed and feces and the diluted extracts were read at 406 mu using a Beckmann spectrophotometer. The authors reported nearly 100 per cent recovery of the plant pigment from the feces in a large number of trials. The calculated digestion coefficients from the plant pigment technique revealed close agreement with conventional total fecal collection techniques.

Cook et al. (1951) compared the lignin and chromogen techniques with sheep on mature winter range pasture and reported negative digestion coefficients and low estimates of dry matter intake. However, when lignin and chromogen techniques were used with alfalfa hay they reported no significant difference between the two methods. It would therefore appear that the chromogen technique may not work with all species of forage. Kane et al. (1953) in a study with lactating cows on orchard grass also found that the chromogen ratio technique did not agree with conventional digestion trials.

Reid et al. (1952) reported that there was a close relationship between forage chromogen concentration and fecal chromogen concentration and presented regression equations for the predication of forage digestibility. Different relationships were found to exist for dry hays and their corresponding fecal chromogen concentration than that reported with fresh grasses. Soni (1954) presented favorable results using Reid's et al. (1952) formula to determine dry matter digestibility of pasture forages with grazing sheep and cattle.

Noller *et al*. (1951) used the chromogen ratio technique with milk cows on pasture to determine forage intake and digestibility.

Chromic Oxide Ratio Technique

Chromic oxide, an inert tracer material, has been one of the most widely used "external" indicators of digestibility. Edin (1918) first reported the feeding of chromic oxide, impregnated in blotter paper as a tracer substance and reported almost complete recovery.

Kane *et al*. (1950, 1953a, 1953b, 1957) reported no significant difference between digestion coefficients using chromic oxide and the total collection method with cows. Smith and Reid (1955), Crampton and Lloyd (1951) and Putnam *et al*. (1957) also reported satisfactory results with the chromic oxide ratio when used with cows.

In other studies satisfactory results were reported with steers, by Brannon *et al*. (1954) and Hardison and Reid (1953); with sheep by Anderson (1934), Woolfolk *et al*. (1955), Skulmowski *et al*. (1943), and Pigden and Brisson (1956); with goats by Chanda *et al*. (1951); and with swine by Schurch *et al*. (1952) and Lloyd (1951).

Archibald *et al*. (1958) reported chromic oxide gave more uniform results than lignin in determining digestibility of forage with cows.

Crampton and Lloyd (1951) and Schurch (1952) reported a low recovery of chromic oxide when fed to sheep on an all roughage ration; however, when a concentrate was added to the ration satisfactory results were obtained.

Variability in the recovery of chromic oxide from feces of cattle have been reported by Kane *et al*. (1950, 1952), Reid (1952), Lancaster and Coup (1953), Hardison *et al*. (1953), Oldfield *et al*. (1956), Putman *et al*. (1957), and Murdock *et al*. (1957).

Smith and Reid (1955) and Hardison (1956) reported the time chromic oxide was given did not affect the excretion curves with cows. Hardison (1956) reported twice a day administration of chromic oxide to cows resulted in a more even rate of excretion.

Reid *et al*. (1952) reported greater diurnal variation when using gelatin capsules than when chromic oxide was given in the feed.

Kameoka (1956) reported results which showed that when sampling the feces twice a day for several consecutive days it was adequate for obtaining a representative sample. He also reported daily variation of chromic oxide excretion a normal occurrence amounting to 10 per cent.

Davis, Byers and Luber (1958) reported considerable variation in the excretion of chromic oxide at various hours of the day regardless of when administered. However, they indicated this variable could be overcome in digestion studies by sampling the feces for a ten-day period. Putnam et al. (1958) concluded that time of chromic oxide administration was of primary importance in respect to time-concentration relationships of fecal chromic oxide, and that diurnal effects were of little importance. They suggested that a 12-hour sampling procedure would be more accurate than unequal fecal sampling times.

Bloom et al. (1957) and Putnam (1958) reported no significant effect of proportion of hay and grain intake on chromic oxide excretion curves for cows.

Kane et al. (1953a, 1953b) reported favorable results were obtained in estimating pasture digestibility and dry matter intake when using chromic oxide in conjunction with the chromogen ratio technique.

Artificial Rumen Technique

One of the first serious attempts to duplicate the conditions of the rumen was made by Marston (1948), in studying cellulose digestion. Louw et al. (1949), Burroughs et al. (1950), and Huhtanen et al. (1952) further developed the artificial rumen and thus several types were sufficiently improved for use in attempting to evaluate forage quality.

Numerous studies have been reported on the modification and development of the artificial rumen and the reader is referred to Pigden (1955) and Salsbury (1955) for a more complete review.

Baumgardt and Hill (1956) reported on a study of dry matter digestibility of various forages in the artificial rumen and sources of error or bias. Dry matter losses noted were: Ladino clover, 65 per cent; alfalfa (bud stage), 58 per cent; Kentucky bluegrass (mature), 40 per cent; and wheat straw, 30 per cent. Kamstra _et_ _al_. (1958) reported on the effect of stage of maturity upon plant cellulose digestion in the artificial rumen. Orchard grass, alfalfa, and timothy were harvested at 3 stages of maturity with orchard grass decreasing as much as 50 per cent in rate of cellulose digestion, and timothy and alfalfa 30 per cent between the first and third stage of maturity.

Quicke _et_ _al_. (1959), in a study of milling by-products, reported higher digestibility _in vitro_, than in a conventional sheep digestion trials. However, they attributed this to the rapid passage of the feed through the rumen and therefore lower cellulose digestion in the rumen.

Kamstra (1955) in a preliminary experiment found that in a 24-hour _in vitro_ digestion period on orchard grass-alfalfa mixture, a pelleted feed mixture, and a corn and cob meal-poor hay mixture cellulose digestion of 60.33, 48.00, and 31.66 per cent, respectively. These same feeds showed *in vivo* digestion coefficients with cattle of 68.17, 46.10, and 41.61, respectively.

Pigden and Bell (1955) reported the TDN values estimated, by the use of a formula applied to the *in vitro* results of 11 forages, fermented for 48 hours, compared favorably with the TDN values obtained *in vivo* with sheep.

EXPERIMENTAL PROCEDURE

The purpose of this study was to evaluate the use of the artificial rumen in estimating pasture forage digestibility.

The experiment was designed in two parts. Part I was designed to evaluate the pasture forage digestibility, with dairy cows on pasture, calculating forage intake and dry matter digestibility with the chromic oxide technique of Hardison et al. (1953) and the chromogen (s) ratio of Reid et al. (1952). Part II consisted of calculating the dry matter digestibility of these same forages using the artificial rumen technique as modified by Pigden (1955).

Part I

Nine pasture forages differing in botanical composition or stage of maturity were compared. The nine pasture forages were as follows:

Trial I	Alfalfa-Ladino-Brome. 6 to 8 inches high. Preliminary period started May 22, 1956.
Trial II	June Grass. 8 to 10 inches high. Preliminary pasture period started May 22, 1956.
Trial III	Alfalfa-Brome (bud stage). 14 to 16 inches high. Preliminary pasture period started June 5, 1956.

Trial IV	June Grass (heading out). 15 to 17 inches high. Preliminary pasture period started June 5, 1956.
Trial V	Alfalfa-Ladino-Brome. (Clipped). 6 to 8 inches high. Preliminary pasture period started June 29, 1956.
Trial VI	Ladino Clover. (Recovered). 6 to 8 inches high.
Trial VII	Sudan Grass. 14 to 18 inches high (weedy). Preliminary pasture period started July 12, 1956.
Trial VIII	Kentucky bluegrass (clipped). 4 to 6 inches high. Preliminary pasture period started July 19, 1956.
Trial IX	Rye (poor condition). 8 to 12 inches high. Preliminary pasture period started July 19, 1956.

Three, mature, dry, Holstein cows were used on each pasture forage studied, using a seven-day preliminary period and five-day experimental, or collection period for each trial. The cows received no grain or feed supplements throughout the trial. Each cow received five grams of chromic oxide in a gelatin capsule administered orally with a balling gun twice daily at 5:30 a.m. and 5:30 p.m.

Rectal "grab" samples were taken from each cow at twelve-hour intervals, at 5:30 a.m. and 5:30 p.m. each day during the five-day experimental period and composited. The fecal samples were stored at 4 to 5° C. until the completion of the experimental period, when a total aliquot sample for each cow was frozen until analyzed.

Part II

Plucked forage samples were collected from each trial pasture on the second and fourth day of the experimental period, stored in sealed polyethylene bags and frozen immediately after collection. Every attempt in the collection of each forage sample was made to duplicate the kind and quality of forage consumed by the cows.

Preparation of Forage for Fermentation

Considerable difficulty was experienced in reducing the green frozen forage to a uniform particle size, adaptable for use in the artificial rumen and in securing duplicate forage samples. The technique which appeared to give the best results consisted of a hand model food chopper, maintained at or below a temperature of 0° C. Grinding of the frozen forage was completed in a walk-in freezer, to eliminate the thawing of the forage or the loss of plant juices and to facilitate a uniform mixing of the two-pound composite forage samples.

Micro-Artificial Rumen Technique

A description of the procedure as adopted as the standard procedure in the fermentation of the nine pasture forages is as follows:

Fifty ml. Erlenmeyer flasks, each equipped with a rubber stopper and a hypodermic needle plugged with cotton were employed as the fermentation vessels. Five grams of green ground forage was weighed into each flask. Five ml. of distilled water was added to each flask and autoclaved at 20 pounds pressure for 15 minutes. The flasks were allowed to cool and 0.5 grams of calcium carbonate and 3.7 ml. of complex salt solution were added to each. The flasks were then placed in a constant temperature water bath and aerated with carbon dioxide for thirty minutes. During this gassing process the flasks were connected in series and equipped with stoppers with gas inlet and outlet tubes. The flasks were swirled gently every eight to twelve hours to remove any forage particles which were deposited on the sides during the active fermentation period.

At the completion of the 48-hour fermentation period the flasks were placed in a drying oven at 105° C. to drive off the moisture. When the residues were nearly dry they were stirred thoroughly with a glass rod to prevent the residual calcium carbonate from caking on the bottom of the flask. Drying was then completed at a lower temperature of 50 to 60° C. When the fermentation residues were dry they were hydrolyzed with 20 ml. of 72 per cent sulfuric acid for two hours at room temperature. Acid and residue were carefully mixed with a glass rod three times, once when the acid was first added and twice more during the first hour. At the end of the hydrolysis period the contents of each flask were diluted to 500 ml. and allowed to set for approximately one hour to allow the insoluble residue to separate out. Anthrone carbohydrate determinations were then made on the supernatant.

Two extra flasks of each forage were prepared and dried immediately before fermentation occurred to obtain the carbohydrate present in each forage. These flasks were hydrolyzed with acid and analyzed for carbohydrate with their corresponding fermentation residues. The breakdown of carbohydrate in the fermentations was expressed as percentage carbohydrate fermented of the total initially present.

The composition of the complex salt solution was as follows:

Ingredients	Gm./2000 ml. Distilled Water
Sodium phosphate (monobasis)	52.50
Sodium bicarbonate	52.50
Potassium chloride	7.50
Sodium chloride	7.50
Magnesium sulphate	2.25
Calcium chloride	0.75

Ferrous chloride	0.15
Manganous sulphate	0.08
Zinc sulphate	0.08
Copper sulphate	0.04
Cobalt chloride	0.02

Inocula

The rumen fluid, obtained for the first run with the artificial rumen, was obtained from a fistulated Holstein cow fed a ration of June grass pasture. Rumen fluid from the same cow was obtained for the second run, however she had been returned to an alfalfa hay ration three days before collection of the fluid.

Analytical Methods

The method used to determine chromic oxide of the fecal samples was that of Gehrke and Baker (1954) and the Chromogen (s) technique was that of Reid *et al*. (1952). The chromogen unit was standardized against Reid's chromogen unit used in his laboratory and the samples were examined for light absorption with a Beckman B Spectrophotometer at 406 mu.

The anthrone carbohydrate determinations were made as used by Pigden (1955). A standard anthrone carbohydrate curve was prepared with glucose using a Beckman Model B Spectrophotometer at a wave length of 540 mu.

RESULTS AND DISCUSSION

The moisture content of the nine pasture forages used in this study are shown in Table I. There is a possibility that the low moisture values recorded for some of the forages may be due to the loss of moisture and dehydration of the forage samples during storage.

Table I

Moisture of Pasture Forages

Trial No.	Forage	% Moisture
I	Alfalfa-Ladino-Brome	74.3
II	June grass	68.5
III	Alfalfa-Brome	80.4
IV	June grass	71.3
V	Alfalfa-Ladino-Brome	77.1
VI	Ladino clover	80.5
VII	Sudan grass	65.4
VIII	Kentucky bluegrass	68.7
IX	Rye	76.0

The coefficients of digestibility of the forages in digestion trials with dairy cows as estimated by the

chromogen ratio technique of Reid et al. (1952) was employed as the criteria for evaluating the results obtained by the artificial rumen technique. For the purposes of this study the assumption was made that a close relationship exists between the per cent anthrone carbohydrate digested and the per cent dry matter digested.

The dry matter intake as determined by a combination of the chromogen and the chromic oxide technique is reported for each cow in Table II. The per cent digestible dry matter as determined by the chromogen technique for each forage is reported in Table III for each cow. The variability between cows on the same forage is less than the expected individual cow variation of 5 to 8 per cent generally reported.

Duplicate trials using the micro-artificial rumen technique were run for each forage and the results are recorded in Table IV. A high correlation coefficient was found to exist between runs for the anthrone carbohydrate present at the zero hour ($r = 0.822$) and 48 hour ($r = 0.973$) fermentation periods. On the basis of this close correlation the laboratory techniques employed in determining anthrone carbohydrate were considered satisfactory and reliable.

The correlation coefficient between runs for the per cent anthrone carbohydrate digested for each forage was not

Table II

Dry Matter Intake Per Cow

	Cow Number	Pounds D.M./Day
Trial I	103	13.2
Alfalfa-Ladino-Brome	19	17.3
	106	23.6
Trial II	20	21.4
June Grass	80	26.6
	107	29.6
Trial III	19	16.8
Alfalfa-Brome (Bud stage)	103	23.5
	106	23.1
Trial IV	107	24.8
June Grass (Heading out)	20	28.9
	80	26.0
Trial V	19	24.0
Alfalfa-Ladino-Brome (Clipped)	107	27.7
	103*	
Trial VI	20	24.3
Ladino Clover (Recovered)	107	31.4
	134	31.0
Trial VII	19	26.2
Sudan Grass	106	23.9
	24	20.8
Trial VIII	134	23.8
Kentucky Blue Grass (Clipped)	20	26.3
	106	28.1
Trial IX	94	24.3
Rye (Poor condition)	22	18.0
	20	17.1

* Removed from experiment due to calving.

significant (r = 0.598). This low correlation between runs must in part be attributed to an accumulation of errors resulting from the laboratory procedures employed in deter-

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Table III

Per Cent Dry Matter Digested

	Cow Number	% Digestion	Average
Trial I Alfalfa-Ladino-Brome	19 103 106	65.8 63.5 69.5	66.24
Trial II June Grass	20 80 107	66.6 71.0 72.1	69.91
Trial III Alfalfa-Brome	19 103 106	66.1 69.1 67.9	67.69
Trial IV June Grass	20 80 107	59.9 64.6 63.6	62.65
Trial V Alfalfa-Ladino-Brome	19 107 103*	70.1 72.2	71.13
Trial VI Ladino Clover	20 107 134	71.9 72.9 72.6	72.44
Trial VII Sudan Grass	19 24 106	69.3 67.6 69.4	68.76
Trial VIII Kentucky Blue Grass	20 106 134	62.5 62.5 58.0	60.90
Trial IX Rye	20 22 94	65.5 65.4 63.2	64.71

* Removed from experiment due to calving.

mining anthrone carbohydrate; however, the variability of the digestion coefficients observed between runs of the same forage must also reflect the errors incurred in

Table IV

Comparison of Duplicate Artificial Rumen Runs

	First Run			Second Run		
Trial	Hours Fermented	Average* ug Anthrone (0000)	% Digest.	Average* ug Anthrone (0000)	% Digest.	Average *In Vitro*
1	48 0	37 79	53	34 166	80	66
2	48 0	143 258	44	148 219	32	38
3	48 0	52 90	42	43 90	52	47
4	48 0	152 200	24	145 217	33	29
5	48 0	88 167	47	78 161	52	49
6	48 0	39 97	60	26 118	78	69
7	48 0	135 234	42	130 256	49	46
8	48 0	98 219	55	113 214	47	51
9	48 0	26 166	84	46 129	64	74

* The values in this column represent the average ug of anthrone carbohydrate from duplicate flasks.

obtaining uniform five gram forage samples and the errors resulting from the variability of the anthrone carbohydrate digested in the micro-artificial rumen. A major source of this error and one that apparently varies between different forages is the variability due to different lots

Table IV

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6	48 0	39 97	60	26 118	78	69
7	48 0	135 234	42	130 256	49	46
8	48 0	98 219	55	113 214	47	51
9	48 0	26 166	84	46 129	64	74

* The values in this column represent the average ug of anthrone carbohydrate from duplicate flasks.

obtaining uniform five gram forage samples and the errors resulting from the variability of the anthrone carbohydrate digested in the micro-artificial rumen. A major source of this error and one that apparently varies between different forages is the variability due to different lots of inoculum used in each trial. The difference between runs of the same forage, in the per cent anthrone carbohydrate digested, ranged from 4.3 to 26.4 per cent, with a mean difference for the nine trials of 12.7 per cent.

Whether the repeatibility of the digestion coefficients estimated by the artificial rumen can be improved can only be determined by further studies and the development of more accurate and reliable techniques.

The percentage of dry matter digested (chromogen technique) is compared with percentage of anthrone carbohydrate fermented at 48 hours *in vitro* and is reported in Table V. The percentages recorded for the chromogen method represents the average per cent dry matter digested by the three cows for each forage and the *in vitro* results represent the average per cent anthrone carbohydrate fermented in the two runs. The relative difference in per cent digestibility for each forage between the two methods would be of minor significance provided they were in the same relative direction; however, it is evident from Table V that this difference is of major importance as the levels of digestibility between the two methods for each forage varies significantly. The correlation coefficient between the two methods was not significant ($r = 0.158$) and substantiated this observation. The significant difference observed between methods may be due to several possible factors.

The lack of repeatibility between *in vitro* runs must be considered as a major source of this error. As discussed previously, the forage sampling and grinding procedures, and the use of different lots of rumen inoculum, may account for the low correlation which existed between the *in vitro* runs of the same forage.

Table V

Comparison of Estimated Dry Matter and Anthrone Carbohydrate Digestibilities

Trial	Name of Forage	% Dry Matter Digested (Chromogen)*	Estimated % Anthrone Digested**
I	Alfalfa-Ladino-Brome	66.2	66.3
II	June Grass	70.0	38.4
III	Alfalfa-Brome (bud stage)	67.7	47.2
IV	June Grass (heading out)	62.7	28.7
V	Alfalfa-Ladino-Brome	71.1	49.4
VI	Ladino Clover	72.4	69.1
VII	Sudan Grass	68.8	45.7
VIII	Kentucky Blue Grass	60.9	51.2
IX	Rye	64.7	74.3

* Average of three cows.
** Average of duplicate artificial rumen runs.

However, another source of error between the two methods may be due to the selective grazing of the cows, and the type and quality of the forage samples collected from the pasture for the *in vitro* studies. It can be observed (Table V, Trials I and VI) that when the type of pasture limited the selective grazing of the forage by the cows, a closer relationship appeared to exist between the digestion coefficients.

The results observed in this study however indicate that the major sources of procedural error must be overcome before the micro-artificial rumen technique can be considered as a reliable and accurate technique for the evaluation of pasture forage quality.

SUMMARY

Three mature Holstein dairy cows using the chromogen ratio technique of Reid _et al_. (1952) were used to estimate the dry matter digestibility of nine pasture forages. The resulting digestibilities were used as criteria for evaluating the results obtained from the same pasture forage using the micro-artificial rumen technique of Pigden (1955) with minor modifications.

Duplicate _in vitro_ runs with the same forage failed to show a significant correlation between runs. The major source of error was attributed to the preparation of forage, the forage sampling procedures, and to the variation in anthrone carbohydrate digestion between runs of the same forage.

No significant correlation was found to exist between the two methods studied. It was therefore concluded that under the conditions of the present experiment that the micro-artificial rumen technique was not a satisfactory technique for evaluating pasture forage quality.

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