A STUDY OF HERBAGE DIGESTIBILITY USING AN *IN VITRO* FERMENTATION TECHNIQUE

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Summary

Using the general method of Tilley and Terry (1963) the effects of the following variables on in vitro dry matter digestibility were investigated:

(i) between days and between sheep variability
(ii) method of sampling rumen liquor
(iii) source of inoculum
(iv) glass compared with nylon incubation vessels
(v) length of fermentation.

All variables except (ii) had a significant effect on in vitro dry matter digestibility. The source of inoculum had a significant effect on the stanadrd error of the estimate and on the predicted in vitro digestibility. ***In vitro*** digestibility increased with length of fermentation up to 96 hours. The ***in vivo*** technique proved to be suitable for estimating the digestibility of pasture material of varying maturity, and of commercial sheep cubes. Marked differences in in ***vitro*** digestibility occurred between glass and nylon incubation vessels.

I. INTRODUCTION

A number of assessments of herbage digestibility have been used to circumvent the need for animal digestibility trials. In this respect, ***in vitro*** studies have been extensively employed. Many, unfortunately, have not been run with animal digestibility trials concurrently to study how applicable the indices from ***in vitro*** studies are to the ***in vivo*** situation. Nor has there been any standardization of the technique between laboratories. This is important, since Minson (1963) has stressed that the relationship between any direct measurement (e.g. apparent ***in vivo*** digestibility) and an indirect measurement (e.g. ***in vitro*** digestibility), is never perfect. However, experiments have indicated that ***in vitro*** techniques may be as effective in estimating the nutritive value of herbage as the more conventional methods based on proximate analyses and digestibility data. It seems highly desirable that the likely errors, and their magnitude, associated with the ***in vitro*** method be defined before this system is adopted for routine analysis of feed materials.

Some of the individual factors influencing ***in vitro*** digestibility have been investigated. For example, Baumgardt, Taylor and Cason (1962) showed that ***in vitro*** digestibility varied from day to day; Baumgardt and Hi Kon Oh (1964) showed that it varied with the length of fermentation and Church and Petersen (1960) showed that it varied with the source of inoculum. Donefer (1962) has observed that proper replication of results, within as well as between laboratories, is still one of the biggest problems in the use of the ***in vitro*** technique.

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The significance of the variations arising from the ***use*** of ***the in vitro*** technique has not been comprehensively investigated. In the absence of this information the validity of predictions of ***in vivo*** digestibility from ***in vitro*** digestibility studies is questionable. It was with this in mind that the causes of variation in ***in vitro*** digestibility studies were investigated further. Results of these studies and estimations of the errors involved in applying ***in vitro*** digestibility indices to the situation ***In vivo*** are discussed in this paper.

II. EXPERIMENTAL

(a) Technique

The ***in vitro*** technique was a modification (Yates 1964) of the Tilley and Terry (1963) method.

Twin Merino wethers, aged about 12 months and individually weighing 25 kg, each fitted with a permanent ruminal cannula (Jarrett 1948), were used as sources of rumen liquor unless otherwise stated.

Samples of feed used in digestibility trials with sheep at the Waite Institute and Roseworthy College were used in the present experiments. These samples were drawn from numerous experiments over a period of years. A full detail of the samples has been given by Yates (1964).

(b) Procedure

(i) Experiment 1

The object of this experiment was to compare the effect of two methods of sampling rumen fluid on the ***in vitro*** digestibility of selected substrates. Two sheep, fed a similar diet, were fasted for 30 hours. Rumen fluid was sampled via the cannula and by stomach tube introduced through the mouth. Two independent samples of rumen liquor were taken by both methods from each sheep and each sample was used for duplicate determinations of the ***in vitro*** digestibility of each substrate.

(ii) Experiments 2 and 3

These experiments were designed to examine the variation in ***in vitro*** digestibility with rumen fluid sampled on different days. Two Merino wethers, selected from a flock grazing mature herbage, were used as sources of rumen liquor in these experiments. The animals were fasted for 8 and 16 hours prior to sampling. Two independent samples of rumen fluid were taken, through the mouth, from each sheep on two days. Each sample was used for duplicate determinations of the *in vitro* digestibility of each substrate.

(iii) Experiment 4

The effect of the diet given to the source animal on ***in vitro*** digestibility was examined in this experiment. Three penned Merino wethers of similar age and weight were used. One was fed mature herbage, one dried young grass and another good quality ryegrass hay. Samples of rumen fluid were obtained, via the mouth, from each sheep after fasting for 48 hours. Each sample was used to inoculate a range of substrates to determine their ***in vitro*** digestibility.

(iv) Experiment 5

The object of this experiment was to examine the effect of length of fermentation on ***in vitro*** digestibility. Ground samples of dried young grass, ryegrass

hay and mature herbage were incubated for 12, 24, 48 and 96 hours with rumen fluid and sampled via the fistula of a wether fed a diet of chaff and fasted 48 hours.

(v) Experiments 6,7, 8 and 9

These experiments were designed to examine the repeatability of ***in vitro*** digestibility. Rumen liquor, taken via the fistula of a wether fed chaff and sheep cubes and fasted 24 hours, was used to inoculate a wide range of substrates in Experiments 6, 7 and 8. In Experiment 9, the source animal was fed good quality ryegrass hay ***ad libitum*** and glass incubation vessels were used, whereas nylon vessels were used in Experiments 6, 7, and 8.

(vi) ***Experiment 10***

A comparison of glass and nylon incubation vessels was made in this experiment. Rumen fluid from a Merino wether, fed ryegrass hay ***ad libitum,*** was used to inoculate similar substrates in both nylon and glass incubation vessels.

III. RESULTS

(a) Experiment 1

No significant differences in ***in vitro*** dry matter digestibility of the substrates used resulted from the use of the two rumen sampling methods. The most important observation was the large variation between sheep compared with the within-sheep variation, i.e. between samples of rumen fluid. The major components of variation in this experiment were due to sheep and the interaction between sheep and the method of sampling. The analytical error was small. The statistical findings are shown in Table 1.

(b) Experiments 2 and 3

The major components of variation in both these experiments were due to sheep, days and the interaction between sheep and days (Table 2). These sources of variation accounted for 90% of the total variation. The analytical error was small, the coefficient of variation of a single determination being 6%.

(c) Experiment 4

Analysis of variance of the results showed significant variations in ***in vitro*** digestibility due to sources of inocula. The regressions of digestible dry matter ***in vitro*** on apparent digestible dry matter ***in vivo*** are shown in Figure 1. Data extracted from Experiment 8, where the sheep was fed chaff and cubes, has been in-

TABLE 1

Analysis of ***variance*** of *wheaten chaff* ***digestibility*** *data*

Variation due to:	df.	S.S.	M.S.	V.R.
Sheep	1	359.03	359.03	48.1***
Sampling method	1	28.25	28.25	3.8N.S.
Sheep x sampling method	1	133.93	133.93	17.9***
Samples	4	20.63	5.16	0.69N.S.
Duplicates	8	59.66	7.46	
Total	15	601.50		

*** $P<0.001$

TABLE 2

Analysis of variance of in vitro ***digestibility data*** **of** ***sample number A93*** (*wheaten chaff*)

Variation due to:	df.	S.S.	M.S.	V.R.
Days	1	73.53	73.53	7.1*
Sheep	1	64.40	64.40	6.2*
Days x sheep	1	539.40	539.40	52.2***
Samples	4	44.73	11.18	1.08N.S.
Duplicates	8	82.80	10.35	
Total	15	804.86		

* $P<0.05$
*** $P<0.001$

cluded in the results for comparison. The regression coefficients were not significantly different from one another and there was no significant displacement of the regression lines from each other. Correlation coefficients were significant at the 0.1% level for regressions 1 and 3 and at the 1% level for regressions 2 and 4. No distinction could be made between the standard errors of estimated ***in vivo*** digestibility coefficients (shown in brackets after the equation) when the sheep was fed hay, dried young grass or chaff plus cubes, but these were all significantly less than that when the sheep was fed mature herbage. If the points used to plot the regression lines 1, 2 and 3 are considered unknowns, in the majority of cases the three lines did not give a significantly different predicated ***in vivo*** value for any one substrate using the appropriate ***in vitro*** digestibility coefficient.

(d) Experiment 5

The ***in vitro*** digestibilities of dried young herbage, ryegrass hay and mature herbage increased with length of fermentation time up to 96 hours (Figure 2). However 50% or more of the dry matter loss at 96 hours occurred in the first 12 hours with all substrates. Using the figures for the dry matter digestion ***in vitro*** after 12 hours incubation and the average daily apparent dry matter digestion of the same material from ***in vivo*** trials, the correlation coefficient was high ($r = 0.981$).

(e) Experiments 6, 7, 8 and 9

The regressions. of dry matter digestibility ***in vitro*** on apparent, dry matter digestibility in ***vivo*** are shown in Figure 3. The regression coefficients for Experiments 6, 7, and 8 were not significantly different and there was no significant displacement of the regression lines from each other. The two notable differences between these experiments and Experiment 9 were that, in the latter experiment, the regression coefficient was 0.997 and the regression line virtually passed 'through the origin. Although two variables, viz. source of inoculum and type of incubation vessel, were changed in Experiment 9, the results of Experiment 10 suggest that the change in type of incubation vessel was the main reason for these differences. It is thought that a mercurial deposit on the walls of some of the nylon vessels may have affected bacterial activity and hence dry matter digestibility .

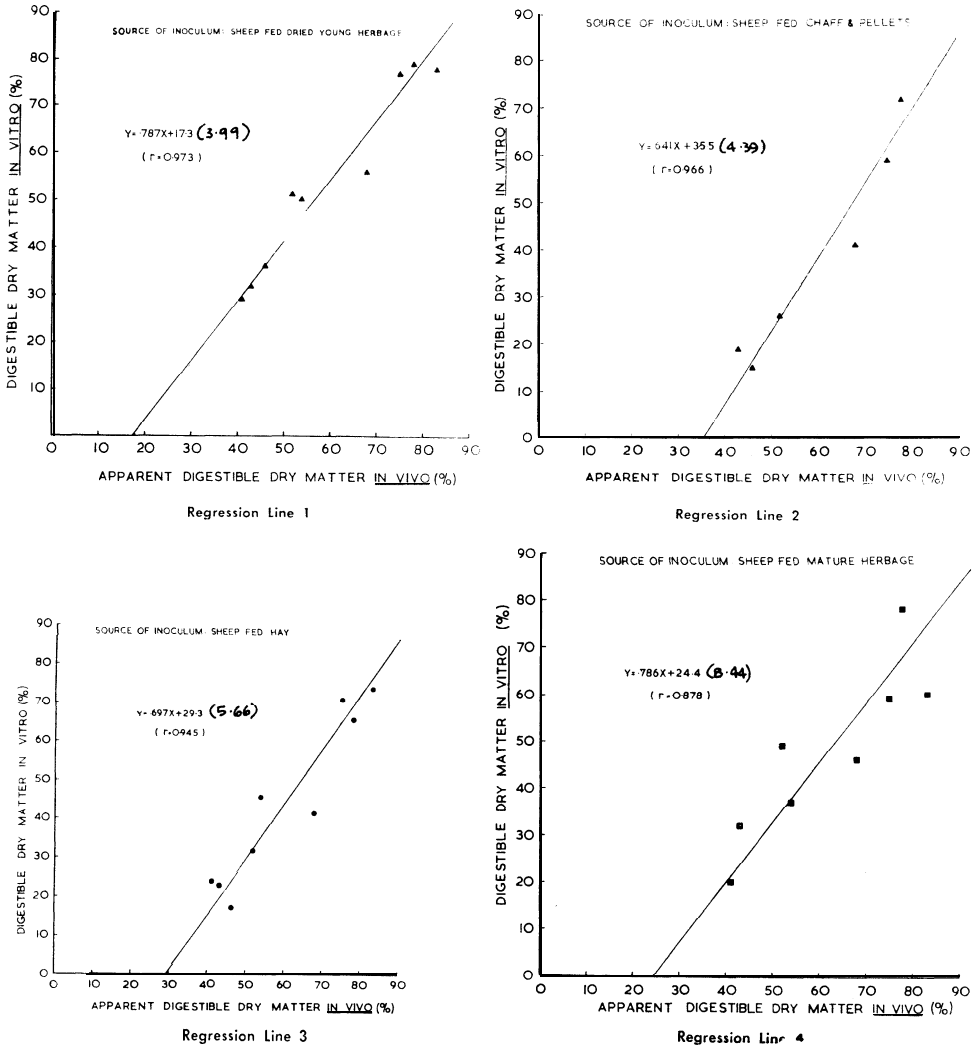

Fig. 1.—Regressions of digestible dry matter ***in vitro*** on apparent digestible dry matter in vivo for four sources of inoculum:-sheep fed dried young herbage (regression line 1), sheep fed chaff and pellets (regression line 2), sheep fed hay (regression line 3), and sheep fed mature herbage (regression line 4). (Experiment 4).

(f) *Experiment 10*

The regressions of dry matter digestibility ***in vitro*** on apparent dry matter digestibility ***in vivo*** using glass and nylon incubation vessels are shown in Figure 4. It is apparent that the regression line obtained using nylon vessels is similar to that of previous experiments where nylon incubation vessels were used, but different from that when glass vessels were used for the incubation. Both correlation coefficients were highly significant but the standard error of the estimate was significantly less for glass vessels than for nylon vessels.

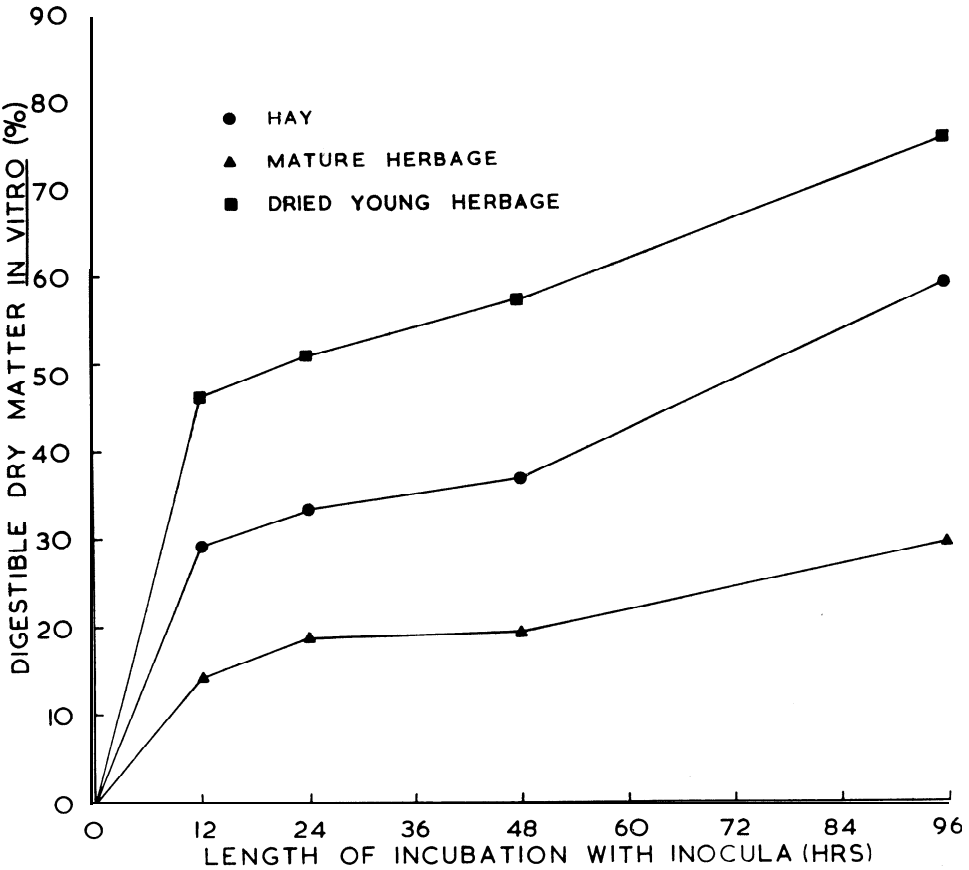

Fig. 2.—Regression of digestible dry matter in vitro on length of incubation time (Experiment 5).

IV. DISCUSSION

Consideration of the results of other workers (Asplund et *al.* 1958.; Reid et ***al.*** 1959; Clark and Mott 1960; Bowden and Church 1962b) indicate that a simple ***in vitro*** technique is capable of providing accurate estimates of ***in vitro*** digestibility.

The precision of the results obtained ***in vitro*** is affected only by the accuracy and reproducibility of the method itself, but other factors have to be taken into account in establishing relationships between ***in vitro*** and ***in vivo*** digestibilities. The fact that similar ***in vitro*** digestibilities were obtained when rumen liquor was sampled either via fistula or stomach tube indicated that it is not necessary to have a rumen-cannulated sheep to provide the inoculum for ***in vitro*** experiments. Experiments 1, 2, and 3 also showed that only one sample of inoculum need be taken at any one time and that there can be marked differences between sheep in the digestive power of their rumen liquor. The day to day variation in ***in vitro*** digestibility shown in Experiments 2 and 3 confirms the results of other workers. Simkins and Baumgardt (1963) reported a daily variation in the

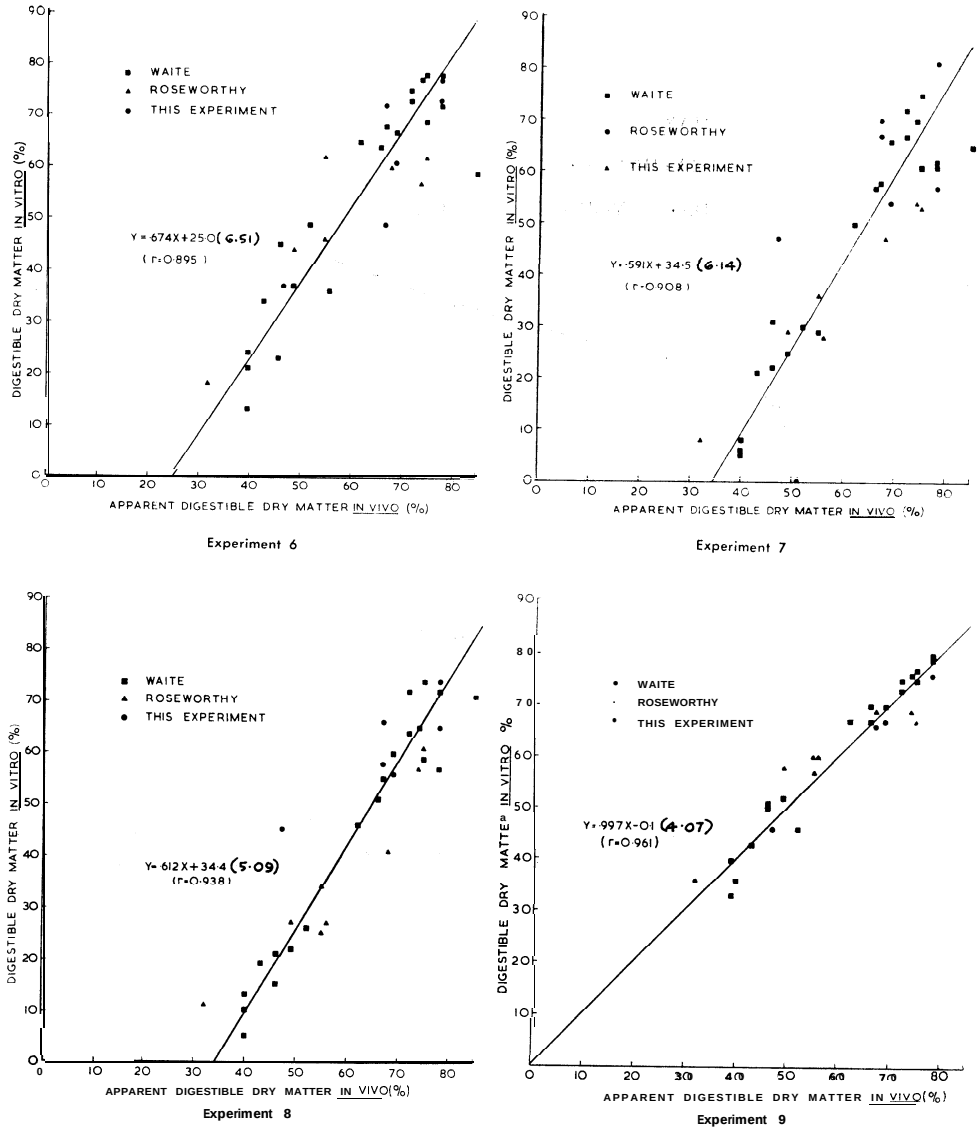

Fig. 3.—Regressions of dry matter digestibility *in* vitro on apparent dry matter digestibility *in* vivo using different substrates (Experiments 6, 7, 8, 9).

digestion of silage cellulose and that the day to day repeatability of ***in vitro*** dry matter digestibility in silages was better than that of ***in vitro*** cellulose digestion. Baumgardt, Taylor and Cason (1962) showed daily variations in ***in vitro*** cellulose digestion of alfalfa and ladino clover which were reduced however when the values were adjusted using a standard forage. Bowden and Church (1962a) noticed a significant difference in the digesting power of inoculum on different days and they ascribed this to the variable water intake of the steer they were using. Variation in amount and quality of feed consumed by the animal providing the inoculum may cause excessive day to day variation in ***in vitro*** digestibility

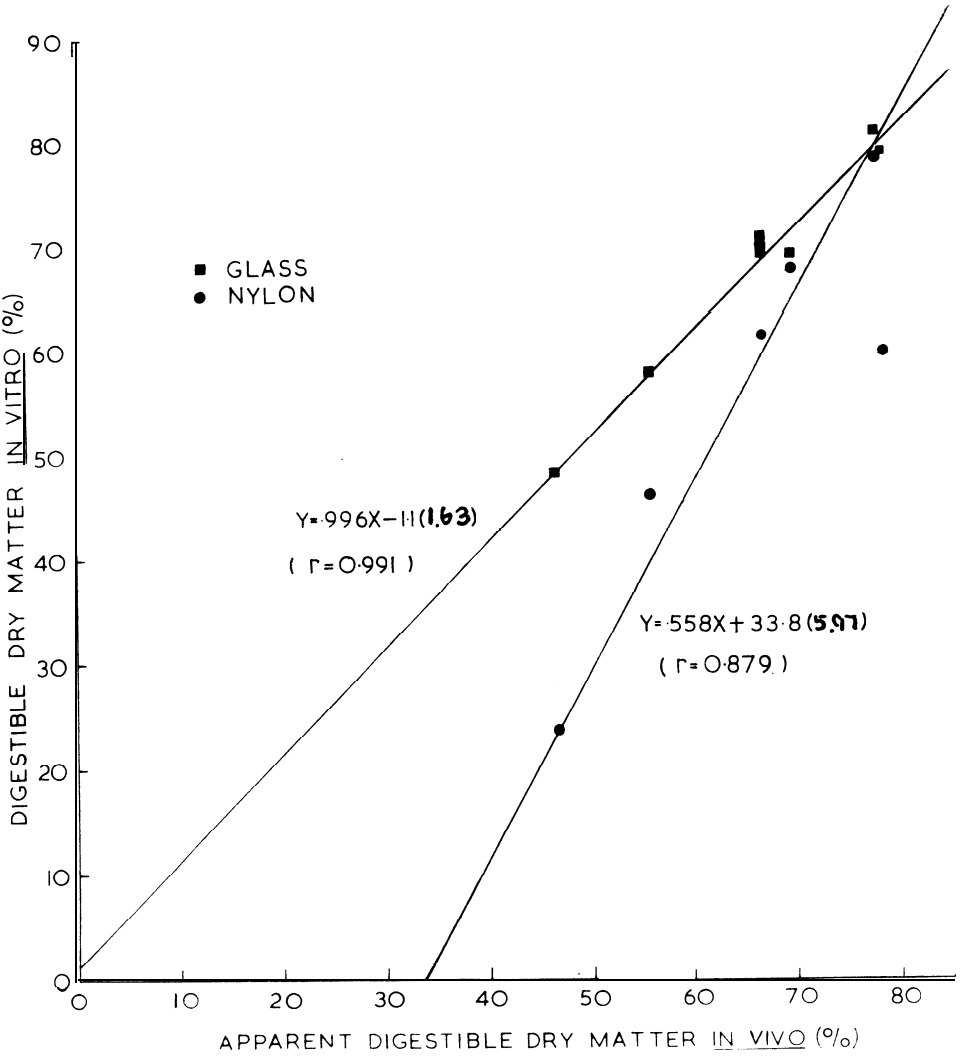

Fig. 4.—Regressions of dry matter digestibility in ***vitro*** on apparent dry matter digestibility in ***vivo*** using glass and nylon incubation vessels (Experiment 10).

(Simkins and Baumgardt 1963). However, Tilley and Terry (1963) fed ***a d*** libitum the sheep from which their rumen inoculum was taken and obtained reproducible results. None of the above factors was studied in the present work but their importance should not be overlooked.

Ideally the source animal should be fed the same ration as that studied ***in vitro*** (Warner 1956; Bowie 1962). Church and Petersen (1960), Asplund et *al.*(1958) and Reid et ***al.*** (1960) have all shown that the relationship between ***in vitro*** and ***in vivo*** digestibilities varies with the diet of the animal providing the inoculum. However, Stewart and Schultz (1958), Salisbury et ***al.*** (1958) and Quicke et ***al.*** (1959) failed to demonstrate large differences in cellulose digestion by inocula from sheep on different diets. Furthermore it is not practicable to

feed the source animal on the in ***vitro*** substrate in many instances where this technique could be particularly valuable, e.g., estimations on a wide range of forages or on small samples. The result of Experiment 4 showed once again that if one is only concerned with ***in vitro*** digestibility significant differences are obtained from the inocula of sheep fed on different diets, but if one is primarily interested in the prediction of apparent digestibility ***in vivo*** then the source of inoculum has no effect.

It is now generally agreed that dry matter loss or cellulose digestion increases with length of fermentation ***in vitro,*** but what is more important is that short fermentation periods, e.g. 12 hours (Johnson ***et al.*** 1962; Donefer, Crampton and Lloyd 1960) or 18 hours (Baumgardt and Hi Kon Oh 1964), have given significant correlations between ***in vitro*** and ***in vivo*** measurements.

The versatility of the ***in vitro*** technique has been successfully demonstrated in the present experiments with a wide range of substrates which included ground samples of sheep cubes and high and low quality forages. The method was reproducible on each of three occasions, but when both the diet of the source animal was changed and the incubation vessels were changed from nylon to glass, a significantly different ***in vitro-in vivo*** relationship was obtained. The results of Experiment 10 (where the diet was kept constant and a comparison of incubation vessels was made) suggested that the latter was the most important factor contributing to this change.

It should be remembered that the ***in vitro*** system does not reproduce that in the animal and is therefore an empirical method. It can however be very useful for agronomic work and is suitable for estimating the digestibility of the small samples of herbage collected in oesophageal fistulae or available in plant breeding studies.

The main point which arises out of the present work is that, provided the source animal is fed a reasonable quality diet and a standard forage is used to account for day to day and sheep to sheep differences, the standard error of prediction of ***in vivo*** digestibility from ***in vitro*** techniques is quite small. With the substrates under study, the ***in vitro*** method was sufficiently accurate to detect differences of 2-4 and 4-6 units in digestibility using glass and nylon vessels respectively.

V. ACKNOWLEDGMENTS

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