THE *IN VITRO* PREDICTION OF HERBAGE DIGESTIBILITY

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SUMMARY

Experiments have been conducted to determine the most suitable *in vitro* method for predicting herbage digestibility. Incubation of the test forage with a measured quantity of rumen fluid from a grass fed fistulated animal for 72 hours followed by a further 24 hours in acid pepsin gave *in vitro* digestibilities very close to *in vivo* figures. A relationship of $y = 1.014\ x$ where y = *in vivo* organic matter digestibility and x = *in vitro* organic matter digestibility was established. Week to week variation was greatly reduced when this method was used rather than a 48-hour micro-organism digestion followed by a 48-hour pepsin digestion. Duration of incubation and source of rumen liquor are examined as major factors affecting the *in vivo* : *in vitro* relationship.

INCREASING INTEREST has been shown in techniques for predicting the apparent digestibility of forages largely from the microbial degradations of small samples in the laboratory. This has been stimulated by the development of a number of different types of artificial rumen, by recognition of the importance of indexes of nutritive value in agronomic as well as animal feeding studies, and through a rapidly-growing appreciation of the restrictions which sole dependence on animal trials place on this work.

For critical biochemical and physiological studies the "artificial rumen" is required to simulate as closely as possible normal rumen function, *i.e.*, maintaining the numbers, proportions and activity of the micro-organisms, and preserving the rates of digestion and the balance of carbohydrates and proteins which occur *in vivo*.

Although most laboratory or *in vitro* methods fall short of these requirements, depending on the type of information which is sought, much can be derived from somewhat empirical techniques which do not fully simulate conditions in the intact rumen.

Most *in vitro* procedures involve the incubation of a test feedstuff with a source of micro-organisms, a buffer solution to maintain pH constant and/or a nutrient medium. For estimating the apparent digestibility of a feedstuff, they

* This work was carried out at Ruakura Agricultural Research Centre.

generally require a small, carefully measured sample to be subjected to breakdown under anaerobic conditions by a culture of rumen micro-organisms either alone or in conjunction with one or more enzymes. The digested portion is subsequently estimated as the difference between the intial weight of sample and the final residue.

(1) FACTORS AFFECTING THE RESULTS OBTAINED FROM *IN VITRO* STUDIES

(a) TYPE OF *in vitro* SYSTEM

Semi-permeable Artificial Rumen

Many workers have used semi-permeable membranes in basic studies of the nature and rates of formation of end products of rumen digestion (Louw *et al.*, 1949; Huhtanen *et al.*, 1954; Huhtanen and Elliott, 1956; Warner, 1956a; Adler *et al.*, 1958; Davey *et al.*, 1960; el Shazly *et al.*, 1960). The object is to remove by dialysing the products of rumen fermentation as they are produced. Substrate and rumen fluid are suspended in a semi-permeable membrane suspended in a mineral mixture. This method is not well suited to estimation of the apparent digestibility of feedstuffs.

Closed "All glass" System

A closed glass vessel, generally a centrifuge tube, is used to incorporate substrate, buffer and rumen flora, and in this pH, temperature and anaerobioses are maintained. This is a simple, widely-used method, and both Johnson *et al.* (1958) and el Shazly *et al.* (1961) have shown that the accumulation of end products of digestion achieved in this system do not deleteriously affect cellulolytic fermentation. This method has been used widely for forage evaluation (Donefer *et al.*, 1960; Baumgardt *et al.*, 1962; Bowden and Church, 1962; Tilley and Terry, 1963; Van Dyne, 1963).

(b) TREATMENT OF SUBSTRATE PRIOR TO DIGESTION

Drying of Samples

Although Tilley and Terry (1963) could find no differences between freeze drying, heat drying at 40°C and heat drying at 100°C on subsequent *in vitro* digestion, other workers obtained greater digestion with freeze dried samples (Reid *et al.*, 1959; Clark and Mott, 1960).

Grinding of Samples

Very fine grinding (ball milling) will greatly increase digestion *in vitro* (Dehority and Johnson, 1961; Tilley and Terry, 1963). When comparing samples ground through 40 and 60 mesh screens, Baumgardt and Hi Kon Oh (1964) found a 4 to 10% increase in digestion with the finer material. Using similar screen sizes, Tilley and Terry (1963) recorded virtually identical digestibilities for all samples. Part of this discrepancy may lie in the differing *in vitro* methods.

(c) Source and Preparation of Inoculum

Between-and Within-Species Variation of Donor Animals

In general, whether rumen liquor is derived from sheep or cattle appears to have had little influence on either the rate or extent of digestion where both species have been on the same feed (Van Dyne and Weir, 1964a; Le Fevre and Kamstra, 1960). Within-species variation has been reported by Bezeau (1965) and Van Dyne and Weir (1964a) who also found greater variability amongst steers than sheep.

Type of Feed Consumed by Donor Animals

Evidence on the importance of this factor is conflicting. Salsbury *et al.* (1958), Stewart and Schultz (1958), Quicke *et al.* (1959), and Tilley and Terry (1960) have been unable to show substantial differences in feed digestibility attributable to varying sources of inocula. In contrast, Reid *et al.* (1959, 1960, 1964), Asplund *et al.* (1958) and Church and Peterson (1960) obtained a range of estimates of percentage apparent digestibility depending on the source of inoculum used, and Van Dyne and Weir (1964b) have reported that both qualitative and quantitative variations in the diet produced 15 to 20% differences in *in vitro* digestions of the same forage.

(d) Length of Fermentation Period

Rate of digestion varies considerably for different *in vitro* methods and may be in part due to seasonal fluctuations in rumen flora. Moir (1951) considered that the numbers, but not the type of micro-organism varied greatly according to the stimulus of adequate green forage. Minimum numbers were present in the autumn and twice the number per ml of

rumen liquor in the spring. It is suggested that this type of phenomenon could have been responsible for the progressive decline in estimates of *in vitro* digestion reported between summer and late autumn by Clark and Mott (1960). Seasonal fluctuations were also reported by Nottle (1956) for sheep pen-fed a uniform ration of oaten chaff and concentrates, and variation in light and temperature has been suggested as possible causes of these.

Much between-trial, and some within-trial variability is directly attributable to length of the fermentation period.

Kamstra *et al.* (1958), Dehority *et al.* (1960), Donefer *et al.* (1960), Le Fevre and Kamstra (1960) and Van Dyne (1962) consider that fibre digestion in the "all glass" system is almost complete after 24 hours. However, Tilley and Terry (1963) have shown that marked additional digestion occurs between 24 and 48 hours, and Barnett (1957) and Quicke *et al.* (1959) recommend a 60 hour fermentation period, particularly for mature forages.

In contrast, Le Fevre and Kamstra (1960) and Wallace *et al.* (1965) report from *in vitro-in vivo* comparisons of a range of hays using sheep as donor animals, an underestimation of dry matter digestibility after 24 hours', and an over-estimation after 48 hours' fermentation.

(2) VARIABILITY OF *IN VITRO* ESTIMATES AND ACCURACY OF PREDICTION OF APPARENT DIGESTIBILITY

Because of the variations among laboratories in procedural details, it is not surprising that published estimates of the accuracy with which the digestibility of feedstuffs is predicted *in vitro* vary markedly.

Most workers who have used an *in vitro* method to estimate the nutritive value of feedstuffs have been concerned to predict the apparent digestibility of cellulose, dry matter, or organic matter. The latter two are generally predicted with greater accuracy than is cellulose. Thus, Bowden and Church (1962) reported little between-trial and between-year variation in the prediction of digestible dry matter, but considerable variability for cellulose. Coefficients of variation for 13 replicates of the same feed were 3.3% for digestible dry matter, and 5.3% for digestible cellulose. The authors postulate that the week to week fluctuations in cellulolytic activity were due to an interaction between substrate and inoculum. The same workers, and Baumgardt and Hi Kon Oh (1964), report consistent within-trial coefficients of variation of 3 to 4%.

To minimize between-period variation, Tilley and Terry (1960) proposed continuous use of a test forage as a standard during all trials. The prediction equation published by Tilley and Terry (1963) has a smaller standard error (± 2.31) than any general relationship for predicting herbage digestibility from chemical composition. It was suggested by these workers that use of the standard feed would improve the accuracy of prediction, but this was not confirmed by Baumgardt and Hi Kon Oh (1964).

(3) THE NEED FOR EXPERIMENTAL INVESTIGATION OF *IN VITRO* PROCEDURES FOR APPLICATION TO NEW ZEALAND CONDITIONS

Because of New Zealand's almost complete dependence on pasture herbages, the wide range of environments in which these grow, the paucity of information on the nutritive value of grasses and clovers within and between areas, and the impossibility of obtaining all of these data directly from animal trials, a relatively precise *in vitro* procedure for feedstuff analysis would be useful in experimental and advisory work.

The conflicting nature of results reviewed indicates the importance which should be attached to local evaluation of techniques, even though the procedures under test may appear relatively standard, before applying them extensively to any new set of conditions. The work to be reported in the present paper was an investigation of the application to New Zealand conditions of the *in vitro* digestion procedure reported by Tilley and Terry (1963). This method was chosen because of its application to forages similar in type to those available in this country and because the accuracy claimed for the prediction of the apparent digestibility of herbage dry matter was high.

EXPERIMENTAL

The standard Hurley method used in the preliminary investigations and followed elsewhere, except where modifications are recorded, was as follows:

Feed samples were dried in a forced draught oven at 100°C and ground to pass a 0.8 mm mesh.

Three non-lactating adult cows and three adult wether sheep fitted with rumen fistulae were available to supply rumen liquor* derived from either a grass or hay diet according to requirements.

* Grass inoculum describes rumen fluid derived from free grazing animals; hay inoculum from stock stall fed on hay.

Rumen liquor was strained through muslin into a bottle previously flushed with CO_2, and was stored during transport to the laboratory at 38 to 39°C in a vacuum flask.

0.6000 g of air-dry samples (dry matter determination done at the time of weighing) were incubated anaerobically at 39°C in 100 ml polypropylene centrifuge tubes for 48 hours with 40 ml of artificial saliva (McDougall, 1948) and 10 ml of strained rumen fluid per tube. During the first 48-hour period, pH was maintained between 6.85 and 6.95. As long as the intial pH of the buffer solution was 6.9 to 7.0, little subsequent adjustment in the tubes was necessary. After this first stage digestion, pH was lowered to 1.2 to 1.3 with 20%v/v HCl, and aqueous pepsin added. The second phase digestion of 48 hours in the incubator was required for completing the protein degradation. At the conclusion of this stage, filtration of the contents of the tubes on to fibreglass pads was accomplished with the help of a filter aid. The pads and residues were dried at 100°C before weighing (W1). They were then ashed at 500°C for 2 hours, cooled and weighed (W2). To determine the fraction of the final residue which originated from the rumen liquor, blank tubes were run in triplicate. The percentage apparent digestibility of the feed organic matter was calculated by subtracting the residual organic matter, less the blank organic matter, from the feed organic matter, and expressing this as a percentage of the feed organic matter, i.e., % of digestibility of O.M. *in vitro*

$$= \frac{\text{O.M. from 0.6 g air-dry} - ((\text{W1} - \text{W2}) \text{ for samples} - (\text{W1} - \text{W2}) \text{ for blank}) \times 100}{\text{O.M. in 0.6 g air-dry}}$$

The trial work was divided into four major sections:

(1) Preliminary investigations.

(2) An extensive analysis of the method of Tilley and Terry (1963) applied to herbage and hay samples ranging widely in apparent digestibility.

(3) A more basic investigation of factors affecting the method because of dissatisfaction with results in section (2).

(4) The development of an alternative to the method of Tilley and Terry (*loc. cit.*) and detailed analysis of this as for (2) above.

In all trials, two "standard" feeds, one of grass and the other a good quality hay, were digested as part of each set of *in vitro* determinations to provide a measure of between-set variability. In some sections, feeds additional to the two mentioned were included.

(1) Preliminary Investigations

These were considered necessary because of the lack of unanimity among published data on the importance of factors which could produce errors in the use of any *in vitro* method.

Comparing Sheep with Cattle as Donors of Rumen Liquor

The three dry adult cows and three adult wethers previously referred to were stall-fed on good quality hay to appetite for a period of 6 weeks. Following this preliminary period, the rumen liquors of the cattle and sheep were compared by measuring fermentation losses using both highly digestible herbage and hay samples as substrates.

Assessing the Importance of Time of Rumen Liquor Collection Relative to Feeding

Good quality lucerne-cocksfoot barn-dried hay having an organic matter *in vivo* digestibility of 71.6% was fermented in duplicate with samples of rumen liquor obtained from a dry cow fed hay twice daily between 10 a.m. to 12 a.m. and 4 p.m. to 6 p.m. The samples compared were drawn after 15 hours' fast, at the end of the morning feed, 2 hours after completion of the morning feed and again 2 hours later.

Assessing the Effect of Method of Drying Forage Samples on the extent of in vitro *digestion*

Oven drying at 100°C was compared with freeze drying using eight feeds digested in the same batch of determinations.

Comparing Pronase with Pepsin as the Proteolytic Enzyme

In four experiments with 18 feedstuffs ranging in digestibility from 56.9 to 82.0, pronase was used to replace pepsin in the second phase of the *in vitro* procedure. Pepsin has an optimum pH requirement of about 1.2 to 1.5, but the addition of HCl to achieve this at the end of the fermentation period is time consuming, and can induce violent

frothing. Pronase has a pH requirement of 7 to 8 and can be added directly to the tubes when required.

(2) Analysis of the Method of Tilley and Terry (1963)

This involved comparing *in vitro* measurements of the organic matter digestibility of pasture herbage, various hays and red clover straw with *in vivo* measurements previously made with sheep and cattle. Most of the test samples were derived from a series of continuous digestibility trials with lactating dairy cows fed pasture herbage in the 1961–2 dairying season. Fifty-one samples were analysed and the feedstuffs they represented ranged in apparent organic matter digestibility from 45.5% to 85.5%. All *in vitro* estimations were done in duplicate, and all samples were fermented with two types of inocula, one derived from sheep fed continuously a hay diet, the other from grass-fed cattle.

(3) Investigations of Factors Affecting the Standard Hurley Method

The effect of increasing the phase of microbial digestion from 48 to 72 and 96 hours using both grass and hay inocula on three feeds was studied in three experiments. The first two were carried out with three feeds and used only 48 and 96 hours' fermentation with and without a subsequent 48 hours' pepsin digestion, while the third experiment used two feeds digested for 48, 72 and 96 hours with and without subsequent pepsin digestion.

(4) The Development of an Alternative Method

The analyses from sections (2) and (3) above suggested that increasing phase one digestion from 48 to 72 hours might be a considerable improvement. At the same time, the pepsin digestion was reduced from 48 hours to 24 hours, thus keeping total incubation time constant at 96 hours. Twenty forages, ranging in digestibility from 61.7 to 82.2%, were digested using the following methods:

Method	*Microbial Fermentation Time (Hr)*	*Pepsin Digestion Time (Hr)*	*Inoculum*
(a)	48	48	hay
(b)	48	48	grass
(c)	72	24	hay
(d)	72	24	grass

The last method (d), proving most satisfactory, was followed for five consecutive *in vitro* experiments on the same two feeds investigated originally with the standard Hurley procedure.

RESULTS

(1) Preliminary Investigations

Comparison of Sheep and Cattle as Donors of Rumen Liquor

The mean *in vitro* estimate of the apparent digestibility of the organic matter in the pasture herbage sample under test was 81.3±0.8% when rumen liquor from sheep was used, and 81.7±1.0% for the dry cows (Table 1). Corresponding coefficients and standard deviations for the hay samples were 71.4±0.4% and 71.5±0.8%. These findings, which demonstrate the similarity in results obtained by using rumen liquor from either species provided they are fed the same diet, agree with the conclusions of Van Dyne and Weir (1964a). With the sample of each donor species used there was slightly less between-animal variability for sheep than for cows.

Time of Rumen Liquor Collection in Relation to Feeding

Mean *in vitro* estimates of the digestibility of the organic matter in the hay sample under test, after inoculating this with rumen fluid drawn from a cow after 15 hours' fast, at the end of feeding, and again 2 and 4 hours post-feeding,

TABLE 1: VARIATION WITHIN AND BETWEEN COWS AND SHEEP AS A SOURCE OF INOCULUM FOR *in vitro* DIGESTION

	Test grass sample	*Test hay sample*
In vivo Digestibility	82.0 (O.M.)	71.6 (O.M.)
SOURCE OF INOCULUM		
Sheep 1	80.9	70.8
Sheep 2	82.3	71.4
Sheep 3	80.6	71.9
Mean	81.3±0.80	71.4±0.44
Cow 071	80.5	70.5
Cow 079	81.5	72.2
Cow 080	83.1	71.9
Mean	81.7±1.03	71.5±0.75

were 68.0, 69.3, 69.7 and 69.0 percentage units, respectively. The slightly lower value obtained following fasting agrees with results of studies of fibre digestion reported by Tilley and Terry (1963).

The Effect of Oven Versus Freeze Drying on in vitro *Digestion*

The comparison of eight samples ranging from highly digestible pasture herbage to material of moderate digestibility showed that freeze-dried substrate gave an increase of 1.0 to 1.5 units of digestibility compared with comparable material oven dried. Similar differences were reported by Clark and Mott (1960) and Reid *et al.* (1964), but Tilley and Terry (1963) found no differences when samples were freeze dried or oven dried at either 40°C or 100°C.

Use of Pronase as the Proteolytic Enzyme

The results of four experiments with 18 feedstuffs show that on seven occasions there was good agreement between *in vivo* estimates of digestibility and those obtained with pepsin and pronase. In all others, pronase appeared to give variable results, and its use made the final filtration much slower. This was also a feature of some earlier work at the Grassland Research Station, Hurley, England (1964), in which an attempt was made to replace pepsin with trypsin at neutral pH.

(2) Results of Applying the Method of Tilley and Terry (1963)

The results of subjecting both the "standard" hay and grass samples to 21 repeat analyses during a period of 8 months and using the 48+48 hour *in vitro* procedure are shown in Fig. 1. The principal source of variation introduced into this phase of the trial was the rumen fluid used for each determination. This was obtained from three rumen fistulated cows which, during the trial, were always fed pasture.

Not only is the prediction of *in vivo* digestibility low in most cases, but the between-week differences are large, particularly those for hay. Tilley and Terry (1963) recommend the use of standards to correct for this type of between-period variability. The markedly different corrections derived in this work from the use of standards of

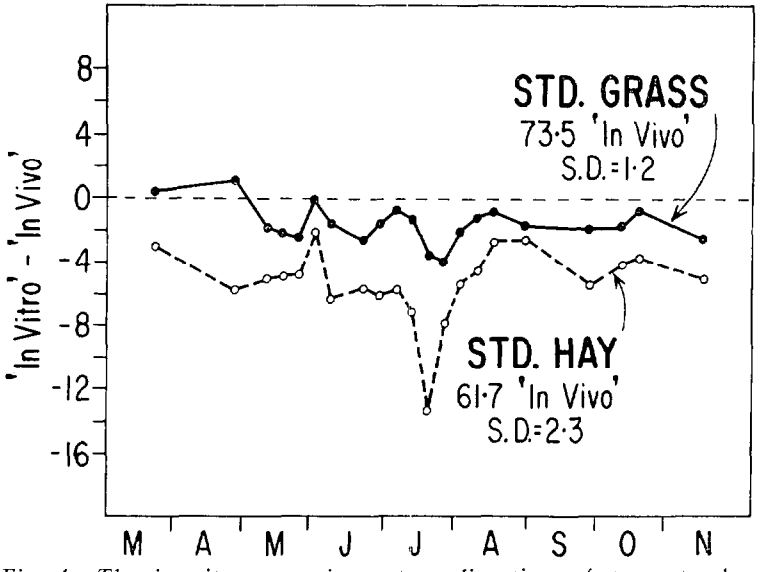

Fig. 1: *The* in vitro *organic matter digestion of two standard forages from March to November, 1964. (Grass inoculum, 48 + 48, i.e., 48 hr microbial digestion followed by 48 hr pepsin digestion.)*

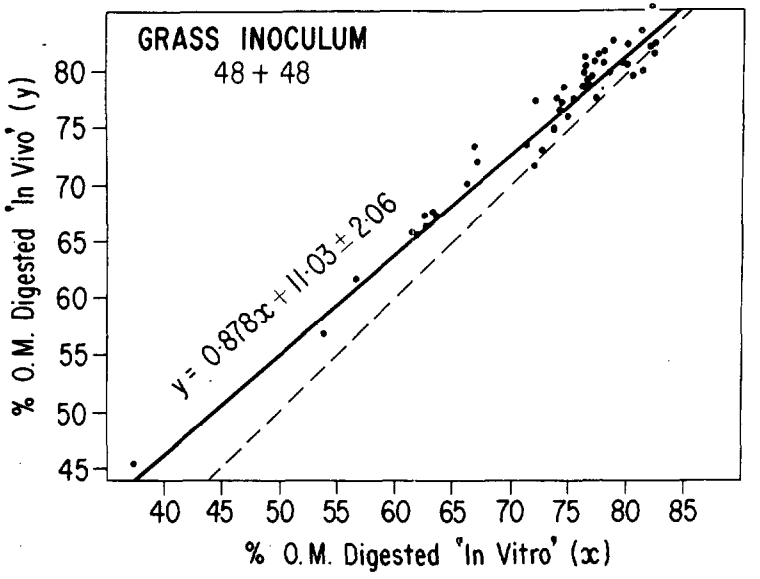

Fig. 2: *Regression of* in vivo *digestibility on* in vitro *digestibility using 51 test forages. Dotted line represents* y = x.

differing digestibility indicate the problems which can be encountered in their use.

Hence, in the more extensive comparison of the *in vitro* with *in vivo* estimates of digestibility, the results of which are summarized in Fig. 2, corrections based on between-period changes in the standards were not included. The regression of the *in vivo* coefficient of digestibility of the feed organic matter on the *in vitro* estimates which included observations on 51 feedstuffs gave the following equation:

$$y = 0.88x + 11.0 \pm 2.1\ (2.7\%)$$

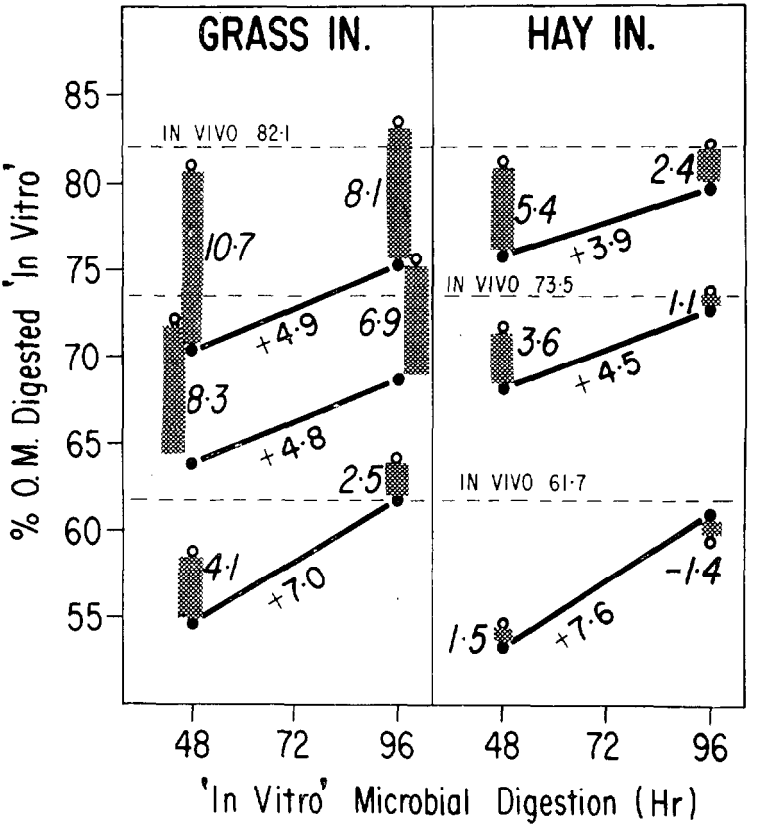

Fig. 3: The effect of 48 or 96 hours' fermentation period on in vitro *digestibility — three forages, using two inoculums (mean of two experiments). The vertical colums show the increase due to 48 hours with pepsin at the conclusion of the microbial fermentation.*

Although the overall estimate of the accuracy of prediction was similar to that of Tilley and Terry (1963), Fig. 2 shows that the error associated with individual estimates becomes progressively larger as apparent digestibility falls.

(3) Results of Investigating Factors affecting the Standard Hurley Method

Data summarized in Fig. 3 show that the most important factor determining this progressive error was the duration of the first phase digestion. Increasing this from 48 hours to 96 hours substantially increased the extent of digestion. When the effect of this is considered together with the second phase, pepsin digestion, the progressively greater

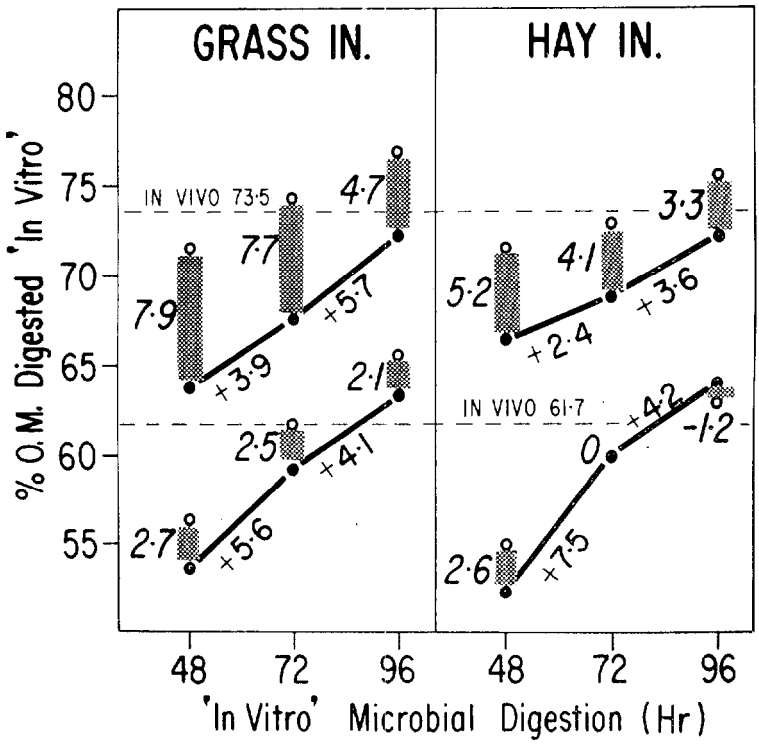

Fig. 4: *The effect of 48, 72 or 96 hours' fermentation period on* in vitro *digestibility — two forages, using two inoculums. The vertical columns shows the increase due to 48 hours with pepsin at the conclusion of the microbial fermentation.*

effect of increased time of fermentation with fall in apparent digestibility of the substrate becomes apparent. This provides a reasonable explanation for most of the differences between the standard grass and hay *in vitro* estimates shown in Fig. 1.

Figure 4 presents similar information, but gives a clearer picture with the intermediate fermentation time of 72 hours, approximately half-way between the 48 and 96 hour treatments. With both feeds, the 72-+48-hour treatment gave excellent agreement with the *in vivo* figures when grass inoculum was used, while hay inoculum produced results slightly below the latter.

(4) Results Obtained using an Alternative Method

Figure 5 shows the results of repeat analyses on twenty forages using the four methods. The progressive increase in

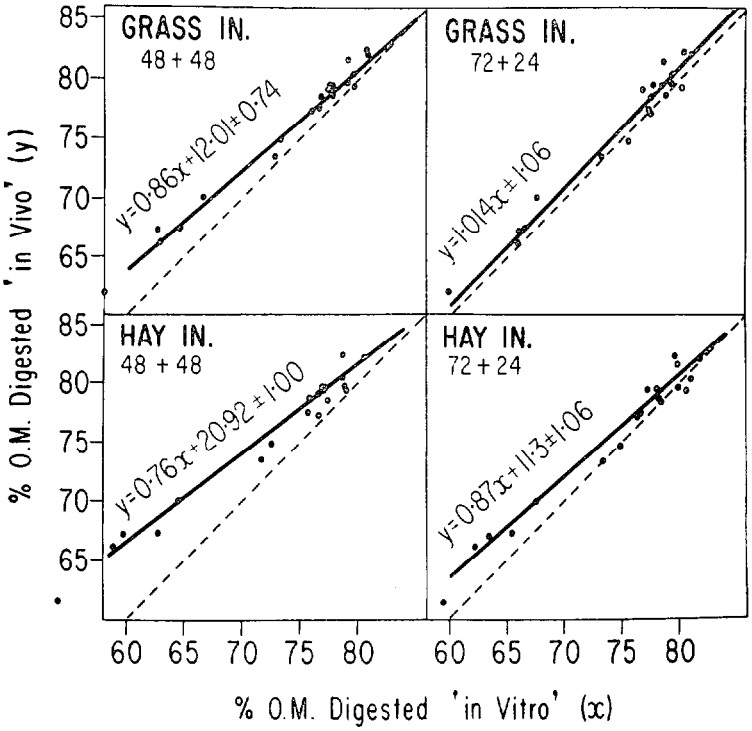

Fig. 5: Four in vitro *methods on the same 20 test forages. The dotted line in each case is* y = x.

the error associated with the estimation of percentage digestibility with decline in feed quality described in Fig. 3 is confirmed for the 48-+48-hour treatment. This occurs irrespective of the source of rumen liquor, and under the conditions of this trial the bias is more marked with hay than grass inoculum. Excellent agreement was achieved between *in vivo* and *in vitro* estimates using the 72-+24-hour combination and grass inoculum for the full range of samples examined, the coefficient of variation of prediction being 1.4%.

Although this is considerably better than most published estimates of this type, it should be appreciated that it represents the results of only one trial. In the case of the 72-+24-hour combination with hay inoculum, an equation was derived almost identical with that for the 48-+48-hour digestion plus grass inoculum. It is suggested, therefore, that most of the bias associated with the *in vitro* estimates from three of the four procedures could be eliminated by appropriate manipulation of the length of the initial fermentation period.

Finally, results of the 72-+24-hour method using grass inoculum on the two original substrates in five consecutive

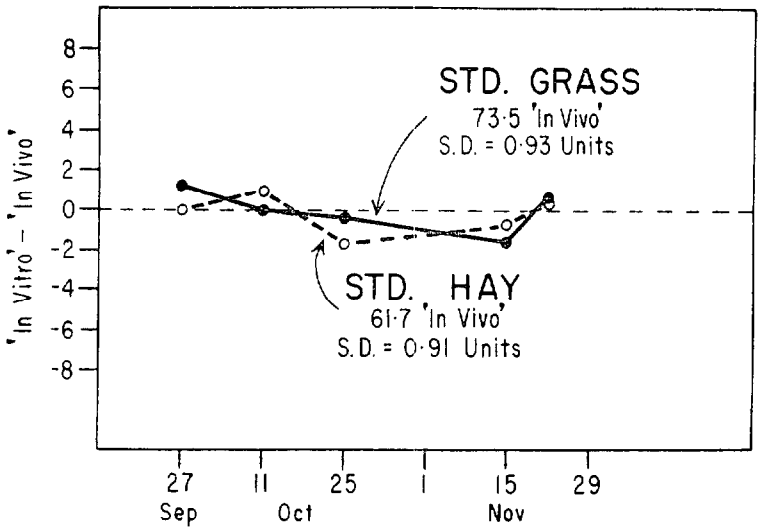

Fig. 6: The in vitro *organic matter digestion of two standard forages using grass inoculum and a 72-hour microbial digestion followed by a 24-hour pepsin digestion. Digestibilities are on organic matter basis.*

experiments are shown in Fig. 6. If in Fig. 1, one examines the situation with the original Hurley method over the same experimental period (September to November), it is obvious that the modifications outlined have markedly reduced between-trial variability, and improved the degree of agreement between actual and estimated values.

DISCUSSION

The *in vitro* digestion procedure developed by Tilley and Terry (1963) and now being used routinely at the Grasslands Research Institute at Hurley appears to require considerable modification for its successful application to evaluation of forages in New Zealand. The most important difference concerns the length of the first stage digestion, which the Hurley workers contend is complete after 48 hours, and which in the present investigation proceeded for at least 96 hours. A second difference involved the source of inoculum used, more consistent agreement between *in vivo* and *in vitro* figures being obtained with grass than hay inoculum. In the present trials, extending the initial fermentation period beyond 48 hours was more important with feeds of low digestibility than for those which were highly digestible. This is perhaps not surprising in view of the different times that feeds of varying digestibility are retained in the reticulo rumen before passing beyond the action of the rumen micro-organisms. What is surprising, however, is the considerable variability in the rate of digestion of the same grass samples occasioned by the use of grass as compared with hay inoculum, and the subsequent markedly different responses to pepsin digestion. As shown in Fig. 3, both the grasses of medium and high digestibility were more completely digested at all times during the first stage digestion with hay than with grass inoculum. Subsequently, the reverse applied in respect of pepsin digestion. This suggests that the proteolytic activity of the hay inoculum was higher than for the grass inoculum. Whether this was a feature of different protozoan or bacterial species present, or merely a difference in numbers, is unknown. Warner (1956b) found that proteolytic enzymes from rumen bacteria were relatively stable in aerobic conditions, and contributed about half the proteolytic activity from the rumen of a sheep fed a high protein diet. In the case of protozoans, however, Johnson (1963) maintains these are very susceptible to changed conditions and would be unlikely to contribute much to any increase in *in vitro* digestion after 24 hours.

One consistent feature of the standard hay substrate plus hay inoculum treatment for which no reasonable explanation can be offered is the decrease in digestibility which occurred after 96 hours' fermentation when the residue was subjected to 48 hours' treatment with pepsin (Fig. 3). The progressive nature of this phenomenon is shown in Fig. 4. It is surprising that the fermentation residue should have been greater after protein degradation than before this. This did not occur when the same feed was fermented with grass inoculum.

Despite these apparent inconsistencies which, in any case, have little influence on the overall degree of digestion, and hence the precision which can be achieved in predicting apparent digestibility, sufficient experience has been gained to indicate that *in vitro* methods can be usefully employed under New Zealand conditions. As in Great Britain, they should find application in the screening of species and strains in plant breeding studies, in comparing and ranking various pasture herbages, conserved fodders and supplementary feeding crops, and developing improved methods for measuring the intake of grazing animals.

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DISCUSSION

J. A. LANCASHIRE: *What are the likely reasons for the differences between the present results and those of Tilley and Terry at Hurley?*

The rumen liquor used is probably the main factor responsible for differences between these and the Hurley results. Tilley and Terry calculated their results on a dry matter basis while experiments reported here are in terms of organic matter. There is an important difference in that, although the percentage digestibility figure *in vivo* shifts up when converting from dry matter to organic matter, the reverse is true when considering *in vitro* figures. The reason for this is that the ash percentage in the final *in vitro* residue is much smaller than in the feed producing that residue. The point to be stressed is that if a laboratory is considering using this type of *in vitro* technique, the one selected must be evaluated carefully in that local situation.

DR G. W. BUTLER: *Could the speaker enlarge on the results obtained with pronase? Since this is a fungal protease preparation with very wide substrate specificity, it is not surprising that better correlations were obtained with the mammalian enzyme preparation than with pronase.*

When pepsin was replaced by pronase as the proteolytic agent, generally lower digestibilities were found. The extent of the decrease varied from ½ to 5 digestibility units and did not appear to fit any pattern. With pronase having a very wide specificity, one might have expected lower *in vitro* residues and hence higher digestibilities than when using pepsin. In fact lower figures were recorded.

J. RUDMAN: *Would the digestibility of the hay used for the source of rumen liquor be considered high, and was the grass fed to the sheep providing rumen liquor of similar quality?*

The hay used would be considered to be of good quality with the digestibility of the organic matter 61.7%. The liquor used in the grass inoculum preparation was obtained from free-grazing animals, so that accurate assessment of the quality of feed eaten was not possible. However, the digestibility of adjacent plots was measured in another experiment and ranged from 70% to above 80% during the *in vitro* investigation period.