A STUDY OF HAY DIGESTIBILITY USING AN IN VITRO FERMENTATION TECHNIQUE

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INTRODUCTION

The methods of determining the nutritive value of feeds can vary in complexity from a single digestibility measurement to a complete appraisal of the energy balance of animals as measured in a respiration calorimeter. It is generally believed that the nutritive value of any feed can be assessed fully only in terms of animal response. Response, as measured by such factors as weight gain or milk production, is controlled largely by intake and by the net energy components of the feed. Although the net energy is the most precise measure of feeding value, it is difficult to measure.

The feeding value of pasture has been expressed by many workers in terms of the voluntary intake of digestible dry matter, organic matter or energy per unit of feed. As Walter (1959) states, there is little doubt that the most useful single measure of the nutritive value of a roughage is the per cent digestibility of the dry matter.

A new feeding system proposed by Blaxter (1962, 1965), is based on the metabolizable energy which can be calculated from digestible energy by multiplying by the factor 0.82.

Swift (1957) concluded that digestible energy may provide a satisfactory criterion of forage quality as digestible energy is highly correlated to T.D.N., D.D.M., and metabolizable energy. The digestible energy is closely correlated with the dry matter and organic matter digestibility, and it was suggested that this relation could be used for predicting digestible energy from the more readily determined dry matter digestibility (Moir, 1961; Heaney and Pigden, 1963).

Regression equations calculated from data published by Armstrong (1964) show that the net energy values for maintenance and production, and digestible energy content are highly correlated. Also net energy can be predicted with a small standard error from the digestible energy content of herbage. Milford and Minson (1966) suggest that when facilities for measuring net energy are not available, there appears to be no advantage in using metabolizable energy in preference to digestible energy as an index of the energy value of pasture.

Since the technique involved in conducting digestibility experiments requires the use of at least three animals, a period of about 20 days in which to conduct the experiment, and a large amount of feed, numerous attempts have been made to evolve simple methods for the prediction of digestibility from readily determined characteristics of forage.

Indirect methods of determining digestibility using faecal nitrogen and lignin have been carried out by many workers such as Lancaster (1959), Raymond (1959), Brisson (1960) and Corbett (1960).

The use of other criteria such as date of cutting, leaf content and dry matter content at time of harvest is suggested by Reid et al (1959). Correlation between leaf percentage and digestibility is also discussed by other workefs such as Sotola (1946), and Minson, Raymond and Harris (1960).

Many attempts have been made to relate chemical composition of feeds to digestibility. As has been shown by Raymond et al (1959, 1960), the standard error of these mathematical relationships for the prediction of the digestibility of herbages from their contents of

nitrogen, crude fibre, cellulose, lignin or chromogen, etc., is large, particularly between species, and this makes them of little use for establishing differences between feeds. Similar observations were also made by other workers such as Forbes (1950), Minson and Kemp (1961) and Kivimae (1960).

Considerable interest has developed recently in the use of the *in vitro* rumen fermentation techniques for the evaluation of forage quality. Although large differences among data published by different laboratories exist, it has been suggested that *in vitro* digestion procedures may give more reliable estimates of *in vivo* digestibility than other short cut measure methods. Pigden and Bell (1955), in a study using 11 forages, obtained a satisfactory regression of per cent organic matter digested by sheep on per cent "anthrone carbohydrate" fermented *in vitro*. Belasco *et al* (1958) used cellulose digestion *in vitro* as a criterion in assessing the effect of fertilizer applications of urea on pasture and forage quality, and Kamstra *et al* (1958) applied this technique in a study of the factors related to the decreased digestibility of a forage, associated with advancing maturity.

Using a two stage *in vitro* fermentation method, Tilley and Terry (1963) predicted dry matter digestibility *in vivo* from that of *in vitro* with an accuracy of $\pm$ 2.0 digestibility units.

Drew (1966), using the same method with a slight modification, presented a regression equation with a standard error of 1.06 as follows:

$$Y = 1.014X \text{ (S.E. } \pm 1.06),$$

where Y is the digestibility of the organic matter *in vivo* and X is the digestibility organic matter *in vitro*.

Oh *et al* (1966) also reported that two stage *in vitro* fermentation gave the most reliable prediction of *in vivo* digestible dry matter compared with chemical constituents such as crude protein, acid detergent fibre, acid detergent lignin and cellulose solubility.

Whilst these reports are encouraging, a number of workers have mentioned the need for caution in the interpretation of results. Clearly the *in vitro* method measures a basic attribute of a feed; it can not take into account changes in digestibility *in vivo* under different regimes of feeding. Despite the close agreement between *in vitro* and *in vivo* values shown above, the relationship cannot be used to give a reliable prediction of the extent to which a given animal will digest a given herbage feed. It can however be very useful if one realizes its limitations, and may be used successfully for agronomic work. It is also suitable for estimating the digestibility of small samples of herbage collected in oesophageal fistulae, (Grimes, Watkin and Gallagher, 1966) or in plant breeding studies and variety testing (Dent, 1963). Thus it was considered worthwhile to conduct *in vivo* and *in vitro* experiments to determine relationships under local conditions.

This thesis includes two parts. In the first part, determination of four different hay digestibilities using sheep, results and problems are presented. In the second part, attempts are made to examine factors affecting *in vitro* fermentation studies, establishment of repeatable technique and relationship between *in vivo* and *in vitro* results.

PART I

THE ESTIMATION OF APPARENT DIGESTIBILITY

CHAPTER 1. REVIEW OF LITERATURE

Introduction

The general principles of conducting digestibility trials are simple but the practical details are more complicated, and various factors such as level of intake, differences between species of animal, length of the experimental period, and number of animals to use, have to be taken into account.

In this chapter some of the factors affecting digestibility, as well as methods of conducting digestibility trials, are reviewed.

1.1. Between species variation in apparent digestibility in ruminants: sheep vs cattle.

There is a considerable divergence of opinion as to the validity of applying results obtained from sheep digestibility trials to those for cattle.

Jordan and Stapler (1951) found that sheep digested roughage better than cattle; similar results were also reported by Ivins (1960) at the 8th International Grasslands Congress (see table 1.1.). Unfortunately in neither case were intake figures or number of animals used available.

Table 1.1. Comparison of abilities of dairy cows and sheep to digest herbage (% D.M. digested). Adapted from Ivins (1960).

	HERBAGE			
	A	B	C	D
Dairy cows	57.8	54.5	55.0	52.7
Wether sheep	59.7	55.4	61.7	59.0

In contrast to these results, other workers have reported the reverse. Morrison (1951) reported that cattle digested low grade roughage to a greater extent than sheep and that there was a slight advantage in protein digestibility by sheep.

Analysing the data which contained 1912 digestion trials from 386 authors (Schneider, 1947), Cipolloni et al (1951) reported that the mean squares after covariance adjustment for composition revealed significant differences between species of animals for the digestibility of O.M., crude fibre, and N.F.E. In the case of silages and concentrates, although significant difference between the two ruminant species was found only with ether extract, the species mean squares for O.M., N.F.E. and T.D.N. were large enough compared to their errors to indicate a trend. They concluded as follows:

> "In general, more accurate assessment of nutritive value will be obtained if, when working with cattle, one uses digestion coefficients obtained with cattle, and if when working with sheep, those obtained with sheep are used".

Their results are summarized in table 1.2.

Table 1.2. The average differences in digestibility by cattle and sheep of three classes of feeds after adjustments for proximate composition[+]. (Adapted from Cipolloni et al, 1951).

FEED CLASS	O.M.	CRUDE PROTEIN	CRUDE FIBRE	N.F.E.	ETHER EXTRACT	T.D.N.
Dry roughage	3**	1	4*	4**	4	3**
Silage	3	1	2	3	8**	3
Concentrates	-2	2	-4	-2	-20**	-3

+Positive values for cattle. Negative values indicate that sheep digest better than cattle.

**Significant at 1% level.

*Significant at 5% level.

Unfortunately, no intake data are available and feed qualities might have been different from sheep to cattle since the results were not obtained in the same digestibility trial.

Forbes et al (1937) compared digestibilities determined with steers and sheep under more controlled and critical conditions. Four rations (i.e. lucerne and concentrate mixtures) were fed to steers and sheep, using 2 to 5 animals and various planes of nutrition in relation to maintenance were used. They found no significant difference between sheep and steers in their ability to digest the rations.

Numerous experiments on this matter carried out during the period from 1890 to 1948 were reviewed in detail by Canadian workers (Watson et al, 1948), who concluded that for all practical purposes any differences in the ability of sheep and cattle to digest food were negligible. Similar observations were also reported by other workers (e.g. Minson, 1958; Jang et al, 1962).

Alexander et al (1962) compared the digestibility of two silages and coastal Bermuda-grass hays by cattle and sheep to see whether digestion coefficients of each species could be calculated from data obtained from the other. The correlation coefficient (r) was highly significant between sheep and cattle in terms of D.M. digestibility.

The similarity of the digestive coefficients of a herbage when fed to sheep and cattle is important. Experiments with sheep are

much more convenient; the use of larger animal numbers and treatment replication permit a better experimental design, while the need for expensive equipment, large amounts of feed and the higher labour demand, which are necessary for cattle digestion trials, are greatly reduced.

1.2. Within species variations

Individual variations between animals in their ability to digest food have often been noted. These are usually small, rarely exceeding two units of digestibility.

Age of animal was found to be a factor which might have affected within species variation. Reports from Hurley (1951) suggested that between the ages of one and four years, older sheep digested herbage more efficiently than younger animals. Raymond *et al* (1954) also showed that the digestive efficiency of sheep increased by approximately 1% per year as the animals grew older. It is, however, now recognised that this increasing efficiency results largely from the effects of gastro-intestinal parasites (Spedding, 1954): as sheep become older they become increasingly immune to these parasites and their depressing effect on digestive efficiency decreases. Further, the claim by Kellner (1915) that the age of animal, apart from the affects of development in the young ruminant, does not influence the digestibility of food is supported by McArthur (1957), who found no significant differences between digestibility coefficients obtained when high quality ryegrass was fed to adult cows and to 8 week old calves.

Whilst Kellner (1915) reported that activity of the animals increased digestibility by up to 2% when animals were subjected to

exercise compared with when they were tied in the stall, experiments at the Hannah Institute with sheep (Blaxter, 1962) and in Czechoslovakia with sheep (Herzig et al, 1963) showed a negligible effect.

Level of milk production had no effect on digestibility (Ivins, 1960).

Excluding extreme cases such as tropical climate (Sharma and Kehar, 1961), the environmental temperature has been reported to have little or no effect on digestibility of food by sheep and cattle (Blaxter, 1962).

Watson et al (1936) are of the opinion that digestibility coefficients of a feed may vary for different experimental periods. They suggested that if the digestibility of a certain feed is to be compared with that of another feed, the experiment should be so designed that all feeds which are to be compared are tested in the same experimental period. A further study at the Grasslands Research Institute, Hurley (1953), however, reported only minor differences due to different time of the year (table 1.3.).

Table 1.3. Effect of different time on O.M. digestibility (average of 6 sheep). Adapted from the Grasslands Res. Inst. Rept. (1953).

	OCT.	JAN.	MARCH	MAY	JULY	SEPT.
Digestibility of frozen grass	79.0	78.0	79.0	78.4	79.5	80.0

Similar results are also reported by Groenewald et al (1950). Five steers were used to determine the digestibility coefficients of

the D.M., crude protein and crude fibre of lucerne hay during five separate periods. They found that period had no influence on the digestibility coefficients of crude protein and little effect on digestibility of dry matter.

It appears that the effects of age, activity, lactation, or period, on digestibility of food within a species of ruminant are probably negligible.

1.3. The effect of level of intake on apparent digestibility.

It is generally believed that the apparent digestibility of mixed ration decreases as the level of intake increases (Forbes et al, 1928, 1930; Mitchell and Hamilton, 1932; Reid, 1956), although cases where level of intake did not affect digestibility are also reported using mixed rations (Lassiter et al, 1957, 1958).

Watson et al found that the digestibility of corn silage (1939), linseed oil (1949a), barley (1949b) and oilcake ration (1949c) decreased when increasing quantities were fed with a constant proportion of hay to steers. Similar results were also reported by other workers such as Forbes et al (1928, 1930), and Mitchell and Hamilton (1932). Blaxter and Wainmann (1961) compared a mixture of dried grass and oats at 5 levels (half maintenance to twice maintenance) and 6 levels (half maintenance to three times maintenance) using three steers and sheep. The results indicate that in both steers and sheep the energy lost in the faeces per unit energy intake rose in a similar manner as the level of feeding was increased.

With roughage alone, results reported from various workers have been conflicting. Watson et al (1935) found no decrease in

digestibility of dry matter when the amount of hay was increased from $4\frac{1}{2}$ kg to 9 kg per day. Although in some of the experiments dealing with this problem the range of intake imposed was small, many workers reported that the level of intake had no or only little effect on digestibility, e.g. with cattle fed hays (Armsby and Fries, 1905, 1908, 1911; Hale et al, 1940), with sheep fed hays (Woodman et al, 1937; Blaxter et al, 1954, 1956; Ivins, 1960), and fresh grass (Raymond et al, 1955; Anderson et al, 1959; Hutton, 1963).

On the other hand, Raymond et al (1959) using sheep fed cold stored pasture, found an average increase of 1.5 units of digestibility between levels from 1190 gm., to 790 gm., of dry matter intake. An experiment of three different levels of feeding with ryegrass of different maturities on digestibility, carried out at the Hannah Institute (Blaxter, 1962) showed that the faecal loss of early cut grass increased only slightly (16% to 20%) when the feeding levels increased from sub maintenance to 3 times maintenance whereas the faecal energy loss of the late cut grass increased from about 33% to 45% when the feeding level increased over the same range.

Blaxter (1962) summarised twenty-one experiments, involving a total of 194 determinations of digestibility, which showed decreases of digestibility with increases in feeding level. The depression is greater for those rations which had the lower apparent digestibility, and is greater for those roughages which had been finely ground and made into pellets than those given in their natural state.

The regression of the digestibility depression per unit increase in feeding level on digestibility determined at the maintenance level was given by Blaxter (1961) as follows:

$$\Delta A = \text{Depression in apparent digestibility of energy on increasing food intake from maintenance to twice maintenance} = 11.9 - 0.119\ Am$$

where Am is the apparent digestibility of the energy of feed determined at maintenance.

A similar equation was also given by Armstrong (1964), working with twelve grasses cut at different stages of maturity as follows:

$$\Delta A = 10.1 - 0.11\ Am$$

1.4. Associative digestibility.

As early as 1926, Titus stated that

> "the experimental evidence is that the combining of feeding stuffs may affect their digestibility and that the effect will depend upon the kinds and amounts of feed combined; in some cases the effect may be almost negligible and in others quite large. At present, accurate knowledge regarding the mutual influence of the proportion of the several nutrients on their digestibility and regarding associative digestibility is quite meager."

Watson (1949) reported at the 5th International Zootechnie Congress that when mixtures of different feeding stuffs are given to ruminants, the apparent digestibility of the mixture is not necessarily the same as the weighted sum of the apparent digestibilities of its components.

It has been generally agreed that the associative effect on apparent digestibility is due to microbial activity in the rumen. The most striking example of this effect is the depression of the digestibility of cellulose by the addition of starch or glucose to the diet (Hamilton, 1942). Another important associative effect is that

addition of protein-rich food to a roughage diet low in nitrogen can often cause an increase in the apparent digestibility of roughage. Possible reasons and factors affecting this matter have been discussed by various workers, such as McDonald (1952), Annison (1956) and Lewis and McDonald (1958).

1.5. Grinding and pelleting

Although there is debate, and the question has not been settled as to whether the net energy content of finely ground pelleted roughage is greater than long or coarsely ground roughage, most research workers agree that the physiological response of growing and lactating ruminants is altered.

In this section, only the influence of finely ground and pelleted roughage on apparent digestibility is discussed.

The grinding of feeds has not been found to increase their digestibility by ruminants as shown by earlier workers (Forbes, Fries and Braman, 1925; Kellner, 1926).

Meyer *et al* (1959) and Paladines *et al* (1964) have shown no difference in gross energy digestibility of finely ground and pelleted roughage compared to long or coarse roughage. Heaney *et al* (1963) and Lloyd *et al* (1960) have shown both a decrease and little change in energy digestibility in similar experiments. Blaxter and Graham (1956) and Johnson *et al* (1964) have shown large and consistent decreases in apparent digestibility when roughages are finely ground and pelleted.

Blaxter, Graham and Wainman (1956) compared three levels of feeding with three different forms of feed, i.e., long form, cubes made from medium and cubes made from fine particles. The results are

summarized in table 1.4.

Table 1.4. Effect of level of intake and physical form on apparent digestibility with sheep. Adapted from Blaxter, Graham and Wainman (1956).

LEVEL OF FEEDING (gm/day)	PHYSICAL FORM OF DRIED GRASS	APPARENT DIGESTIBILITY (%)	MEAN LENGTH OF TIME STAYED IN GUT (hr)
600	long	80.3	103
	medium	76.9	74
	fine	75.9	53
1200	long	79.1	72
	medium	71.5	53
	fine	68.8	38
1500	long	79.4	68
	medium	69.9	42
	fine	65.4	34

The results suggest that the physical form of food, and the quantity given, influence the length of time it stays in the gut, and if the time is short a decrease in the digestibility of food is apparent.

Hopkins et al (1960) reported similar results. Meyer et al, (1959), however, reported a faster rate of passage but no change in digestible energy content. Recently Johnson et al (1964) showed a general decrease in digestible energy, crude fibre, and dry matter when roughages were finely ground and pelleted, while, earlier, Lindahl and

Reynolds (1959) showed no change in energy or crude fibre digestibility when finely ground dehydrated alfalfa was pelleted.

Determination and description of fineness of grind needs more attention (Meyer et al, 1965). Forages from different species and stages of maturity ground through the same screen varied greatly in particle size when examined by mechanical analysis with sieves (Heaney et al, 1963). The result reported by Rodrique and Allen (1960) described this clearly.

It appears from these reports that the physical form to be used in digestibility trials may affect the digestibility of the feed, particularly with low quality roughage.

1.6. Variations in apparent digestibility as affected by experimental technique.

1.6.1. Length of preliminary- and collection periods.

When it is necessary to determine the losses of a particular nutrient in the faeces, two techniques are employed to ensure that the faeces collected are representative of the intake of food. The first consists of feeding a marker substance, such as carmine, immediately before and at the end of the experiment. With ruminants, however, the use of markers does not result in a clean separation of the faeces because of their more complicated digestive structures. The method employed to obtain accurate digestion coefficients with ruminants, then, is to give the experimental ration in constant amounts for long periods, in order to ensure that a steady state of faecal excretion is reached, and then to collect the faeces excreted during a measured interval of

time. The periods used by various workers in their digestibility trials vary from 5 days to 30 days for preliminary periods, and 5 days to 20 days for collection periods.

In 1915, it was suggested by a special nutrition committee that for ruminants preliminary periods should be 10 to 12 days and collection periods, 12 to 15 days. Schneider and Ellenberger (1927) have indicated the need for periods of 7 to 30 days for adjustment and 7 to 14 days for the collection of faeces. Mitchell and Hamilton (1932) and King (1943) have shown that 14-day collections are adequate when compared to 20 or more days. Other workers have used periods ranging from only 5-day experimental period (Eheart, Holaway and Pratt, 1945), 7-day preliminary and 12-day collection period (Raymond et al, 1953), 10-day preliminary and 10-day collection period (Swanson and Herman, 1944), 13-day preliminary and 10-day collection period (Bechtel, Shaw and Atkenson, 1945) to 10-day preliminary and 15-day collection period (Hodgson and Knott, 1932).

Comparisons of shorter collection periods were made by various workers. Staple and Dinusson (1951), who used 10-day preliminary periods, compared 7- with 10-day collection periods. Losses in efficiency for the digestibility of the nutrients studied were less than 1.1% in all cases except that for nitrogen-free extract, which showed an efficiency loss of 6.83%. Axelsson and Kivimae (1951) compared different lengths of collection period from 2 to 44 days preceded by 10 days preliminary periods and showed that shorter collection periods were associated with larger standard deviations of digestion coefficients of O.M., i.e., 2-day collection period, 1.70; 10-day, 0.65 and 42-day, 0.25. Similar results were also reported by

Gainger et al (1960), who compared 3- and 7-day collection periods preceded by a 21-day preliminary period and found a significant difference between 3 and 7 days. King et al (1960), however, found no differences between 6- and 10-day collection periods on digestibility coefficients in their 6 digestion trials with 3 heifers fed oat hays cut at different stages of growth, supplemented by cotton seed meal.

Because of the irregular passage of food through the gut, even on a constant level of food intake, errors are likely to arise during faecal collection periods of only a few days. Further uncertainty may arise through "end-period errors", i.e., whether faeces passed just at the beginning or end of the faecal collection period should be included and are representative of amounts eaten. These problems have been reviewed by Schneider and Ellenberger (1927), Raymond et al (1953) and Blaxter et al (1956).

Lloyd et al (1956) concluded from their results, in which comparisons of preliminary periods of zero to 60 days were made, that a preliminary period of 10 days was sufficient with the feeds they used, which were Timothy hay and Timothy and concentrate mixture. Blaxter et al (1956) also suggested that a 10-day preliminary period was sufficient with the roughages they tested.

Nicholson et al (1956) made a study of the length of preliminary feeding periods and concluded that longer adjustment periods are needed when the hay-to-grain ratio fluctuates widely. The optimum period found for such trials lay between 16 and 30 days. However, for rations in which the basic components and the hay-to-grain ratio were constant, a preliminary period of 7 days was found adequate.

From the results of various workers on digestibility trials,

it appears to be sufficient to have 10-day preliminary and 10-day collection periods.

1.6.2. Number of animals required to determine digestibility.

It is generally recognised that a digestion trial on a single animal is quite inadequate for an accurate determination of digestibility, but only limited data are available on the number of animals required for a given degree of precision.

Fraps (1925), in a discussion of the use of average digestibility data in a compilation of the productive energy of feeding stuffs, considered that the variation in the digestive efficiency of different animals affected the quoted digestibility somewhat less than the variation between samples of the same feed. Number of animals used in digestibility trials vary from one to six or sometimes more than six.

Hodgson and Knott (1932) used three heifers. Three steers were also used by Neal, Becker and Dix-Arnold (1935). Four heifers were used by Swanson and Herman (1944) and French (1956) used six steers, while Lansbury (1958) used two to three heifers on each feed.

Forbes et al (1946) studied relationships between the variability of the observed values for digestibility of the nutrients of clover-timothy hay and the numbers of sheep represented. The results are presented in table 1.5.

Table 1.5. Relationship between the variability of the observed value of digestibility of dry matter and the number of sheep represented.

NUMBER OF SHEEP IN EACH GROUP	DIGESTIBILITY OF D.M. S.E.+	SIG. DIFF. BETWEEN MEANS*
1	1.09	3.0
2	.77	2.1
3	.63	1.7
4	.55	1.5
5	.49	1.4
6	.45	1.2
7	.41	1.1
8	.39	1.1
9	.36	1.0
10	.35	1.0

Adapted from Forbes et al (1946)

+The standard errors of the means.

*Minimum difference required between mean values for odds of 19:1.

They concluded that five sheep per treatment are a sufficient number for usual purposes if the experimental technique is efficient. Schneider and Lucas (1950) have estimated, from digestibility data on 194 feeding stuffs by 94 authors, the relative proportion of "within feed" variance due to authors, batches, and animals. They concluded that the largest portion of error in digestibility data were those associated with authors and batches. The portion associated with animals (including chemical and minor sampling error) was relatively small. Bredon et al (1961) used 6 steers for each digestibility trial with ground nutcake, maize meal and hay to find the errors associated with number of animals. They presented the following table (table 1.6).

Table 1.6. Relationships between the variation of D.M. digestibility and the number of animals.

NO OF STEERS	D.M. DIGESTIBILITY MEAN	S.D.*
1	46.60	10.2
2	54.07	4.2
3	53.75	3.6
4	53.49	3.1
5	52.94	2.0
6	50.57	2.5

Adapted from Bredon et al (1961)

*Standard deviations.

They concluded from their previous experimental results together with the data presented above that, although standard deviations of the coefficients obtained from individual steers are approximately consistent, using one steer may lead to as much as 10.2% error in determination of dry matter digestibility if the average figure for 6 steers is taken as the true figure.

Forbes et al (1946) have given an estimate of variance of dry matter digestibility from a single experiment in which 22 sheep were fed the same hay (mean digestibility of dry matter = 57%). They estimated:

$$S^2 = 1.191$$

$$S = 1.091 \text{ (21 d.f.)}$$

Raymond et al (1953) also estimated variance from an analysis of fifty-three digestion trials carried out at Hurley over a period of 4 years. The estimate obtained was:

$$S^2 = 1.73$$

$$S = 1.315 \text{ (with 132 degrees of freedom)}$$

Using this figure and the formula given by Cochrem and Cox (1957), $r \geq 2 (\frac{\sigma}{\delta})^2 (t_1 + t_2)^2$, they calculated the following table (table 1.7.).

Table 1.7. Number of animals required for a given probability of obtaining a significant result.

TIME DIFFERENCE (δ) AS PER CENT OF THE MEAN	PROBABILITY OF DETECTING σ	
	80%	90%
	NUMBER OF REPLICATIONS	
1	28	38
1.5	14	17
2	8	11
2.5	6	7
3	4	6
4	3	4
5	3	3
10	2	2

Adapted from Raymond et al (1953)

It appears to be important to have at least three animals per treatment and increasing the number of animals in digestibility trials reduces the standard error. However, in practice, other factors such as feed quantity, limitation of labour and facilities should also be considered in relation to deciding the number of animals per treatment.

1.6.3. Drying of feed and faeces samples

The accuracy of the determined digestibility depends very

much on accurate measurements of the dry matter content of feed consumed and faeces voided. There are two major sources of possible inaccuracy in determining dry matter of feed and faeces. The first is associated with freshly harvested material and fresh faeces, in which continuing metabolic activity causes loss of material during drying. This problem has been dealt with by McRostie and Hamilton (1927), Raymond (1951) and Davies et al (1948). The second possible error arises from the difficulty of completely removing the water from herbage at temperatures which will not cause serious decomposition of the herbage or faeces and loss of volatile materials (Leroy, 1954; Greenhill, 1960).

Various drying methods have been used by different investigators. Dry weights have been determined by oven-drying at 105°C (Couchman, 1959; Thomas and Smith, 1955), 100°C (Raymond et al, 1953), 80°C (Hirst et al, 1959), 70°C (Stickler and Johnson, 1959), 65°C (Forbes et al, 1946) and 38°C (Thomas and Smith, loc. cit.).

The duration of the drying is given as "to constant weight" or "overnight". The Analysis of Fodders Sub-Committee (1944) recommended:

> "that a 2 g sample of air-dried food be dried in an oven at a temperature between 95°C and 105°C until the loss in weight does not exceed 1 mg per hour".

In the case of drying faeces 10% of whole faeces was used for dry matter determination by various investigators.

Greenhill (1960) compared faeces drying methods for dry matter determination and suggested that drying over P_2O_5 at 40°C was the best method. For a less accurate method, oven-drying at 80°C for

16 hours was proposed in practical use.

CHAPTER 2. MATERIALS AND METHODS

2.1. Animals

From a flock of New Zealand Romney sheep, eight 2-tooth wethers were selected for the experiment. In order to reduce individual variability as much as possible, the animals were chosen for similarity in age, weight and general condition. They spent about a week in larger crates before they were put into metabolism crates.

Internal parasitic control was carried out a week before the digestibility trial began by dosing the sheep with thiobendazole. The live weight of each sheep was obtained at the commencement of the experiment, at the end of the digestibility trial with mixed hay and the end of the whole experiment. All weights were recorded at 8:00 a.m. prior to the morning feeding.

The sheep had previously been grazing ryegrass-clover pasture and all appeared to be in good health.

2.2. Feeds

The hays were selected according to appearance from the University farms. Descriptions of the hays are given in table 2.1., and chemical composition of the hays is given in table 7.4., (Part II, 7.6.2.).

Since the particle size of feed may be an important factor affecting digestibility, it was decided to coarsely chop each hay by Hammer mill to pass through a 1 inch diameter sieve rather than grinding and pelleting them. The hays were chopped to facilitate feeding and sampling. After chopping, each hay was well mixed on a

Table 2.1. Description of hays.

	HAY A	B	C	MIXED HAYS
Date closed	25/12/1965	7/11/1964	30/10/1965	
Date of cutting	5/ 2/1966	9/ 1/1965	18/12/1965	
Period closed	6 weeks	9 weeks	7 weeks	
Components and colour	Young, leafy ryegrass and good proportion of clover light green	Cocksfoot with ryegrass. Some mould, greenish brown	Fairly mature and stalky, rained during harvesting, ryegrass straw, light brown.	
Length of storage	4 weeks	56 weeks	12 weeks	
Crude protein (%)	15.96	14.47	11.74	15.61
Crude fibre (%)	28.26	31.32	35.17	30.22

Table 2.2. Experimental design used in digestibility trial for the mixed hay and hay A, B and C.

FEED	NUMBER OF SHEEP	AD LIB. PERIOD (DAYS)	PRELIMINARY PERIOD (DAYS)	COLLECTION PERIOD (DAYS)
a) Mixed hay	8	14	10	10
b) Individual hays (hays A, B and C).	2	7	a) 7	10
			b) 10	7
			c) 4	14
			d) 7	7

concrete floor and a sample was taken for chemical analysis and for *in vitro* digestion studies (see Part II, section 6.6.1.).

Approximately one third of each hay was taken and mixed together thoroughly on a concrete floor (hereafter called mixed hay).

2.3. Experimental Design

To correct any variations in individual animal difference, a covariance design (Snedecor, 1965) was utilized. The eight experimental sheep (nos 1 to 8) were fed with the mixed hay to determine the digestibility (independent variable). After completion of the mixed hay digestion trial, 6 out of the 8 sheep were paired and randomly allocated to the individual hays, and their digestibilities were determined (dependent variable).

An attempt to compare different lengths of preliminary and collection period was made to see any effect on digestibility of hays A, B and C.

Experimental procedure in more detail are described in the following sub-sections and summarised in table 2.2.

2.3.1. Training period

The main objects of the training period were to accustom the animals to indoor feeding, to establish satisfactory feeding routines and techniques for collecting faeces and sampling materials. During this period, internal parasitic control and live weight measurements of the sheep were carried out. This training period lasted from 26th February, 1966 to 5th March, 1966. Hay A was used for this period.

2.3.2. Period for establishing voluntary intake

The object was to establish voluntary intake as a guide to the level of hay to feed over the experiment. The allowances of feed were arrived at by the modified sequential system of feeding developed for sheep by Blaxter, Wainman and Wilson (1961).

The daily feeds were weighed to the nearest 1 gm., and given at 8:00 a.m., and 4:30 p.m. Water was provided separately from the feed and changed once a day. Refused feed was collected and D.M. determined each day.

2.3.3. Preliminary period

After establishing voluntary intake for each animal, a constant feeding level was maintained during the whole experimental period by reducing the average intake of the last 7 days by 10%.

2.3.4. Collection period

Feed offered, refused, and faeces produced, were obtained during this period (see also 2.4.2., and 2.4.3.).

O.M. digestibility of the hays was calculated using the following equation:

$$\text{Digestibility } \% = \frac{\text{Amount of feed eaten (O.M.g)} - \text{Faeces produced (O.M.g)}}{\text{Amount of feed eaten (O.M.g)}} \times 100$$

2.4. Sampling of feeds and faeces

2.4.1. Chemical analysis

Analysis of the hays and grasses used in the *in vivo* and *in vitro* experiments for moisture, ash, crude fibre, crude protein, ether

extract and nitrogen-free extract were made by methods recommended by the A.O.A.C., (1960) with the modification described by Meeker and Wagner (1933) and Hiller *et al* (1948) for crude protein determination.

2.4.2. Feed D.M. and O.M. determinations

About 100 gm., duplicate samples of each hay fed were taken every two days and the whole refused feed was collected every day for D.M., determination. The samples were dried in an air drought oven at 85°C for 16 hours and were weighed within 10 minutes of removal from the oven. As there were no significant differences in O.M., content of feed offered and feed refused, O.M., determinations were made using the samples which were taken for chemical analysis and *in vitro* studies (see 2.2.).

2.4.3. Faeces D.M. and O.M. determination

Faeces was collected from each sheep at 9:00 a.m., during the collection period, weighed and about 200 gm., duplicate sub-samples were taken for D.M., determination. They were dried for 48 hours and weighed, using the same method described in 2.4.2. About one-tenth of the dried faeces samples from each day were stored at -12°C. Composite samples were then ground to pass through 1.0 mm., sieve and 4 replicates of about 2 gm., of faeces samples from each sheep were weighed out for O.M., determinations.

CHAPTER 3. RESULTS

3.1. Animals

Animals soon became accustomed to indoor feeding conditions. Sheep no. 3 scoured considerably over the training period so feed intake was reduced, although the condition cleared up by the end of the training period.

Live weights of the animals are summarised in table 3.1.

During the mixed hay digestibility trial, all the sheep except sheep 3 and 6 gained in live weight. Sheep 1 and 2 gained about 3 to 4 kg., while others gained only 0.5 to 2.0 kg.

During the digestibility trial with the individual hays, sheep fed hay A gained a little live weight, excluding sheep 3 and 6, and sheep fed on hays B and C maintained their live weight, except sheep 2 and 5 which gained slightly.

3.2. Digestibility of the mixed hay

3.2.1. D.M. intake during ad lib. feeding period

Daily mean and range of D.M. intake during the period are given in table 3.2.

There was a significant difference in D.M., intake between days ($P < 0.01$) and between sheep ($P < 0.01$) when intake was expressed in terms of the 0.75 power of liveweight (appendix 1). Although there was no significant difference in D.M. intake between the 1st and 2nd week, D.M. intakes during the 2nd week were slightly lower for all sheep than those of the first week.

Table 3.1. Live weights of experimental sheep (means of 2 successive measurements).

SHEEP NO	DATE 4-5/3 (kg)	DATE 4-5/5 (kg)	DATE 26-27/5 (kg)	FEED
1	31.3	35.4	36.9	mixed hay, hay A
2	34.6	37.4	38.1	" " B
3	31.0	30.2	26.3	" " A*
4	34.5	35.6	36.4	" " A
5	33.6	35.4	36.4	" " C
6	38.1	36.5	34.1	" " A*
7	35.7	35.6	35.5	" " B
8	35.7	36.1	35.6	" " C

*Excluded from the individual hay digestibility trial after the mixed hay digestibility trial.

Table 3.2. D.M. intake during ad lib. feeding period with mixed hay.

SHEEP NO	FOR THE WHOLE PERIOD				FOR THE LAST 7 DAYS			
	D.M. INTAKE(g)		INTAKE/kgW$^{.75}$(g)		D.M. INTAKE(g)		INTAKE/kgW$^{.75}$(g)	
	mean	range	mean	range	mean	range	mean	range
1	1,056	959-1,126	79.9	73.4-85.2	1,035	972-1,074	78.4	70.2-81.3
2	1,124	972-1,153	78.8	68.2-87.9	1,101	972-1,188	77.2	68.2-83.3
3	740	538-1,011	56.7	41.2-77.4	738	649-806	56.5	49.7-61.7
4	877	717-994	61.7	50.4-69.9	847	781-906	59.6	54.9-63.7
5	824	625-1,051	59.0	44.8-75.3	781	625-957	55.9	44.8-68.6
6	1,006	843-1,254	65.5	54.9-81.7	973	843-1,053	63.4	54.9-68.6
7	969	896-1,043	66.3	59.4-71.3	972	917-1,043	66.5	62.7-71.3
8	999	828-1,165	68.3	56.6-79.7	959	828-1,054	65.6	56.6-72.1

Table 3.3. Mean daily D.M. intake of mixed hay during the preliminary period.

SHEEP NO.	1	2	3	4	5	6	7	8
Mean (g)	830.7	951.1	613.8	647.7	661.2	814.2	783.2	808.6
+S.E. of mean	6.3	2.1	8.7	5.1	8.5	3.6	7.8	3.6
*C.V. (%)	2.4	0.7	4.5	2.5	4.1	1.4	3.1	1.4

+Standard error

*Coefficient of variation

3.2.2. D.M. intake during preliminary period

An attempt was made to keep the animals to a constant intake throughout the experiment by feeding them approximately 90% of the amount of feed consumed during the last 7 days of ad lib., feeding period. Mean daily D.M. intake, the standard error of mean and coefficient of variation (C.V.) are given in table 3.3.

When an analysis of variance was made for differences between sheep and days, there was a highly significant difference between sheep ($P < 0.01$), even when the intake was expressed in terms of metabolic body weight ($Wkg^{0.75}$). As expected there were no significant differences in intake between days (appendix 2).

3.2.3. Feed consumed and faeces produced during collection period

The difference in O.M. intake between sheep were highly significant ($P < 0.01$) but there were no significant differences in intake between days when the amount of O.M. eaten was expressed in terms of metabolic body weight (see appendix 3).

Mean daily O.M. intake and faeces voided over the collection period are shown in table 3.4.

The digestibility of mixed hay was 58.62 ± 0.13. No relationship was found between level of intake of individual sheep and digestibility under the conditions described.

3.3. Digestibility trial using individual hays

3.3.1. Ad lib. feeding period

The mean daily D.M. intake is given in table 3.5.

Table 3.4. Mean daily O.M. intake and faeces produced during the collection period and O.M. digestibility of mixed hay.

SHEEP NO.	1	2	3	4	5	6	7	8
1. Feed intake Mean (O.M.g)	751	846	536	590	635	711	712	725
S.E. of mean	1.9	2.4	12.0	3.3	5.3	9.9	2.4	3.9
C.V. (%)	0.8	0.9	7.1	1.8	2.7	4.4	1.1	1.7
2. Faeces voided Mean (O.M.g)	308	349	225	244	266	291	297	299
S.E. of mean	3.6	6.8	5.6	2.1	5.4	14.9	4.2	7.8
C.V. (%)	3.7	6.2	7.9	2.7	6.4	16.3	4.5	8.2
3. Digestibility (O.M. %)	58.99	58.81	58.07	58.67	58.17	59.12	58.28	58.83
Mean				58.62				
S.E. of mean				0.13				

Table 3.5. Mean daily D.M. intake during ad lib. feeding period with hay A, B and C (mean of 7 days).

HAYS	SHEEP NO.	D.M. INTAKE (g)	D.M. INTAKE $KgW^{0.75}$ (g)	AVERAGE OF 2 SHEEP $g/KgW^{0.75}$
A	1	967	66.6	63.0
	4	866	59.4	
B	2	799	52.9	49.4
	7	668	45.8	
C	5	863	59.4	51.9
	8	654	44.4	

Table 3.6. Feed consumed, faeces voided and digestibilities of hay A, B and C.

	HAY A		HAY B		HAY C	
	SHEEP 1	SHEEP 4	SHEEP 2	SHEEP 7	SHEEP 5	SHEEP 8
1. D.M. intake during the preliminary period						
Mean (D.M.g)	882	770	743	614	748	565
S.E. of mean	2.1	5.4	9.8	2.8	8.8	6.6
C.V. (%)	0.6	1.8	3.5	1.6	3.1	3.1
2. O.M. intake during the collection period						
Mean (O.M.g)	771.0	668.0	685.2	555.9	689.5	478.5
S.E. of mean	3.1	5.5	2.9	2.6	4.3	13.6
C.V. (%)	1.3	2.6	1.3	1.5	2.0	9.0
3. Faeces voided						
Mean (O.M.g)	264.8	231.1	312.7	262.5	273.7	183.9
S.E. of mean	4.7	5.5	4.4	2.8	4.9	4.6
C.V. (%)	5.5	7.5	4.5	3.4	5.7	7.9
4. O.M. digestibility	65.66	65.43	54.37	52.78	60.30	61.57
Average	65.55		53.58		60.94	
5* Adjusted mean digestibility	66.02		53.43		60.90	
6+ Difference	+0.47		-0.15		-0.04	

*O.M. digestibility was adjusted using covariance analysis, taking digestibility of the mixed hay as Xs and digestibility of hay A, B and C as Ys.

+'5' - '4'

Significant difference in D.M. intake between sheep ($P < 0.01$) and between days ($P < 0.01$) were found (appendix 4a). Although there was no significant difference between hays, the order of intake in terms of D.M. in gm. per 0.75 power of live weight (kg) was 63.0, 49.4 and 51.9 for hay A, B and C respectively (table 3.5., and appendix 4b).

3.3.2. Digestibility trial period

Mean daily D.M. intake during the preliminary period and O.M. intake during the collection period with measures of variability are given in table 3.6.

Intake of hay A was greater than hays B and C (see 3.3.1.), and the difference was highly significant ($P < 0.01$) (see appendix 5).

Daily O.M. intake was maintained fairly constant throughout the whole digestion period except for sheep 8 (see figure 3.1.).

Faeces produced fluctuated more than feed consumed in terms of coefficient of variation. The amount of O.M. consumed had no effect on digestibility under the limited replication possible, i.e. two sheep per treatment.

3.4. Comparisons of different lengths of preliminary and collection period and their effect on digestibility

Different lengths of preliminary and collection period were compared. Daily mean amounts of O.M. consumed, faeces produced and digestibilities adjusted by covariance analysis are given in table 3.7 (see also appendix 8).

Effect of lengths of experimental period on daily O.M. intake are analysed and presented in appendix 6, and effect on digestibility

is given in appendix 7. No significant differences were found on digestibility due to different lengths of preliminary and collection period.

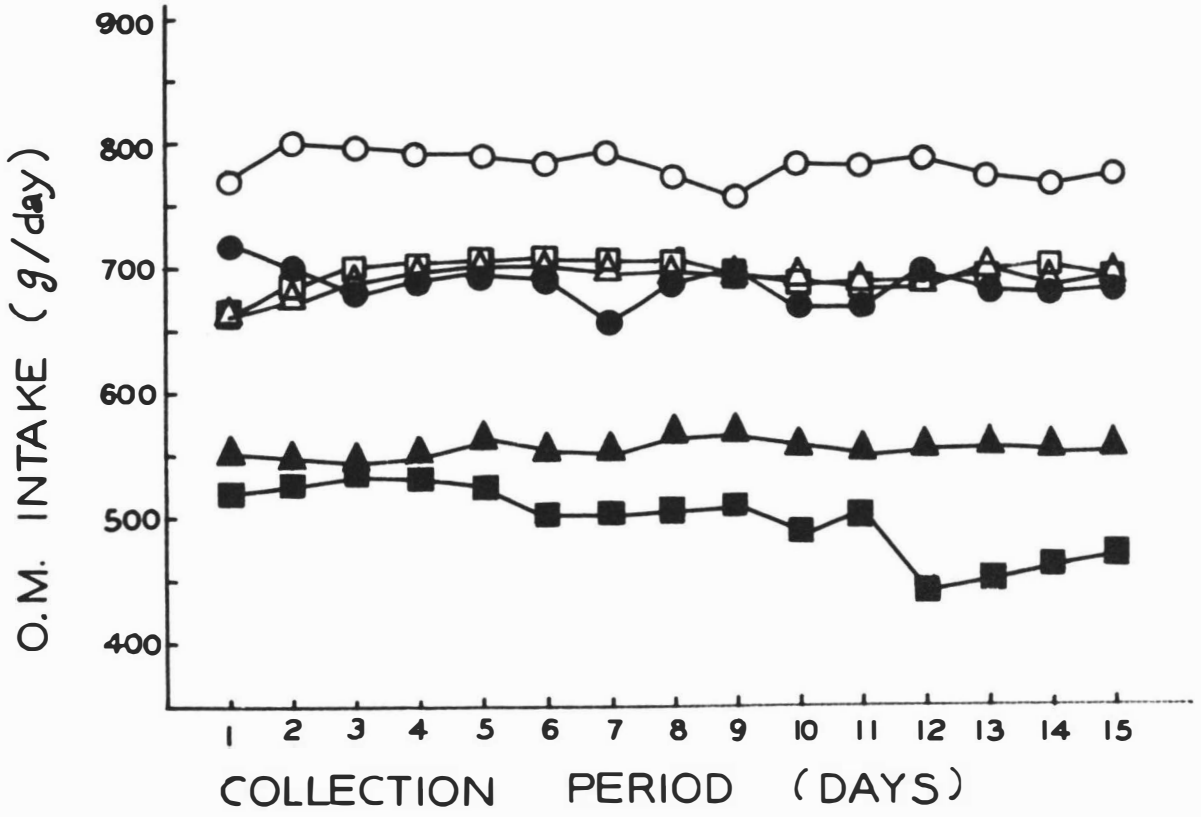

FIG. 7. O.M. INTAKE DURING COLLECTION PERIOD

HAY A ○ SHEEP No. 1
HAY A ● " 4
HAY B △ " 2
HAY B ▲ " 7
HAY C □ " 5
HAY C ■ " 8

Table 3.7. Comparison of different lengths of preliminary and collection period

		HAY A		HAY B		HAY C	
	PERIOD*	SHEEP 1	SHEEP 4	SHEEP 2	SHEEP 7	SHEEP 5	SHEEP 8
Daily O.M. intake ± S.E. (g)	1	771 ± 3.2	668 ± 5.5	685 ± 2.9	556 ± 2.9	689 ± 4.3	479 ±13.6
	2	771 ± 3.9	666 ± 6.8	682 ± 3.3	554 ± 3.4	682 ± 2.5	471 ±11.1
	3	778 ± 9.6	673 ± 4.6	686 ± 2.4	556 ± 2.1	693 ± 3.4	495 ± 9.3
	4	773 ± 3.9	671 ± 7.6	688 ± 2.7	558 ± 1.1	695 ± 4.6	489 ±10.6
Daily faeces collected ± S.E. (g)	1	264.8 ± 4.7	231.1 ± 5.5	312.7 ± 4.4	262.5 ± 2.8	273.7 ± 4.9	183.9 ± 4.6
	2	263.9 ± 6.0	223.8 ± 4.8	316.5 ± 3.0	259.2 ± 2.7	268.3 ± 4.7	183.3 ± 3.8
	3	268.4 ± 1.2	232.2 ± 4.3	313.6 ± 3.4	263.4 ± 2.4	276.4 ± 4.3	189.7 ± 4.4
	4	267.5 ± 3.4	230.3 ± 7.8	310.0 ± 5.5	263.6 ± 3.1	277.5 ± 5.9	187.1 ± 5.9
Digestibility (O.M. %)	1	65.66	65.43	54.37	52.78	60.30	61.57
	2	65.77	66.41	53.59	53.22	60.66	61.05
	3	65.51	65.52	54.25	52.62	60.10	61.66
	4	65.39	65.70	54.89	52.75	60.09	61.77
Adjusted mean digestibility	1	65.19		53.72		61.15	
	2	66.02		53.43		60.90	
	3	65.12		53.59		61.13	
	4	65.09		54.01		61.21	

*Period 1 7 day-preliminary and 10 day-collection
2 10 " " " 7 " "
3 4 " " " 14 " "
4 7 " " " 7 " "

CHAPTER 4. DISCUSSION

4.1. Animals

It has been noticed that it is difficult to obtain accurate measurements of live weight of animals, particularly with ruminants.

Although the time of weighing was standardized in the present experiment, the level of intake differed between periods and sheep. Furthermore, the live weight was obtained by averaging only measurements of two successive days.

With these limitations, however, the data indicate some clear pictures; that sheep 3 and 6 lost live weight considerably during the whole experiment period possibly due to reduced intake, and that sheep fed with hay A gained live weight considerably because of the quality of feed and intake.

4.2. Digestibility of the mixed hay

4.2.1. D.M. intake during ad lib. feeding period

An interesting point raised in this period was that there was significant difference in D.M. intake between sheep, even when the intake was expressed in terms of metabolic body weight ($Wkg^{0.75}$). Blaxter et al (1961) reported that voluntary intake increased with increasing size of sheep.

However, they used a larger range of body weight (42 kg to 74 kg) than that of the present experiment (31 kg to 38 kg), and when their intake figures were taken around the body weight of 42 to 54 kg, the voluntary intake varied from 780 to 960 gm. /day which is similar

to the range found in the present experiment. The other possible reason, as has been mentioned in the previous section, is the difficulty of measuring body weight precisely.

4.2.2. D.M. intake during preliminary period

D.M. intake of individual sheep during the preliminary period was fairly constant, however the animals did not eat the whole feed offered. Three possible reasons can be discussed. Firstly, animals still select the material even under restricted conditions, although the pattern of selection differed from sheep to sheep, since some sheep always left finer materials while others left coarser materials.

Secondly, the animals could not get feed from the corners of feeding bins.

Thirdly, the voluntary feed intake might have been reduced. No clear reason is known, but this is probably due to reduced maintenance requirement.

4.2.3. Feed consumed and faeces produced during the collection period

As in the previous period, the O.M. intakes of sheep were fairly constant throughout, except sheep 3 and 6. The reason is not known and they were discarded from the digestibility trial with the individual hays.

Faeces produced varied more than feed consumed from day-to-day and this fact was probably due to end-period errors (Blaxter et al, 1956). The present result confirms the result reported by Blaxter et al,(loc. cit.) that the error attached to a collection period of faeces

was about ± 23 gm. dry matter irrespective of the length of the collection period.

An interesting point raised in the present experiment is that the standard error of mean digestibility of O.M. was low (± 0.13), although the difference between sheep on O.M. intake was highly significant when expressed in terms of metabolic body weight ($Wkg^{0.75}$).

The level of feeding on digestibility has been very confusing and is still to be solved. Recently Blaxter et al (1966), using 5 different age levels and 6 different intake levels, i.e. 1.2 kg to 7.2 kg per day fed to cows, found no systematic change of digestibility with intake of diet. Probably the finding was due to the diet which had high energy digestibility of 70%.

The present digestibility trial was not designed to find the effect of level of feeding with these particular feeds, however, the range of feed intake occuring in the experiment had no effect on O.M. digestibility.

4.3. Digestibility trial using individual hays

4.3.1. Ad lib. feeding period

Although the statistical analysis of variance showed no significant differences between hays on D.M. intake by sheep, the intake of good hay was highest and the poorest hay gave the lowest intake figure on the basis of digestibility. The limitation of the present experiment was only two sheep per treatment. Feed consumption of hay A by sheep 1 and 4 was lower than that of the same period of the previous experiment with the mixed hays, although the O.M. digestibility

of hay A was about 6% higher than the latter one. No reason is known. With hay C, digestibility was similar to that of the mixed hay. Sheep 5 improved the feed consumption considerably while sheep 8 lowered feed consumption when they were compared to the same period of the previous experiment. Again no reasons are known.

4.3.2. Digestibility trial period

Daily feed intake was maintained fairly constant throughout the whole period except for sheep 8. No reason is known. Although there were large differences of intake between sheep within a feed, no systematic effect of level of intake on digestibility was found (see section 4.2.3.).

Use of covariance analysis for adjustment of digestibility is questioned since the digestive power of individual sheep may not be the same to different quality feeds, however, it may be useful to eliminate environmental variation and helpful in finding any abnormal animals, such as sheep 3 and sheep 6 in the present experiment. It was also useful to adjust the intake at the start of the digestibility trial with individual hays since capacity of intake of individual sheep was available.

4.4. Comparison of different lengths of preliminary and collection period on effect of digestibility

There were no significant differences in O.M. digestibility due to different lengths of preliminary and collection periods in the present experiment. The periods compared, however, were not greatly different, i.e., 14 days to 18 days.

It was concluded from the result that a preliminary period of 10 days and a collection period of 10 days with the feeds used were satisfactory, although a shortening of a few days is possible.

PART II

IN VITRO FERMENTATION STUDIES

CHAPTER 5. REVIEW OF LITERATURE

Introduction

The concept of studying digestibility of feeds by the rumen micro-organisms *in vitro* is not a recent one. However the use of the *in vitro* fermentation technique for estimating herbage feed value is relatively recent. Inevitably two schools of thought developed on the use of *in vitro* fermentation techniques. The first school argue that it is not yet possible to simulate the conditions of the rumen artificially, especially in relation to removal of the end products of digestion - the volatile fatty acids.

Theoretically, the ideal *in vitro* system provides fermentation conditions identical to those occuring in the rumen of the animal. In practice, as the second school emphasizes, the development of such a system is impossible. Consequently it is necessary to define the conditions that should be present *in vitro* in order to obtain a satisfactory imitation of the rumen.

In this section, factors affecting *in vitro* fermentation studies and relationships between *in vitro* and *in vivo* are reviewed.

5.1. Type of in vitro system

It is well known that there is normally present in the rumen a large number of microbial species of widely differing physiological needs and capabilities. Some of these microbial species may be expected to be able to multiply more readily than others in given conditions. When, however, the environment is not that found in the rumen of the animal from which the rumen liquor was taken, the results

of this multiplication and metabolism may bear little or no relation to events in the rumen *in vivo* since the organisms which multiply may be simply those most suited to the experimental conditions.

The principal object of recent investigations has been to devise a system that closely imitates the rumen. Five main types of artificial rumen systems have been described for *in vitro* digestion studies.

(1) Undiluted or only slightly diluted rumen liquor is incubated with substrate in an all glass system used principally by Pearson and Smith (1943), Quin (1943) and Gray, Pilgrim and Welden (1951).

(2) Whole rumen liquor is diluted with a mineral solution resembling ruminant saliva (McDougal, 1949), and incubated with substrate in an impermeable membrane system (all glass), as first described by Burroughs, Frank, Gerlaugh and Bethle (1950).

(3) Various fractions of rumen liquor are used in an impermeable system, such as rumen liquor freed from protozia by centrifuging (McNaught, 1951) or suspension of all rumen micro-organisms in a mineral solution (Marston, 1948).

(4) Rumen liquor, usually whole and undiluted, with substrate in a semipermeable container, is dialysed against a mineral solution, as described by Louw *et al* (1949), Huhtanen and Gall (1952) and Wasserman *et al* (1952). A further developed method of semipermeable membrane system is the continuous flow type developed by Adler *et al* (1958), and used by Davey *et al* (1960) and Bowie (1962).

(5) Two stage fermentation technique in an all glass system, firstly by rumen liquor, followed by pepsin digestion, is originally described by Tilley *et al* (1960) and used by other workers such as Tilley and

Terry (1963), Alexander *et al* (1961, 1964), Yates (1964) and Oh *et al* (1966).

The main limitation of a glass fermentation vessel is that it does not permit continuous removal of fermentation products which occurs *in vivo*. Attempts have been made to simulate this phenomenon *in vitro* by the use of a semipermeable membrane (Louw *et al*, 1949), whereby a good rumen population was maintained during a 24 hour fermentation period (Gall and Huhtanen, 1951). However, this type of artificial rumen is large and rather cumbersome for use in routine work.

El-Shazly *et al* (1960) made a comparison of three different types of apparatus commonly used in *in vitro* fermentation studies, semipermeable membrane, continuous type of apparatus and the all glass system. Purified wood cellulose and several different types of hay were used as substrates, with cellulose digestion, total VFA production, and ammonia nitrogen production being used as criteria of microbial activity. They found no major differences between the different types of apparatus; however, the all glass apparatus appeared to be advantageous because of its simplicity, and a disadvantage of the semipermeable membrane was the weakness of the membrane used.

Johnson *et al* (1958) demonstrated that the end products such as VFA s were not inhibitory for cellulose digestion where a considerably higher concentration existed. Since then this all glass system has been used widely for forage evaluation (Donefer *et al*, 1960; Baumgardt *et al*, 1962a, b, 1963; Bowden and Church, 1962a; Van Dyne, 1963).

Tilley, Deriaz and Terry (1960) proposed a two-stage *in vitro* fermentation method for use in predicting digestibility *in vivo*. They

found that when using the method described by Walker (1959) good agreement was obtained between *in vitro* and *in vivo* at under 60% of D.M. digestibility, but with the feed having digestibility above 60%, particularly those with a high protein content, *in vitro* digestibility was as much as 10% lower than the corresponding *in vivo* figures. Although most of the digestion by ruminants takes place in the rumen of the animal, further digestion, especially of protein, takes place lower down the digestive tract (Halliwell, 1961; Lewis, 1961). The use of a secondary digestion with the proteolytic enzyme, pepsin, by the workers was designed to breakdown protein. Using the two-stage *in vitro* method, Tilley and Terry (*Loc. cit.*) were able to reduce the standard error of estimate to 2.0% units of digestibility.

5.2. Source and preparation of inoculum

5.2.1. Collection method of rumen liquor

The collection of rumen liquor from a fistulated animal is a matter of some complexity because the rumen contents are by no means homogenous. Smith *et al* (1956) made an examination of the ingesta and their analysis indicated that the content of fibre, total nitrogen, sugars and volatile fatty acids was higher in the dorsal rumen, with pH and the capacity to digest cellulose *in vitro* higher in the ventral levels.

Bryant (1961), using a fistulated cow, reported different levels of pH and VFA concentration in the different regions of the rumen. Davey (1965) showed that a sample taken from the middle of the rumen contents was representative. Yates (1964) showed no significant

differences in *in vitro* D.M. digestibilities of substrates when two different rumen sampling methods were used, i.e., collection of rumen liquor through rumen fistula and collection of rumen liquor by stomach tube introduced through the mouth.

5.2.2. Time of rumen liquor collection in relation to feeding

Time of rumen liquor collection in relation to feeding varies from worker to worker. Hershberger *et al* (1959), for instance, collected rumen liquor prior to morning feeding where the cow had been starved since 5:00 p.m. on the previous day; while Wright *et al* (1963) used inoculum which was collected approximately 4 hours after feeding. Yates (1964) used inocula from sheep which were fasted for 48 hours.

Drew (1966) compared the digestibility of good quality lucerne-cocksfoot hay fermented with inocula drawn after 15 hours of fast, at the end of the morning feed, and 2 hours after and 4 hours after completion of feeding. He found no significant differences of O.M. digestibility of the hay with the inocula drawn at the various times after feeding, except that 15 hours of fast gave a slightly lower O.M. digestibility. Tilley and Terry (1963) also raised the point that when rumen inoculum was taken from animals which had not been recently fed, fibre digestibility was much reduced and was very variable between tubes, and suggested that rumen liquor be taken from actively digesting animals.

5.2.3. Between and within species variation of donor animals

Very little difference between inoculum from sheep and that from cattle was demonstrated by Le Fevre and Kamstra (1960). Means of

22 cellulose digestion coefficients for the 24 hour fermentation period were 31.6 and 30.8, and for the 48 hour period were 50.2 and 49.6 respectively. Unfortunately no standard errors were given.

Van Dyne and Weir (1964a) carried out studies on variations within and between cattle and sheep as sources of inoculum for *in vitro* digestibility using 9 ruminal fistulated steers and 9 wethers in three range grazing trials and one dry lot feeding trial. They found no significant differences *in vitro* cellulose digestibility between and within sheep and cattle.

In general, whether rumen liquor is provided from sheep or cattle, little differences on either the rate or extent of digestion have been noticed where both species have been fed on the same feed (Baumgardt and Oh, 1964; Drew, 1966). Yates and Allden (1966) reported a significant difference due to source of inoculum which was provided from two sheep on the same feed. It was not clear, however, whether this variation was due to sampling error or truly due to individual sheep differences because rumen liquor were collected via a stomach tube.

5.2.4. Type of feed consumed by donor animals

Results are variable and there is lack of agreement between workers as to the effect of feed on rumen inoculum.

Van Dyne and Weir (1964b) reported that both the amount and type of diet eaten by the donor animals produced 15 to 20% differences in *in vitro* digestibility of the same forage. The effect of variation in rumen liquor of feed fed to the donor animals was also demonstrated by Reid *et al* (1959, 1960, 1964) and Shelton and Reid (1960) and the

importance of type of feed has been emphasized by Barnes (1965) and Bezeau (1965).

Barnett (1957), determining the digestibility of cellulose using an all glass type of *in vitro* method, found that his experimental results were unaffected by variations in the diet of sheep, when these were as wide in type as turnips, grass, silage and oats. However, the inocula he used were obtained at the slaughter house and the animal's previous feeding history was not known. Walker (1959) also found no differences in the D.M. digestibility of roughages in *in vitro* experiments due to variations in the diet of sheep which provided the rumen inocula. Similar results have been also obtained by other workers such as Salsbury *et al* (1958), Stewart and Schultz (1958), Church and Peterson (1960), and Drew (1966).

Bowden and Church (1962b) reported that the most accurate *in vivo* estimates of digestibility were made from *in vitro* which used the inocula from an animal on a diet as close as possible to that being tested. Johnson (1963, 1966) concluded that care must be taken to maintain the donor animal on a ration similar to that being tested or at least on a constant ration. Yates (1964), however, demonstrated that, although analysis of variance of the results showed significant differences in *in vitro* digestibility due to the source of inoculum from sheep fed different types of feed, the regression coefficients were not significantly different from one another and there was no significant displacement of the regression lines from each other.

The apparently irregular behaviour of legumes is of considerable interest since it has been reported by Reid, Shelton, Welch and Jung (1959). It was shown in cross inoculation experiments that while

there was no significant difference in the *in vitro* digestibility of a grass hay when inoculated with strained rumen liquor drawn from fistulated lambs maintained on several grass hays of different qualities and species, inocula from alfalfa fed animals caused a marked increase in *in vitro* D.M. digestibility. Hopson *et al* (1963) also found that when alfalfa was fed as a ration to a fistulated animal, digestibility of all 4 forages in dacron bags placed in the rumen increased over the digestibility when three grass hays were fed.

Quicke *et al* (1959) reported that in general the cellulose digestibility of a given forage *in vitro* was the same irrespective of the forage fed to the steer used as a source of inoculum, using 30 and 48 hour fermentation times. Raymond and Terry (1966) have reported similar results. Johnson (1962) and Donefer *et al* (1960) suggest that differences observed after short periods (12 and 24 hours) may disappear in longer fermentations. However, it has been reported by many workers that inoculum for *in vitro* fermentation from alfalfa fed animals gave better digestibilities than those with inoculum taken from animals fed lower quality forages. It has also been observed that alfalfa ash is a growth factor for micro-organisms (Knipfel and Troelsen, 1966).

5.2.5. Inoculum prepared in different ways

Quicke *et al* (1959) compared cellulose digestibilities of brome grass hays using three different types of inoculum, i.e. strained rumen juice, phosphate buffer extract of squeezed rumen contents and resuspended ruminal micro-organisms, all derived from the same sample of rumen contents. They reported that the digestion obtained with the

phosphate buffer extract and resuspended ruminal micro-organisms gave a slightly higher *in vitro* cellulose digestibility than that with strained rumen juice.

When Barnes *et al* (1964) compared three different *in vitro* methods, including method A (Donefer *et al*, 1960), B (Baumgardt *et al*, 1962b), and C and D (Tilley and Terry, 1963) on cellulose digestibilities of lucerne meal, lucerne hay and Brome grass hay, they found methods A and B gave higher cellulose digestibility than methods C and D, and suggested that this might have been affected by different ways of preparing inoculum, as methods A and B included mineral nutrients in their buffer solution.

The use of washed cell suspensions of rumen bacteria has been advocated for certain purposes (Doetsch, 1954). There are limitations to this approach, as the activities of an isolated suspension may not reflect those of the whole rumen population, since it eliminates the protozoa. Growth factors present in the supernatant fluid are lost and enzyme inactivation may occur during the various stages of preparation of suspension.

Barnett and Reid (1961) suggest that strained rumen liquor will supply adequate levels of accessory factors for efficient digestion, such as valeric acid and trace minerals, also protein for bacterial growth.

5.3. Preparation of substrate

5.3.1. Drying of samples

The digestibility of the forage may be reduced if it is held

at a high temperature when dry (Watson and Nash, 1960). Clark and Mott (1960) found that freeze-dried samples gave a higher digestibility *in vitro* than oven-dried samples and the D.M. digestion coefficients of the freeze-dried herbage were comparable to those expected for herbage of such quality digested *in vivo*. Ekern *et al* (1965) also suggested that the energy of fresh herbage was digested better than that of dried herbage. Drew (1966) compared 8 samples ranging from highly digestible pasture herbage to material of moderate digestibility, and showed that the freeze-dried substrate gave an increase of 1.0 to 1.5 units of digestibility compared with comparable material oven dried (100°C).

Reid, Shelton, Welch and Jung (1959) reported that *in vitro* digestibility varied significantly according to whether grass was used in the fresh, freeze-dried or oven-dried state and *in vivo* D.M. digestibility was most accurately predicted from *in vitro* D.M. digestibility with oven-dried samples. Noller *et al* (1964) made comparisons between oven-dried plant material and similar material quick-frozen and freeze-dried on their change of chemical compositions and digestibility. They found that quick-frozen and freeze-drying gave consistently higher levels of soluble carbohydrates and non-protein nitrogen than oven-drying, but the products were less digestible and less acceptable to animals. Bowden and Church (1962a) reported that oven-dried samples provided more suitable results than fresh-frozen samples of tall fescue, based on between trial variability. 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 unless the drying at 100°C was continued longer than four days.

5.3.2. Grinding of samples

Dehority and Johnson (1961), and Dehority, Johnson and Conrad (1962) reported that increasing the ball-milling time increased the *in vitro* digestibility of cellulose, especially at the more mature stages of herbage. Baumgardt and Oh (1964) supported the results of Dehority and Johnson (*loc. cit.*) by reporting that forages ground through a 60-mesh screen had a higher ($P < 0.01$) apparent cellulose digestibility than when ground through a 40-mesh screen. The forage by mesh interaction was significant, because the effect of fineness of grind was much greater with grasses than with alfalfa.

Church and Peterson (1960) compared four different particle sizes (i.e., 20, 40, 60 and 80 mesh Wiley mill screens), using lucerne and peavine hay on their *in vitro* D.M. and cellulose digestibility. They found that decreasing the particle size of lucerne hay depressed the D.M. and cellulose digestibility and that there were no differences when peavine hay was the substrate.

Tilley and Terry (1963) compared four different sizes of grinding and found no differences on their *in vitro* dry matter digestibility. They suggested that samples need only be ground finely enough to ensure good sampling of the small weights of herbage used.

5.4. Length of fermentation period

It is generally agreed that D.M. loss or cellulose digestion increases with length of fermentation *in vitro*, but what is more important is that short periods, e.g., 12 hours (Johnson *et al*, 1962; Donefer, Crampton and Lloyd, 1960) or 18 hours (Baumgardt and Oh, 1964) and 24 hours (Hershberger *et al*, 1959) have given significant

correlation between *in vitro* and *in vivo* measurements.

Baumgardt *et al* (1962b) carried out *in vitro* digestion experiments with high quality lucerne hay and a low-nitrogen more mature cocksfoot hay, to determine the optimum time for the *in vitro* fermentation. They found that the initial rate of digestion of the lucerne was greater and the maximum was obtained sooner (42 hr) than for the grass. Apparently, the maximum cellulose digestion of the cocksfoot had not yet been obtained at 48 hours. This is in agreement with the results reported by Quicke *et al* (1959) in which a longer fermentation period was necessary to obtain maximum cellulose digestion of more mature forages.

In further work, Baumgardt *et al* (*loc. cit.*) indicated that a higher digestibility figure was obtained when fermentation time was increased from 24 hours to 48 hours. However, the correlation between *in vitro* cellulose digestion and *in vivo* digestible energy (D.E.) was highly significant in both cases and it was concluded that there was no advantage in extending the fermentation period from 24 hours to 48 hours, in so far as the estimation of D.E. was concerned.

Kamstra *et al* (1958), Dehority *et al* (1960), Le Fevre and Kamstra (1960) and Van Dyne (1962) suggested that *in vitro* cellulose digestion was almost complete after 24 hours.

To contrast these reports, other workers preferred longer fermentation periods such as 48 hours (Tilley, Deriaz and Terry, 1960; Barnes *et al*, 1964), 60 hours (Quicke *et al*, 1959) and 72 hours (Walker, 1959; Drew, 1966).

The main argument of these latter workers was that longer *in vitro* fermentation periods reduced standard error for estimating *in*

vivo measurements. As was shown by Baumgardt and Oh (1964), although cellulose digestibilities obtained during the periods of 18, 24, 30 and 48 hours of fermentation *in vitro* were all significantly correlated with *in vivo* digestible D.M., the day-to-day variation was significantly less ($P < 0.01$) for the 48 hour fermentation than any of the shorter times. Barnes *et al* (1964) demonstrated that increased fermentation time gave smaller coefficient of variation.

There is no information available for the optimum fermentation length for the second stage digestion with pepsin. Drew (1966) compared different lengths of second stage fermentation period in relation to first stage fermentation and gave no information about optimum second stage fermentation length. However, he showed that the second stage fermentation period might possibly be shortened from 48 hours to 24 hours.

5.5. The amount of substrate and ratio of substrate to artificial mixture (saliva and rumen liquor)

Church and Peterson (1960) found that increasing the volume of rumen liquor resulted in a linear increase in per cent *in vitro* D.M. digestibility, and the rate of increase depended upon the concentration of minerals and substrate, being greater at the higher than at the lower mineral and substrate levels. Barnes *et al* (1964) found no difference because of the total amount of liquor, however, they suggested that combination of substrate size and the preparation of inocula and buffer nutrients might have an effect on cellulose digestibility, particularly at the initial stage of fermentation period.

Kamstra et al (1958) reported, using forage levels supplying 0.30 to 0.80 gm. of cellulose per flask, that the amount of cellulose digested increased in proportion to the amount added. Similar results have been reported by Baumgardt et al (1962b), where forage substrate levels of 0.5, 1.0 and 1.5 gm. per flask were incubated. When these results were interpreted in terms of per cent digested, they remained nearly the same. Quicke et al (1959) and Hershberger et al (1959) also found similar results, i.e., nearly the same per cent cellulose digestion for forage levels from 0.44 to 2.50 gm., (cellulose levels from 0.08 to 0.48).

Baumgardt et al (1962b) concluded that 1.0 gm. of forage would offer a suitable substrate concentration. Other workers used from 0.25 gm. (Barnett, 1957), 0.5 gm. (Tilley et al, 1960; Tilley and Terry, 1963; Dent, 1963, 1966), 0.6 gm. (Drew, 1966), 0.9 gm. (Donefer et al, 1960), 1.0 gm. (Baumgardt et al, 1962b) to 3.0 gm. (Hershberger et al, 1959) of forage substrate for in vitro fermentation study.

5.6. Effect of pH

In many of the in vitro experiments reported, pH adjustments have been made in the range of 6.50 and 7.00. In some of the early literature, Wegner et al (1940) suggested an adjustment of pH between 6.0 and 7.5, whereas Meites et al (1951) have reported an optimum pH between 4.53 and 7.35 for cellulose digestion. More recently, Cline (1956) found pH 6.9 to result in maximum cellulose digestion under the conditions imposed. Kitts and Underkopler (1954), and Stanly and Kesler (1959), working with isolated cell-free rumen liquor

preparations, found optimum cellulose activities at 5.5 and 6.0 where carboxymethylcellulose (CMC) was the substrate used.

Church and Peterson (1960) compared the effect of pH ranging from 6.5 to 7.1 on in vitro cellulose digestibility of different substrates. In one case there was no significant differences in cellulose digestibility between pH 6.5 to 6.8 (alta fescue, Culture No. 232), while in other cases, cellulose digestion was increased by upward adjustment of the pH from 6.6 to 7.0 and the digestion of cellulose from a purified source was depressed by adjustment of pH levels to 6.9 or 7.1 (alta fescue, Culture No. 215, and Solka-Floc 40A). These results confirm the results of Reis and Reid (1959), in that the optimum pH for cellulose digestion would appear to vary according to substrate and/or source of rumen liquor.

The optimum pH for in vitro digestion which has been adopted by many workers is about 6.9 (6.7 - 6.95). Little change of pH occured during the fermentation period when ordinary pasture grass and hay were used as substrates (Drew, 1966; Raymond and Terry, 1966).

5.7. Accuracy of in vitro technique

In reviewing the usefulness of the in vitro fermentation method for the routine evaluation of forage quality, it must be emphasized that the method must be relatively simple in order to allow the rapid analysis of a large number of samples, producing results with a high degree of precision and give an accurate, unbiased estimate of forage quality.

The in vitro methods reviewed thus far essentially fulfil the first requirement. The errors associated with the in vitro procedures

can be considered in two categories; the factors which contribute to the lack of precision in the results from the *in vitro* technique and the failure of the *in vitro* results to estimate the *in vivo* digestibility.

5.7.1. Repeatability

The magnitude of the errors reflecting the precision of the *in vitro* methods are those associated with the within and between trial variability.

A considerable range in standard deviations has been reported in the literature. Huhtanen *et al* (1954), using miniature artificial rumens and 24 hour fermentation periods, reported the following results:

	Fibre digestibility			
	Mean	S.D.	S.E.	CV%
a) within a trial between replicates	47.0%	4	0.905	8.64
b) between trials for 2 months	46.2%	3.8	1.2	8.23

Kamstra *et al* (1958) reported that leafy alfalfa had a lower standard deviation (4.5) and third stage alfalfa had a higher (9.3) standard deviation, while Quicke *et al* (1959), using a similar method, reported no difference in standard deviation due to maturity of herbage, 3.1 for first growth vs 3.2 for second growth.

Hershberger *et al* (1959) noted a greater degree of variation occured with purified cellulose than with forage substrates, 3.63 vs 1.90 standard deviations, respectively. They also found that the within trial standard deviations for *in vitro* and *in vivo* cellulose digestion were quite comparable, 1.30 and 1.44 respectively.

Using purified cellulose with a 12 hour fermentation period,

Donefer et al (1960) found a comparatively larger standard deviation (6.0) than that with 11 forages (2.0). Bowden and Church (1962a) reported the within trial variation of D.M. digestibility and cellulose digestibility to be 1.9 and 2.9 respectively, when a standard alfalfa was digested in vitro for 13 trials. Variability between trials was greater than within trials and analysis of variance revealed significant differences between means of trials, both for D.M. and cellulose digestibility of the alfalfa.

Analysis of duplication results within an experiment, carried out by Tilley et al (1960) showed that, for 80 pairs of measurements of in vitro 'rumen liquor' digestibility and for 100 pairs of in vitro 'rumen liquor and pepsin' digestibility, the standard errors between duplicates were, respectively, $\pm$ 1.3 and $\pm$ 0.9. The reproducibility of in vitro digestion was further tested using rumen liquors prepared on different days and taken from sheep on different feeds. The standard error for in vitro 'rumen liquor' ranged from 2.1 to 2.8 for seven measurements and corresponding data for 8 measurements of in vitro 'rumen liquor and pepsin' digestibility were 2.0 and 2.7. A further study by Tilley and Terry (1963), using 46 grasses, showed a within trial variation (S.E.) of 0.66 and a between trial variation of 1.18 for in vitro digestible D.M.

5.7.2. Prediction of in vivo digestibility from in vitro results

A number of reports outlining various in vitro-in vivo digestibility relationships have been published. In this section an attempt is made to summarize these relationships in tables depending upon whether cellulose or D.M. (or O.M.) was used as the in vitro

criteria (see appendix 24 and 25).

Pigden and Bell (1955) found a good estimate of organic matter digestion *in vivo* could be obtained from the fermentation of anthrone carbohydrate *in vitro* (standard eror of the estimate of 2.76). With hays of separate origin and different quality, Asplund *et al* (1958) found a pooled correlation coefficient of 0.71 between *in vivo* and *in vitro* D.M. digestibility using minaiture artificial rumen. Reid *et al* (1959) reported a correlation coefficient of 0.98 between *in vivo* and *in vitro* D.M. digestibility with Sy.x of 3.6.

Walker (1959) used 72 hours of fermentation period and calculations of his data shows a highly significant correlation coefficient and relatively small standard error of estimate. Clark and Mott (1960) studied 11 forages of known *in vivo* digestibility. They reported that *in vitro* digestibility estimates obtained during the spring were significantly correlated (r = .77) with *in vivo* data. However, when the original data were recalculated, the correlation coefficient was 0.59, not greatly different from the correlation coefficient of 0.49 they obtained during the autumn.

Tilley, Deriaz and Terry (1960) showed higher correlation coefficients of 0.99 and 0.98 to 0.91 and 0.87, and smaller standard error of estimates of 1.96 and 2.0 to 3.6 and 4.4 when they included pepsin digestion stage following rumen liquor fermentation period. Their further results with 148 forages showed a standard error of estimate of 2.31 (Tilley and Terry, 1963).

The digestibility of the organic matter of 12 dried grasses was studied *in vivo* and *in vitro* by Armstrong, Alexander and McGowan (1964). The correlation coefficient and derived regression equation

relating percentage digestibility of organic matter determined *in vivo* with sheep fed at the maintenance level (Y_1) to that determined *in vitro* (X) were 0.986 and $Y_1 = 0.92X + 12.48$. The comparable data for *in vivo* digestibility determined when sheep were fed twice the maintenance level (Y_2) and *in vitro* digestibility were 0.989 and $Y_2 = 1.15X - 6.12$. Regression equations of metabolizable (M.E.) and net energies for maintenance (N.E.) and production (N.E.f.) on digestibility or organic matter determined *in vitro* are also given in appendix 24.

Recent reports on relationships between *in vivo* and *in vitro* digestibility are more promising (Oh *et al*, 1966; Yates and Allden, 1966; and Drew, 1966) except that of Wilkins and Grimes (1966) and those with nylon fermentation vessel of Yates and Allden (*loc. cit.*).

Standard errors of estimate of digestibility ($S_{y.x}$) have ranged from 1.0 to 5.0 for the prediction of *in vivo* digestibility from the most *in vitro* results. As Raymond *et al* (1960) have pointed out, satisfactory results can only be obtained if digestibility coefficients are predicted with an error of 2.5 units (or less) and it has been the goal of animal nutritionists to develop a method with this accuracy.

Although the correlation coefficients may be high, the prediction equations derived from *in vivo*-*in vitro* relationships may have limited value if the errors are large. The *in vitro* results can be no better than the *in vivo* data upon which they are based. Because of the validity associated with the *in vivo* measurements, particularly voluntary intake, caution is needed when interpreting results.

CHAPTER 6. MATERIALS AND METHODS

Introduction

In vitro fermentation techniques for obtaining organic matter digestibility were carried out using the method of Tilley and Terry (1963), as modified by Alexander and McGowan (1961) where centrifuging was replaced by filtration and organic matter was used instead of D.M. for digestibility calculations.

6.1. General description

6.1.1. Preparation of standard grass sample

About 1 kg. (D.M. basis) of leafy spring pasture grass (perennial ryegrass dominant) was cut with hand clippers, dried in a forced draught oven at 65°C. for 48 hours and ground to pass a 2.0 mm. sieve using a Wiley mill. This was then divided into two parts after thorough mixing and one part was again ground to pass a 1.0 mm. sieve. These samples were stored at -10°C. until required. The latter samples were used throughout the experiment unless otherwise stated.

6.1.2. Weighing out

Samples (0.5 gm.) of the standard grass were weighed into 100 ml., glass bottles (diameter 5 cm., height 10 cm., and diameter of neck 4 cm.) with 6 to 10 replicates according to requirements. At the same time triplicate samples were weighed for moisture and organic matter content.

6.1.3. Collection of rumen liquor

The collection of rumen liquor from the cows was standardised as far as possible, i.e., time, type of feed and position of rumen. Rumen liquor was strained through four layers of muslin into a plastic bottle kept at 39°C. immediately before it was required and further filtered through a piece of muslin into a beaker in the laboratory. pH was measured and recorded within 10 min. after the collection of rumen liquor.

6.1.4. First stage fermentation

Forty ml. of artificial saliva (McDougal, 1949), gassed with CO_2 and the pH adjusted to 6.90 to 7.00 by using normal sodium carbonate solution (N - Na_2CO_3), was added to all bottles including blanks, which contained only rumen liquor and artificial saliva. The bottles and contents were warmed in a water bath before the rumen inoculum was added. Ten ml. of rumen liquor was added to all bottles which were gassed with CO_2 for about 20 seconds and closed with a rubber bung fitted with a gas release valve made on the principle of the Bunsen valve. All bottles were incubated in a thermostatically controlled water bath at 38.5°C. $\pm$ 0.5°C. for 48 hours.

During the fermentation period, the bottles were occasionally swirled (5-6 times) and pH was measured after 6, 12, 24 and 48 hours, and if necessary adjusted to pH 6.9 with N - Na_2CO_3 solution. The bottles were again flushed out with CO_2 before returning them to the water bath. It was found, however, that adjusting the pH was not necessary with this experiment and this procedure was not used any further (figure 7.3).

6.1.5. Second stage fermentation

At the end of first stage fermentation, pH was lowered to 1.2 - 1.3 with 20% (v/v)HCl, and 5 ml. of aqueous pepsin was added to each bottle (0.03 gm. of 1:2, 500 pepsin).

All bottles were again flushed with CO_2, mixed thoroughly, capped and returned to the water bath for a further 48 hours. Anaerobic conditions were not necessary at this stage, however, saturation with CO_2 lessened frothing. This second stage digestion was designed to complete protein degradation.

6.1.6. Filtration and final analysis

At the end of 48 hours fermentation with pepsin, approximately 1 gm. of filter-aid (Hyflo super cell) was added to all bottles and the contents of the bottles were filtered on to glass fibre filter paper, using Hortley 3-piece funnels. The residue was washed four times with hot water.

The filter papers with residues were dried at 100°C. for 16 hours and weighed (Wa). They were then ignited at 550°C. for 4 hours in a muffle furnace, and reweighed (Wb). The former represents dry weight of the filter paper, filter-aid and grass sample undigested during the *in vitro* fermentation and the latter represents the weight of filter paper, filter-aid and inorganic matter of grass sample undigested, since no weight changes were obtained before and after ignition of filter paper and filter-aid.

The percentage digestibility of the feed organic matter *in vitro* was calculated by subtracting the residual organic matter (Wa - Wb), less the blank organic matter from the feed organic matter and

expressing this as a percentage of feed organic matter as follows:

$$\% \text{ digestibility of O.M. in } \underline{\text{in vitro}} = \frac{\text{O.M. in 0.5 g sample} - ((Wa-Wb) \text{ for sample} - \text{mean of } (Wa-Wb) \text{ for blank})}{\text{O.M. in 0.5000 g sample}}$$

6.2. Repeatability trial

6.2.1. Inoculum taken from a grazing cow on several days

For this experiment a lactating cow with a rumen fistulae (cow No. 48) was grazed on pasture alone with the rest of the herd. Rumen liquor was collected at 9:00 a.m. from the cow which had been kept in the yards since the morning milking (6:00 a.m.). Eight to ten replicates of standard grass samples and two to four replicates of blanks, according to the water bath capacity, were incubated according to the method previously described. Four runs were made on four separate days and results were analysed to determine the repeatability of the method and day-to-day variation.

6.2.2. Inoculum taken from a cow fed hay on several days

Since no control on intake, time of feeding, water intake and uniformity of diet were possible with grazing animals, a fistulated dry cow (No. 120) was fed with medium quality hay (hay B, see Part I). The cow was fed hay for 10 days before collection of rumen liquor. This cow was taken from the yard where hay was fed ad lib. to shed at 6:00 a.m. and rumen liquor was collected at 9:00 a.m.

Six to ten replicates of standard grass with four blanks were incubated in the water bath for 5 separate runs on different days. The results were analysed to determine the repeatability of the method and

day-to-day variation.

6.3. Comparison of source of inoculum - 'grass inoculum'vs 'hay inoculum'.

To eliminate any day-to-day variation, two sources of inocula were compared within an experiment. Cow 48 and cow 120 were available for this study. Cow 48 was grazed on pasture and cow 120 was fed the hay described previously. The two sources of inoculum were compared in terms of *in vitro* organic matter digestibility of the standard grass. The design of this experiment was as follows:

Sources of rumen liquor (2) x standard grass sample (6) + 2 x 4 blanks = 20 bottles.

6.4. Comparison of two cows as donors of rumen liquor

Two fistulated lactating cows were available for this study (cows No. 48, 45). Both cows were grazed on the same area as other milking cows to ensure that similar quality of pasture was eaten. Rumen liquor was collected by fistulae at 9:00 a.m. from both cows which had been left in the shed since the morning milking. The rumen liquors were compared by measuring fermentation losses using the standard grass as substrate. The design of this experiment was as follows:

Sources of rumen liquor (2 cows) x standard grass sample (10 replicates) + 2 cows x 4 blanks = 28 bottles.

6.5. Time of rumen liquor collection in relation to time since last grazing

A fistulated cow (No. 48) was available for this study. The cow was brought into the shed at 8:00 a.m. and the rumen liquor was collected at 10:00 a.m., 2:00 p.m. and 8:00 p.m., i.e., 2, 6 and 12 hours after grazing. Four replicates of the standard grass sample with triplicate blanks were included in each treatment, i.e.,

three treatments (3) x substrate (4 replicates) +

three treatments (3) x 3 blanks = 21 bottles.

6.6. In vitro digestibility studies with hay A, B and C samples

6.6.1. Preparation of samples for in vitro study

When each hay was chopped and mixed on the concrete floor for the in vivo digestibility trial (see Part I), a bulk sample was taken from 80 different points after thorough mixing. These bulk samples from each hay were thoroughly mixed again separately and finally a sub-sample of approximately 1 kg. was taken from the 10 kg. bulk sample (hereafter called hay samples). The hay samples were dried at 65°C. for 16 hours and ground using the same method for preparation as the standard grass sample (see 6.1.1.).

6.6.2. Source of inoculum

The fistulated cow (No. 48) was used throughout this experiment as rumen liquor donor animal. This cow was grazed on pasture. The preliminary study (6.4.) showed that there was no significant difference on in vitro digestibility of the standard grass when grass inoculum was compared with hay inoculum and using grass inoculum was more convenient in practice than using hay inoculum since no indoor

feeding was required. Rumen liquor was collected 2 hours after the cow was brought into the shed, as no significant difference was obtained *in vitro* digestibility of the sandard grass in relation to time since grazing (6.5.).

Although pasture was restricted because of winter shortage, no hay supplement was fed.

6.6.3. Length of second stage fermentation period

This experiment was designed to see whether the second stage of fermentation could be shortened.

The experimental design was as follows:

a) Fermentation periods compared:

48 hrs (1st stage) + 48 hrs (2nd stage) (pepsin)

48 hrs " + 24 hrs "

b) Feed samples used:

Hay A, B, C, and the standard grass.

c) Replication:

Four replicates for each of the hays

Three replicates for the standard grass and blanks.

6.6.4. Length of first stage fermentation

The aim of this experiment was to determine the effect of length of fermentation on organic matter digestibility *in vitro* and its relation to organic matter digestibility *in vivo*.

The feed samples used in this experiment were hay A, B, C and the standard grass. Samples were removed from the first stage of digestion after 24, 36 and 48 hours and taken through the normal pepsin

digestion for 48 hours; they were allotted a sampling order from a table of random numbers (Cochran and Cox, 1962).

The design was as follows:

a) Fermentation periods:

24 hrs (1st stage + 48 hrs (2nd stage)

36 hrs " + 48 hrs "

48 hrs " + 48 hrs "

b) Feed samples used:

Hay A, B, C, and the standard grass.

c) Replication:

Triplicate for feed samples

Duplicate for blank.

Feed samples (4) x Length of fermentation (3) x

Replications (3) + (3) x (2) blanks = 42 bottles.

Relationships between in vivo and in vitro O.M. digestibility on different length of fermentation were analysed. pH was also measured after 24, 36 and 48 hours of first stage fermentation.

6.6.5. Grinding

Three hays (hay A, B, C) were used to investigate the influence of fineness of grinding. The method used for grinding is described in section 6.6.1. Samples ground to pass through 2.0 and 1.0 mm. sieves were digested in vitro. The 2.0 mm. of fineness of grinding compared was much coarser than those reported in the literature. The coarser sieve was used because it was found when using the finer sieves there was difficulty in recovery of the more fibrous portion of the sample.

The experimental design was as follows:

a) Fineness of grinding:

2.0 mm. and 1.0 mm.

b) Hays:

A, B and C.

c) Fermentation period:

48 hrs (1st stage) + 48 hrs (2nd stage)

72 hrs " + 48 hrs "

d) Replication:

Triplicate for feed samples

Triplicate for blank.

Feed samples (3) x fineness of grind (2) x fermentation period (2) x replications (3) + (2) x (3) for blanks = 42 bottles.

Estimates of within and among trial variation, together with first and second order interactions, were made.

6.6.6. In vitro digestibility of hays A, B, C, and mixed hays

Five replicates of each hay sample and triplicates of the standard grass and blanks were tested for weight separate runs (see table 6.1.)

Table 6.1. The design of the experiment 6.7.

SUBSTRATES	REPLICATES	RUNS
Hay A	5	8
B	5	8
C	5	8
Std. grass	3	8
Blanks	3	8
Mixed hay	5	2

In vitro digestibility of mixed hay was also determined in two separate runs using 5 replicates.

The data were analysed to obtain mean *in vitro* digestibility of each hay, and variations within and between trials. The figures were also adjusted using the digestibility data of the standard grass to see if day-to-day variation could be removed.

Regression of O.M. digestibility obtained with sheep on *in vitro* organic matter digestibility were also made.

6.7. Relationships between chemical components and in vitro O.M. digestibility of feed

All the feed samples studied were chemically analysed. In addition, three more species of ryegrass, i.e., perennial ryegrass (*Lolium perenne*), Italian ryegrass (*L. multiflorum*) and Western Wolths (Grasslands 4707), were obtained from the University farm and incubated *in vitro*. Chemical data of these grass samples were also available.

The *in vitro* O.M. digestibility of the three ryegrasses were determined using 4 replicates with the method described previously.

Relationships between *in vitro* digestibility and chemical components were analysed only using crude protein and crude fibre content of the hays and grass samples.

CHAPTER 7. RESULTS

7.1. Repeatability

7.1.1. Inoculum from cow grazing pasture

The mean *in vitro* O.M. digestibility of the standard grass, standard error of mean (S.E.) for between replicates within trials and S.E. of mean for day-to-day variation are summarized in table 7.1.a.

Analysis of variance of the *in vitro* O.M. digestibility data are presented in appendix 9a.

Despite the fact that day-to-day variation gave a significant result at 10% level, the standard error of mean was only 0.75, when the variation was calculated using the following equation (Snedecor, 1965):

$$\text{variance} = 6^2 + k6a^2$$

$$\text{where } 6^2 = 2.88$$

$$\text{and } k = \frac{1}{r-1}\left(N - \frac{ri^2}{N}\right) = \frac{1}{3}\left(38 - \frac{64 + 100 + 100 + 100}{38}\right)$$

7.1.2. Inoculum from cow fed medium quality hay (hay B)

The mean *in vitro* O.M. digestibility of the standard grass, standard error of mean for within and between trials and coefficient of variation are summarized in table 7.1.b.

The mean *in vitro* O.M. digestibility of the standard grass was slightly lower than that from grass inoculum. Within trial standard error of mean and C.V. were all slightly smaller, while day-to-day variation was larger than that of the previous experiment.

Analysis of variance showed a highly significant day-to-day

Table 7.1. IN VITRO O.M. digestibility of the standard grass and within and between trial variability

	MEAN O.M. DIGESTIBILITY	WITHIN TRIALS		BETWEEN TRIALS	
		S.E.*	C.V.+(%)	S.E.	C.V.(%)
a) grass inoculum	80.08	0.55	2.12	0.75	2.8
b) hay inoculum	79.76	0.45	1.44	1.30	4.0
c) pooled	79.93	0.53	1.85	0.97	3.4

*Standard error of mean

+Coefficient of variation

variation (appendix 9b).

7.1.3. Pooled results

Analysis of variance was made on the combined data from the two experiments on difference due to source of inoculum, day-to-day variation and residual variance (see appendix 9c). There was no significant difference on *in vitro* O.M. digestibility of the standard grass due to source of inoculum but day-to-day variation was highly significant at 1% level ($P < 0.01$)

The mean *in vitro* O.M. digestibility of the standard grass, standard error of mean (S.E.) and coefficient of variation are given in table 7.1.c.

From these results, day-to-day variation appears to be larger than variation within a trial.

7.2. Comparison of source of inoculum

7.2.1. Grass inoculum vs hay inoculum

Since significant day-to-day variations were observed, the two sources of inoculum were compared within a trial.

The results showed that there was no significant difference due to different sources of inoculum (see appendix 10a). The means of *in vitro* O.M. digestibility were 78.6% for hay inoculum and 79.07% for grass inoculum, summarized as follows:

INOCULUM	MEAN O.M. DIGESTIBILITY	S.E.	C.V.
grass	79.07	0.30	0.92%
hay	78.63	0.18	0.71%

7.2.2. Comparison of cow difference

There were no significant differences between cows used as sources of inoculum (see appendix 10b). However, cow 48 gave more variation than cow 45. The results are summarized as follows:

COWS	MEAN O.M. DIGESTIBILITY(%)	S.E.	C.V.
cow 48	80.33	0.7	6.12%
cow 45	81.05	0.3	1.1%

7.2.3. Inoculum collected at different times after grazing

Three different times of collecting rumen liquor were tested with standard grass as substrate. The mean *in vitro* O.M. digestibility of the standard grass for these three different times were 79.08%, 78.72% and 79.60% for 2 hours, 6 hours and 12 hours after grazing and no significant difference was found between them, though the 6 hours gave the lowest *in vitro* digestibility (appendix 10c).

The residue in rumen liquor (blanks) was significantly reduced from 2 hours to 6 hours and from 2 and 6 hours to 12 hours (appendix 11).

7.3. Length of fermentation period

7.3.1. Comparison of different lengths of second stage (pepsin) fermentation period (24 vs 48 hours)

The results are summarized in table 7.2.

Although there was no significant difference between means of two different lengths of fermentation period, variations (S.E. and C.V.)

Table 7.2. Comparison of different lengths of second stage fermentation using four substrates

HAYS	A				B				C				STANDARD GRASS			
STAGE*	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II
LENGTH+	48 + 24		48 + 48		48 + 24		48 + 48		48 + 24		48 + 48		48 + 24		48 + 48	
Mean Digestibility	64.13		65.06		52.39		53.63		59.45		60.01		78.89		78.91	
S.E.	0.40		0.22		0.71		0.43		0.42		0.36		0.47		0.25	
C.V. (%)	1.78		0.94		3.86		2.24		1.98		1.72		1.46		0.77	
Difference	N.S.				N.S.				N.S.				N.S.			

*I 1st stage fermentation (rumen liquor)

II 2nd stage fermentation (pepsin)

+Fermentation period expressed in hours

associated with 24 hour pepsin digestion were greater in all 4 feed samples than those of 48 hour pepsin digestion (see table 7.2., and appendix 12), and *in vitro* O.M. digestibilities of the former were slightly lower than those with the latter one. No difference was associated with crude protein content of feed samples.

7.3.2. Comparison of different lengths of first stage (rumen liquor) fermentation periods

The *in vitro* digestibility of hay A, B, C and standard grass increased with increasing length of fermentation (figure 7.1.). Within any one fermentation time the digestibility of the standard grass was higher than the other three hay substrates and the proportion of O.M. digested was greater with good quality feeds than with poorer feeds (figure 7.2.).

pH was lowered from about 7.0 at the beginning of the fermentation to about 6.75 at the end of the 48 hour fermentation time (see figure 7.3.). Analysis of variance showed that changing of pH during the fermentation period was highly significant for feed samples, i.e., pH of standard grass was significantly lower than the other three hay samples (appendix 13).

Analysis of variance was made on fermentation time, different substrates and first orders interaction of fermentation time by substrates (appendix 14). There were significant differences in *in vitro* O.M. digestibility of substrates due to fermentation time, different feed samples and the interaction of the two, indicating that the rate of digestion differed with different feed samples (see figure 7.2.).

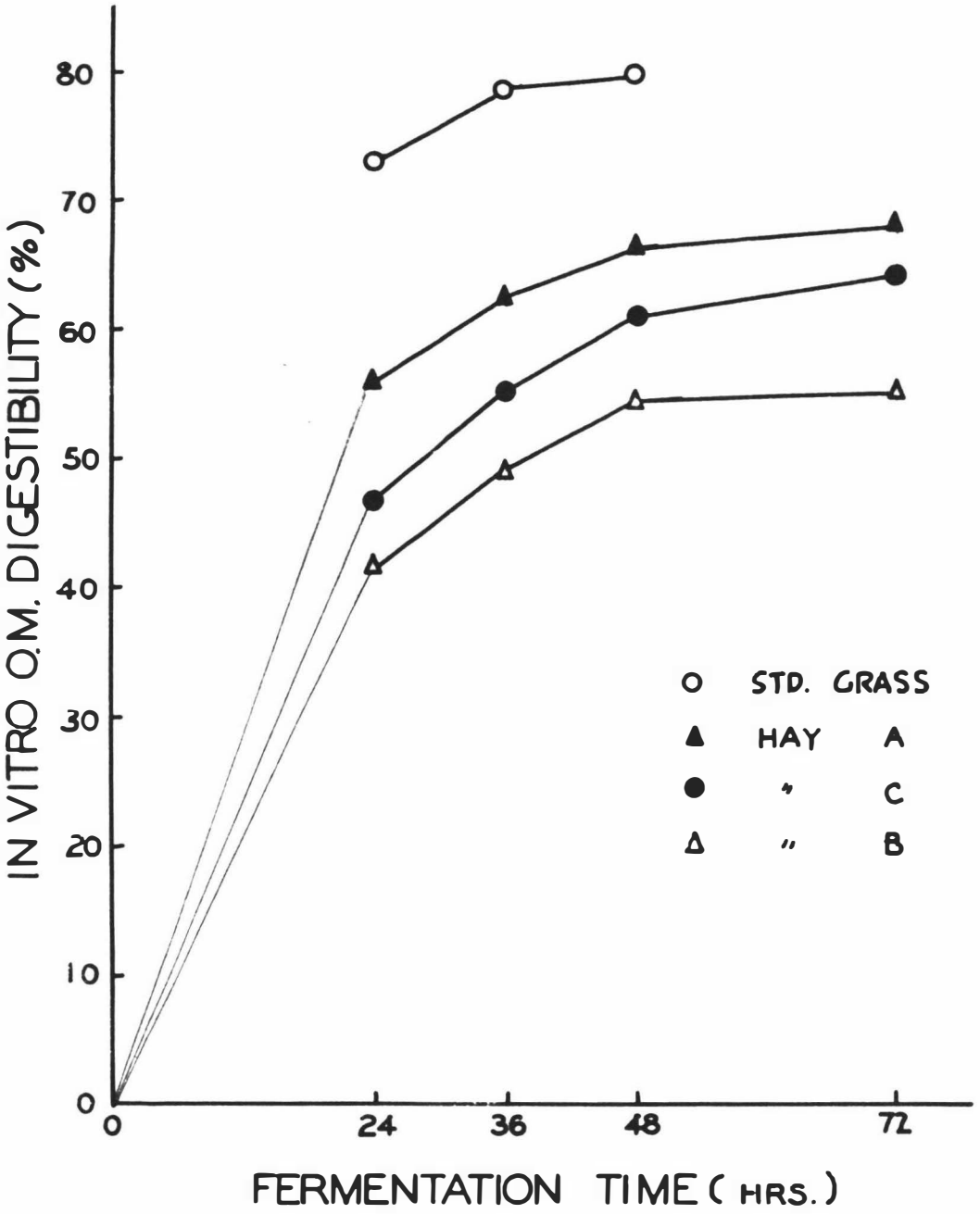

FIG. 7.1 THE EFFECT OF LENGTH OF FERMENTATION ON IN VITRO O.M. DIGESTIBILITY OF HAY A, B, C & STD. GRASS

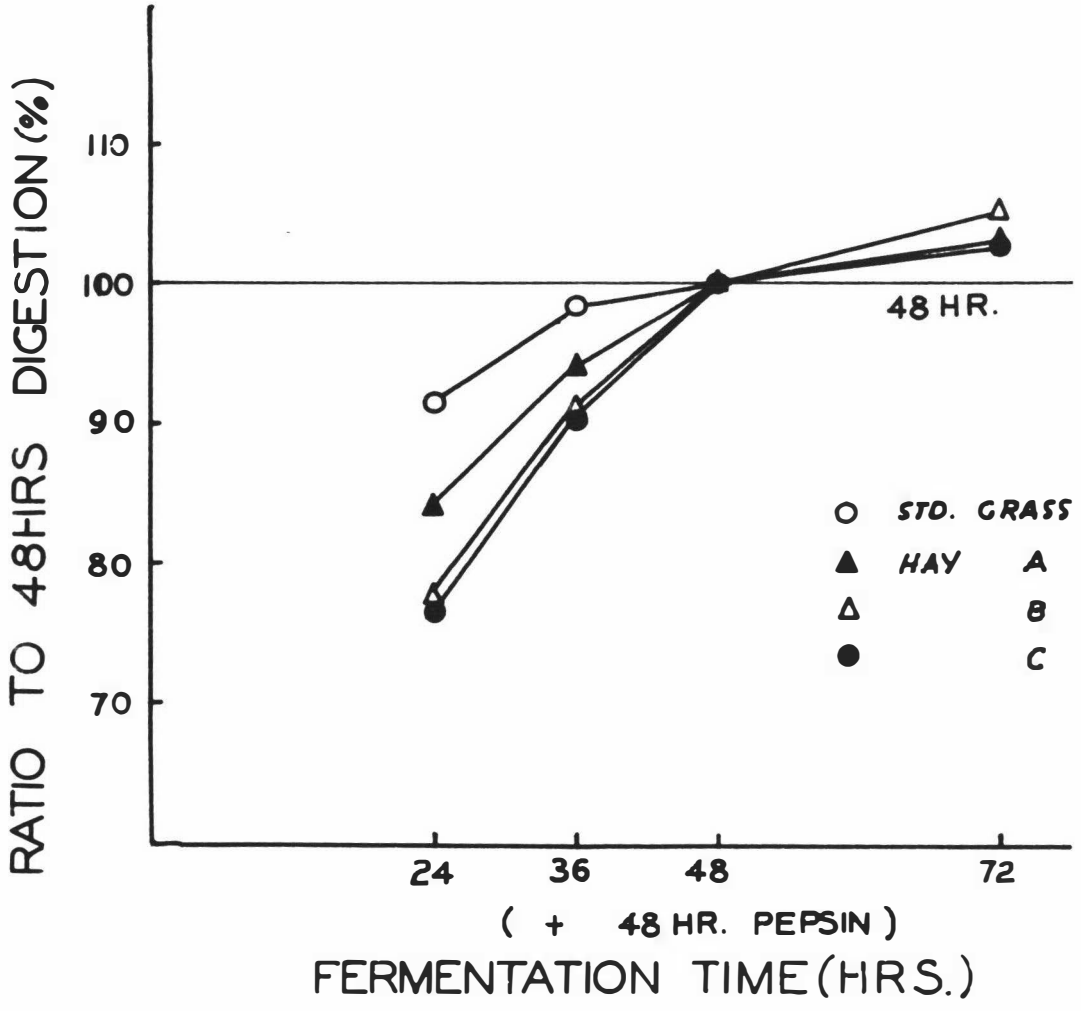

FIG. 7.2 PROPORTION OF O.M. DIGESTED **IN VITRO** FOR DIFFERENT FERMENTATION PERIODS. (DIGESTIBILITY AT 48HRS. IS TAKEN AS 100%)

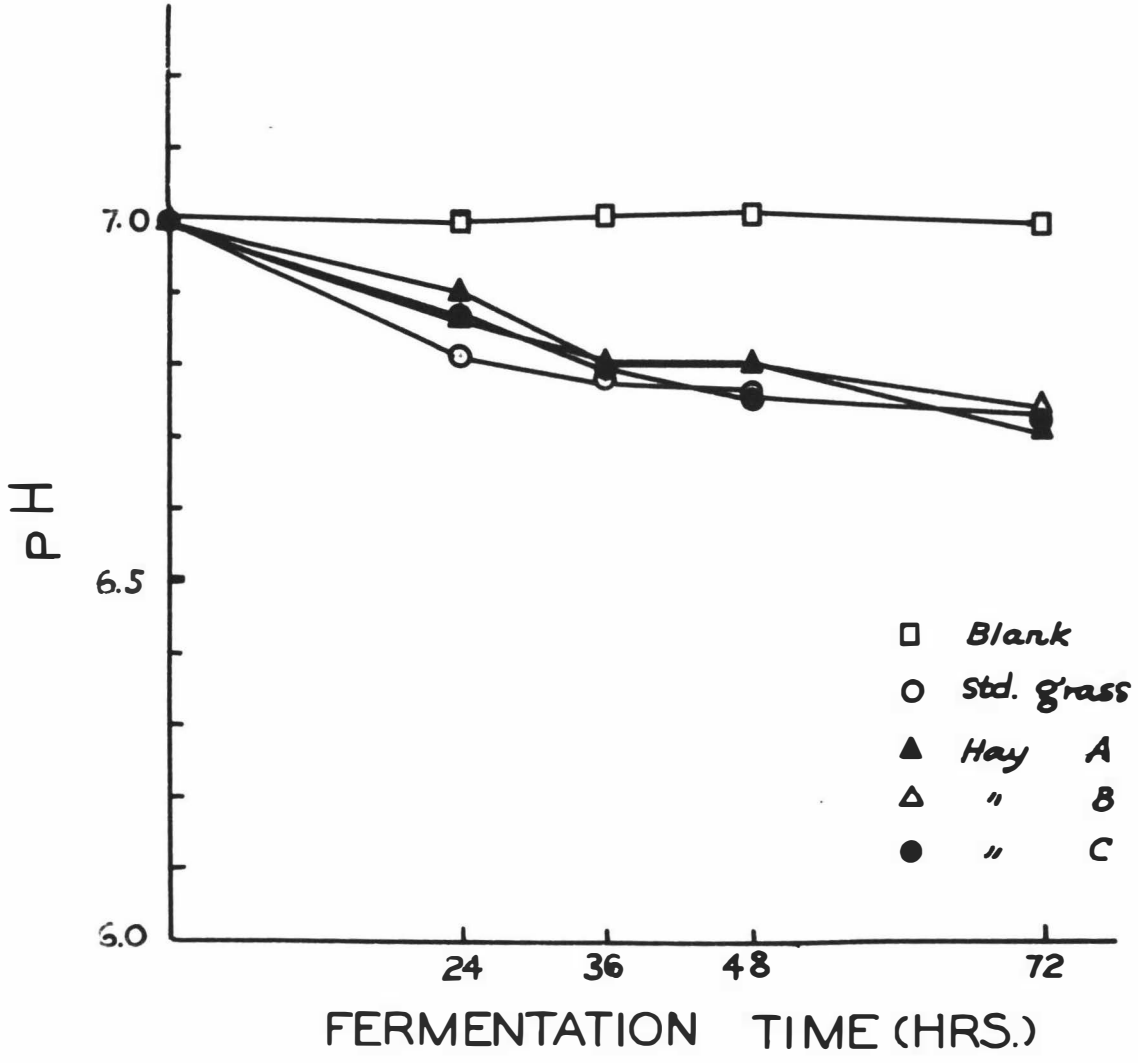

FIG. 7.3 PH CHANGE DURING FERMENTATION WITH HAY A, B, C & STD. GRASS

The regression of *in vivo* O.M. digestibility (Y) on *in vitro* O.M. digestibility (X) are shown in figure 7.4. Even after 24 hours fermentation *in vitro*, there was a fairly good correlation between the two. The limitation of only three points are recognised and the regression coefficient has to be almost 1.0 for it to be significant. Although all the correlation coefficients were significantly high ($P < 0.05$ for 24 hours and $P < 0.01$ for 36 and 48 hours), regression coefficient was highly significant only for the values at the 48 hours fermentation period (appendix 15). Standard error of estimate (Sy.x) was smallest with the values at the 48 hours (0.53) and largest with values at the 24 hours (2.6). Thirty-six hours fermentation with rumen liquor plus 48 hours pepsin digestion gave the intermediate value for the standard error of estimate (1.9).

7.4. Fineness of grinding (2 different fineness of grinding and 2 different lengths of fermentation period)

Analysis of variance of *in vitro* O.M. digestibilities of three hays was carried out on hays, grindings, fermentation times and their interactions (appendix 16).

The major sources of variation were due to substrates and to a lesser extent, to fermentation times. No significant differences were found for different fineness of grinding.

All the first order interactions were significant. Hays x Fermentations ($P < 0.01$) and Grindings x Fermentations ($P < 0.10$), except the interaction of Hays by Grindings. There was no significant second order interactions between hays, grindings and fermentation times.

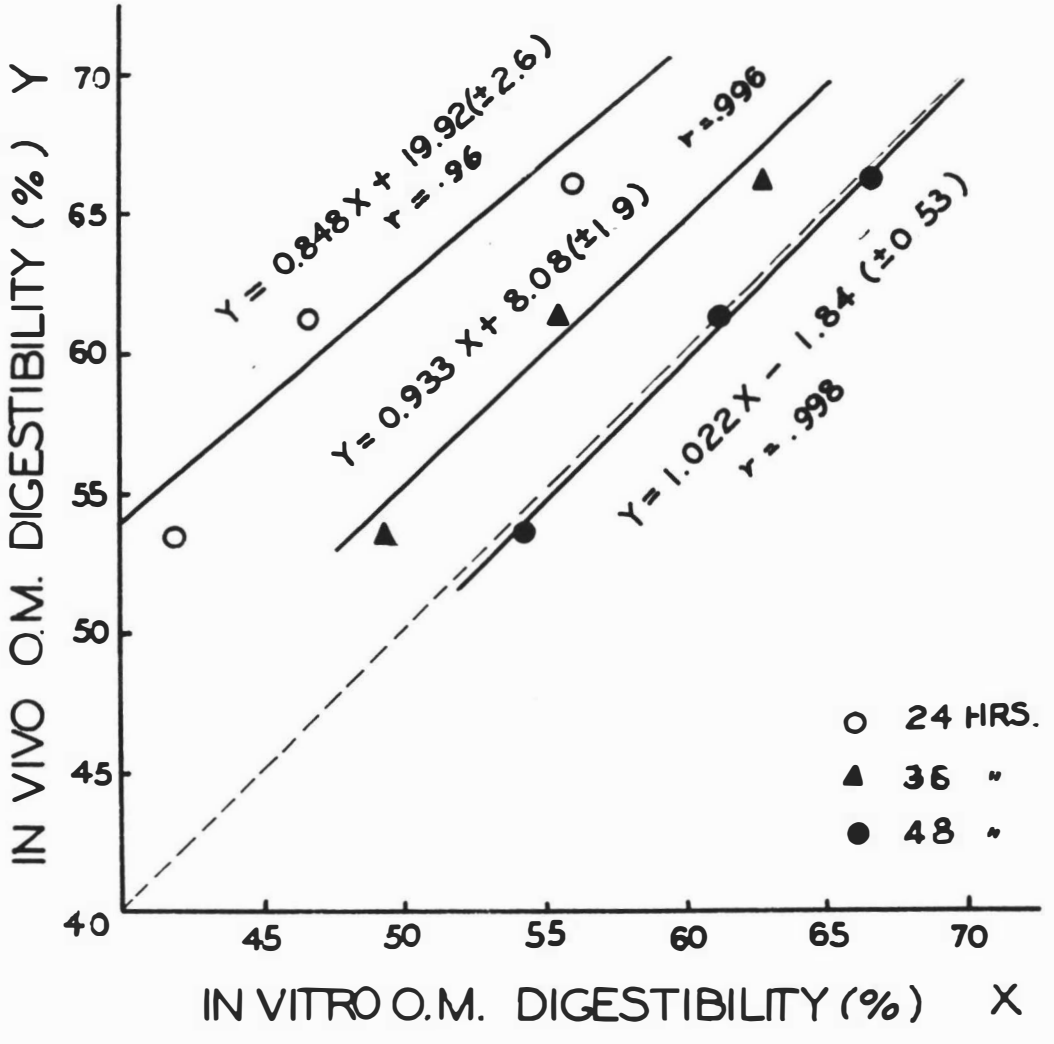

FIG. 7.4 *REGRESSIONS OF* ***IN VIVO*** *DIGESTIBILITY ON* ***IN VITRO*** *DIGESTIBILITY OF THREE DIFFERENT LENGTHS OF FERMENTATION PERIOD USING HAY A, B & C*

– – – – – – : Y = X

Regressions of *in vivo* O.M. digestibility on *in vitro* O.M. digestibility were made using the two grindings and 72 hours (1st stage) plus 48 hours (2nd stage) fermentation period (see figure 7.5.). Regression analysis showed none of the regressions were significant, although the correlation coefficients were highly significant (appendix 17).

7.5. *In vitro* O.M. digestibility of hay A, B, and C using 48 hour rumen liquor fermentation plus 48 hour pepsin digestion

The results of eight separate trials are summarized in appendix 18.

Analysis of variance of *in vitro* O.M. digestibility of hay A, B, and C showed that there were significant differences between hays and days ($P < 0.01$). The mean *in vitro* O.M. digestibility of three hays and variations are given in table 7.3. *In vitro* digestibilities of 3 hays and standard grass on different days are illustrated in figure 7.6.

Covariance analysis was made using the standard grass digestibility as X's and those of individual hays as Y's to see whether there was any trend of changing digestibility according to the digestibility of standard grass (appendix 19). There was a significant difference between hays but no significant difference was found of digestibility according to the digestibility of standard grass. However, when O.M. *in vitro* digestibilities of the test hays were adjusted by the number of precentage units that the standard grass digestibility differed from 79.83%, which was assumed to be the *in vitro* O.M. digestibility of the standard grass, error mean square and

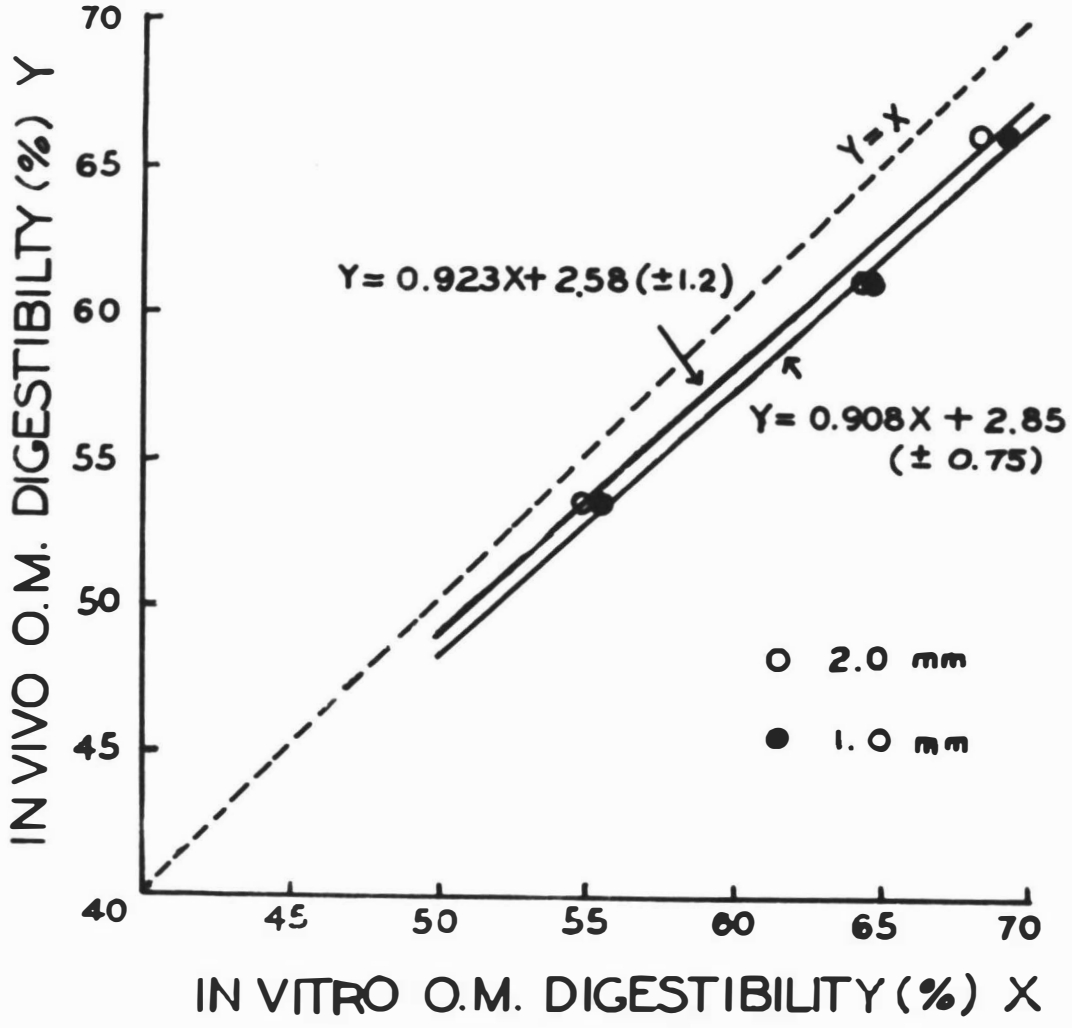

FIG. 7.5 REGRESSION OF IN VIVO O.M. DIGESTIBILITY (Y) ON IN VITRO O.M DIGESTIBILITY (X) OF TWO DIFFERENT PARTICLE SIZES ON 72 HRS (R.L) + 48 HRS (PEPSIN) FERMENTATION PERIODS

Table 7.3. IN VITRO O.M. digestibility of hay A, B, and C and within and between trial variability

HAYS		MEAN DIGESTIBILITY (O.M.%)	WITHIN TRIALS		BETWEEN TRIALS	
			S.E.	C.V. (%)	S.E.	C.V. (%)
A	Original	65.21	0.41	1.41	0.48	2.10
	Adjusted	65.54	0.31	1.10	0.39	1.67
B	Original	53.11	0.57	2.43	0.48	2.58
	Adjusted	53.44	0.58	2.43	0.45	2.38
C	Original	59.28	0.42	1.59	0.62	2.95
	Adjusted	59.63	0.42	1.59	0.61	2.88
Pooled	Original		0.25	0.53	0.94	4.47
	Adjusted		0.46	1.72	0.63	2.97

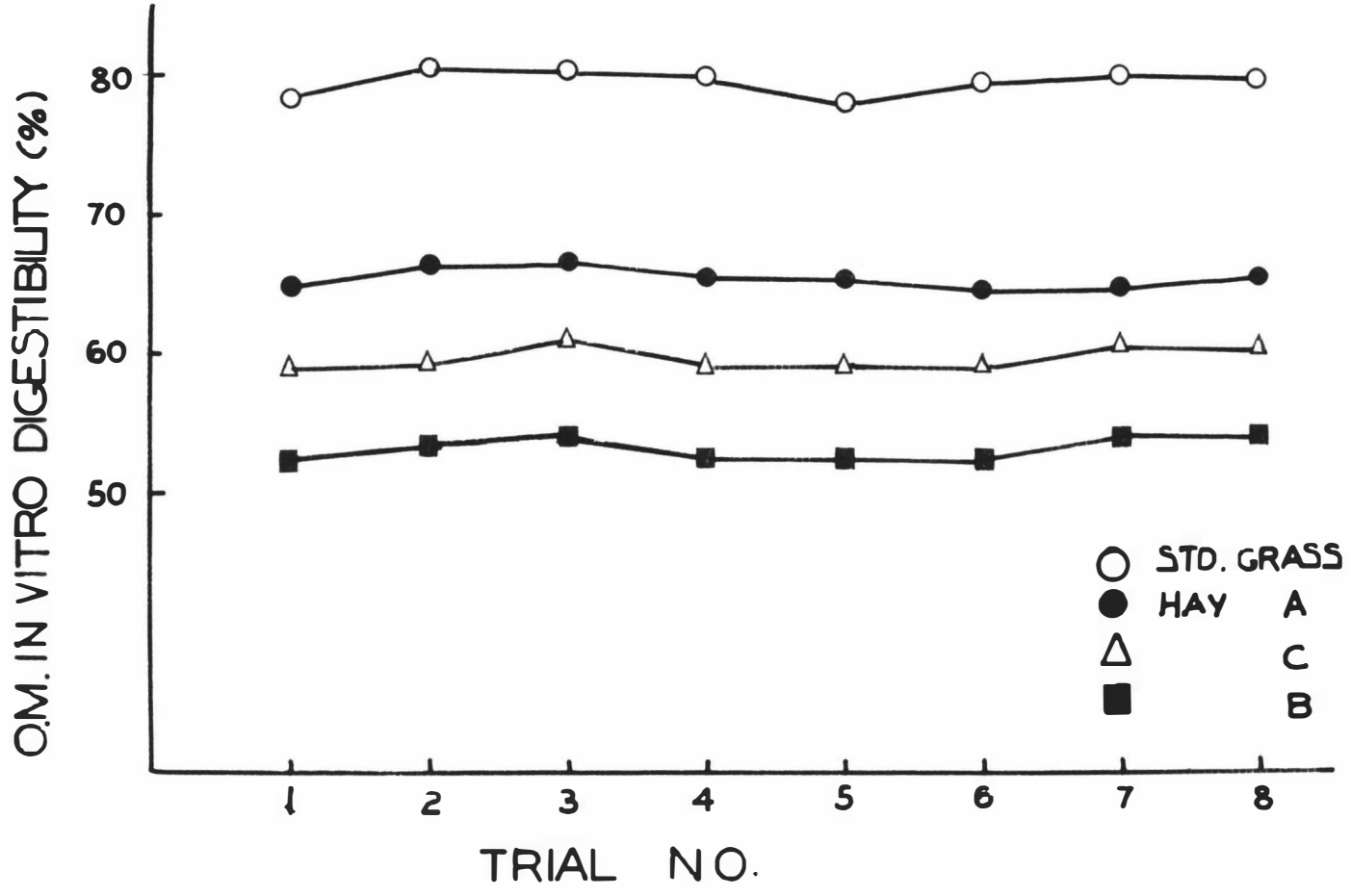

FIG. 7.6 VARIATION OF IN VITRO O.M DIGESTIBILITY OF HAY A, B. C & STD. GRASS BETWEEN TRIALS

(AVE. OF 5 REPLICATES FOR HAY A.B. & C., TRIPLICATES FOR STD. GRASS)

day-to-day variation were decreased from the original data, except error mean square of pooled data (see appendix 17 and table 7.3.).

When regression of in vitro O.M. digestibility of individual hay on in vitro digestibility of the standard grass was made, significant regression was obtained only with hay B ($P < 0.05$). Regressions are shown in figure 7.7., (see appendix 20).

The regression of in vivo O.M. digestibility of 4 hays, hay A, B, C and mixed hay, on in vitro O.M. digestibility is shown in figure 7.8. Regression analysis showed that regression coefficient was highly significant (appendix 21) and gave a regression equation, $Y = 1.047X - 2.07$ (Sy.x, 0.79).

7.6. Relationship between chemical components and in vitro organic matter digestibility of hays and grasses

7.6.1. In vitro O.M. digestibility of three ryegrass samples

In vitro O.M. digestibility of Western Wolths, Paroa (Italian ryegrass) and Ruanui (perennial ryegrass) were 82.52, 80.54 and 80.18, respectively. The digestibility of Western Wolths (W.W.) was significantly higher ($P < 0.01$) than those of Paroa (It.) and Ruanui (Pr.). There were no significant differences between Paroa and Ruanui ryegrass (appendix 22).

7.6.2. Relationship between chemical components and in vitro digestibility

Chemical composition and in vitro O.M. digestibility of hays and grasses are presented in table 7.4.

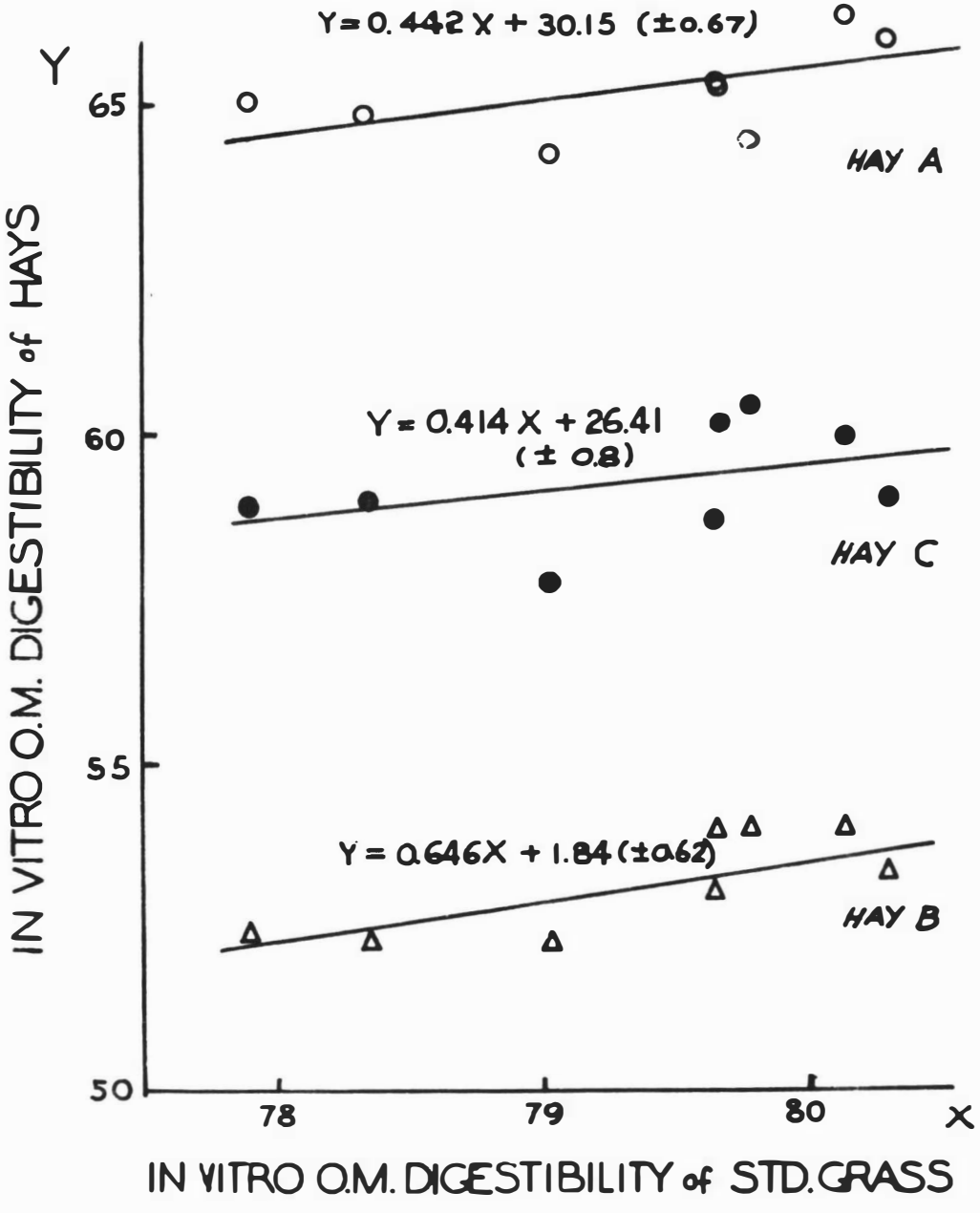

FIG. 7.7 REGRESSION OF IN VITRO O.M. DIGESTIBILITY OF HAYS ON IN VITRO O.M. DIGESTIBILITY OF STD. GRASS

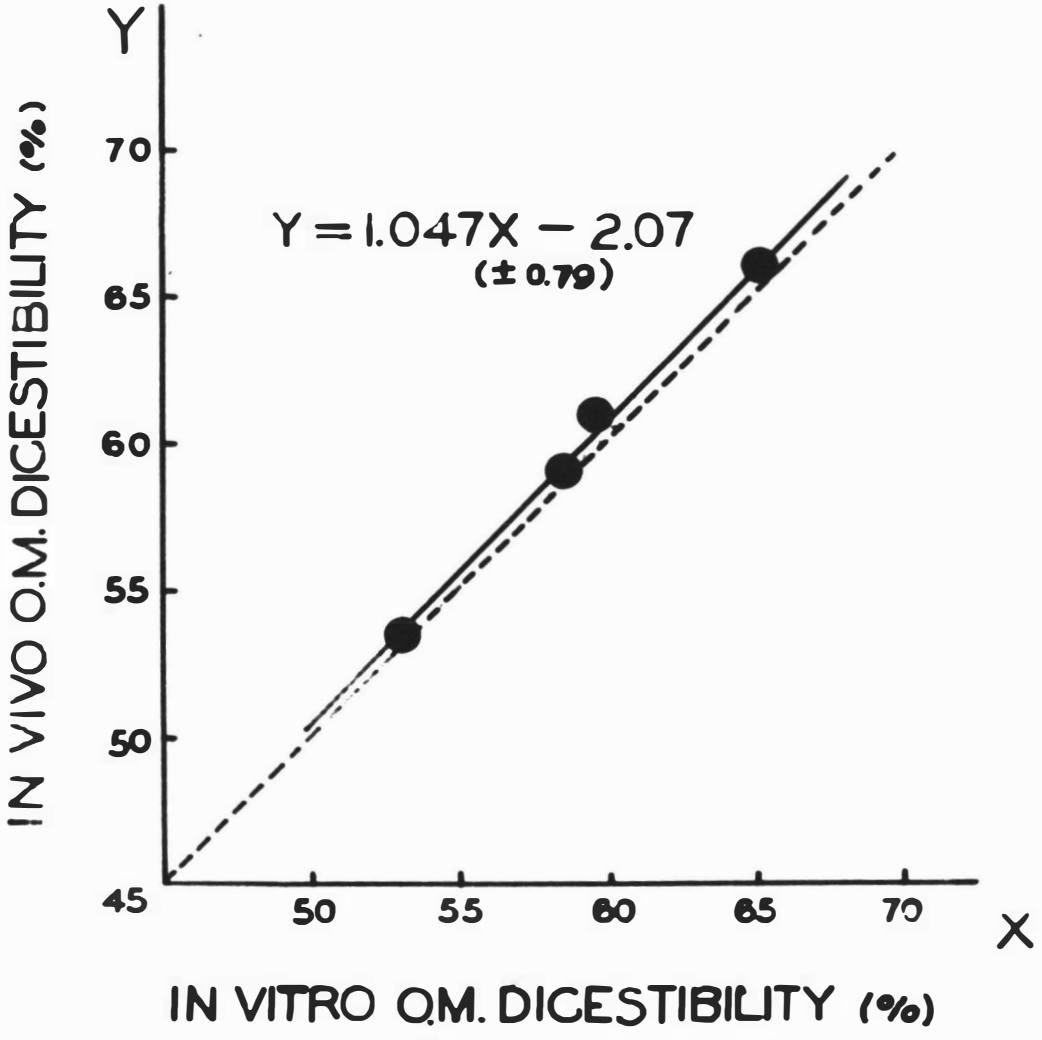

FIG. 7.8 REGRESSION OF IN VIVO DIGESTIBILITY (Y) ON IN VITRO DIGESTIBILITY USING 48 hr. + 48 hr. FERMENTATION PERIODS WITH 4 HAYS

Table 7.4. Chemical composition and *in vitro* O.M. digestibility of hay and grass samples.

FEED	CRUDE PROTEIN (%)	CRUDE FIBRE (%)	ETHER EXTRACT (%)	ASH (%)	N.F.E. (%)	SOL. CHOS (%)	IN VITRO DIGESTIBILITY (O.M. %)
Hay A	15.96	28.26	2.89	10.17	42.72		65.21
Hay B	14.47	31.32	1.78	9.97	42.46		53.11
Hay C	11.74	35.17	1.83	7.41	43.85		59.28
Mixed hays	15.81	30.22	2.42	10.10	41.45		58.55
Std. grass	22.56	20.69	4.44	10.41	41.90		79.83
W.W.	27.60	12.92	5.91	9.90	43.67	23.50	82.52
Pr.	28.80	15.30	5.61	9.12	43.13	18.00	80.54
It.	28.40	13.50	6.44	10.35	41.31	20.60	80.18

Highly significant correlation coefficients were found between crude protein content and *in vitro* O.M. digestibility ($r = 0.91$, $P < 0.01$) and crude fibre content and *in vitro* O.M. digestibility ($r = 0.94$, $P < 0.01$).

The regressions of *in vitro* O.M. digestibility of hay and grass samples on crude protein and crude fibre content were analysed for the both cases, the standard error of estimates (Sy.x) were 5.37 for crude protein and 5.15 for crude fibre content.

When regression analysis was made hays and grasses separately, none of the correlation coefficients and regression coefficients were significant (appendix 23).

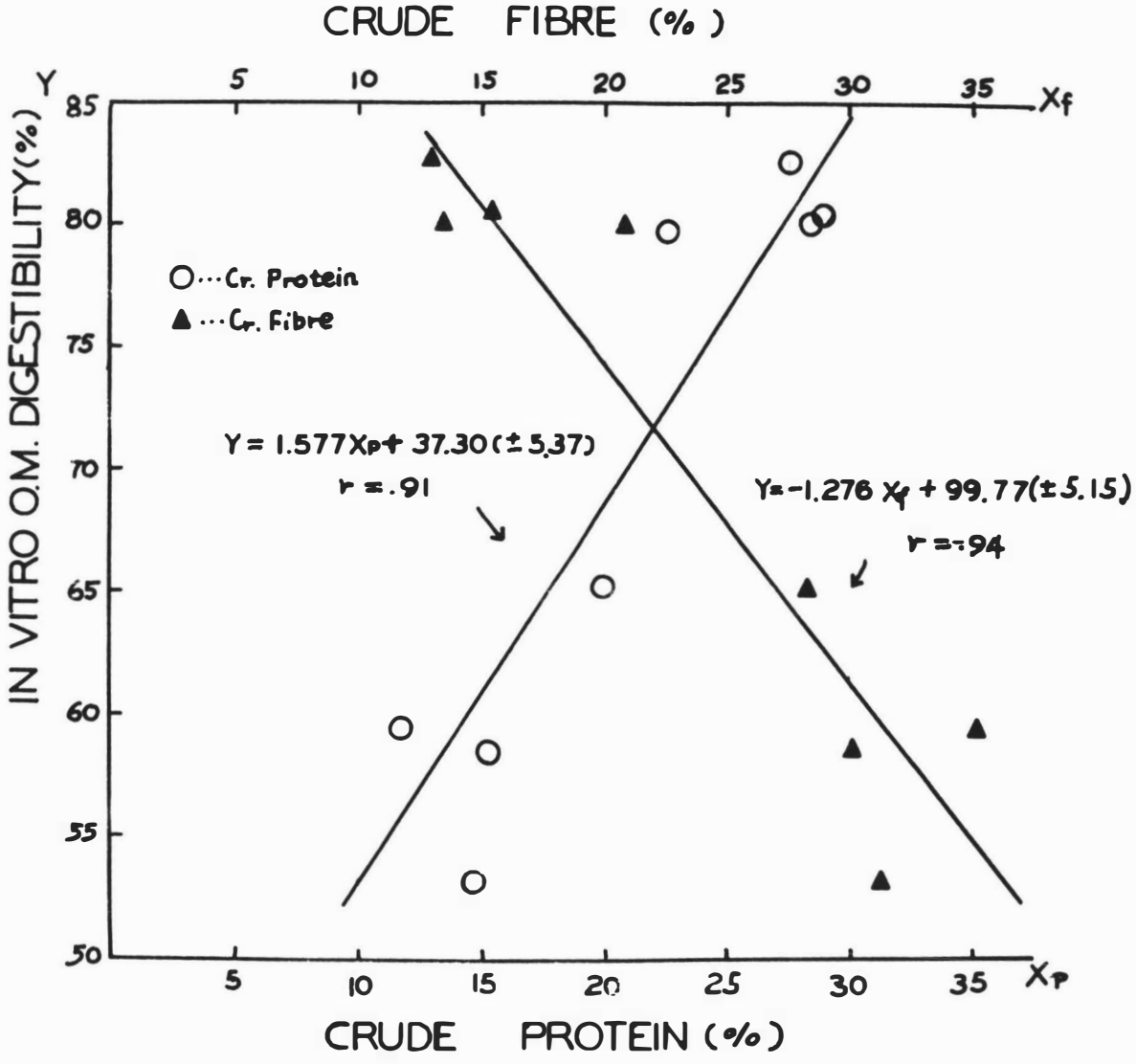

FIG. 7.9 REGRESSION OF INVITRO O.M. DIGESTIBILITY (Y) ON CRUDE PROTEIN CONTENT (Xp) AND CRUDE FIBRE CONTENT (Xf) OF FEED.

CHAPTER 8. DISCUSSION

8.1. Repeatability of in vitro method for estimating digestibility

In using the in vitro fermentation method to estimate forage quality, the results must be repeatable both within and between experiments.

The results obtained both within and between determinations, using both hay and grass inoculum, in the present study were satisfactory and are within the ranges of those reported by other workers, such as Baumgardt et al (1926), Baumgardt and Oh (1964) and Tilley and Terry (1963), and are smaller than those of Bowden and Church (1962a) when standard deviations are compared.

No significant differences with different source of inoculum were found in in vitro O.M. digestibility and in vitro repeatability of the standard grass, despite the fact that differences have been reported. Yates (1964), and Raymond and Terry (1966) reported that the in vitro digestibilities of feed samples they used with inoculum from animals fed poor quality hay gave considerably lower in vitro digestibilities when compared to those with good quality grass or hay inoculum. The hay fed to the inoculum donor animal was the poorest of the three hays used in the present experiment, but was probably not poor enough to alter digestibility, since its crude protein content was reasonably high (see table 7.4.).

The quality of pasture varies as the season advances, however, during the three weeks of the experiment with the cow grazing under a well controlled pasture management system, the change of pasture quality might not have varied to any great extent or else

possible changes did not have any great effect on the microbial population of the donor animal.

This confirms the results of Drew (1966) who reported that grass inoculum from cows grazed on pasture resulted in a smaller day-to-day variation and slightly better *in vitro* digestibilities of two standard feed samples than those with inoculum from hay fed animals.

Using animals grazing on pasture as rumen liquor donor animal is more convenient than animals fed indoors for long periods, particularly where the amount of feed (hay) and labour are limited. However, differences may occur over a period of time where marked changes occurring in the stage of maturity of the pastures in *in vitro* digestibility.

Day-to-day variation of *in vitro* O.M. digestibility of the standard grass was greater than the variation within trials and analysis of variance showed that differences in digestibility between days were significant (grass inoculum, $P < 0.10$; hay inoculum, $P < 0.01$). This result agrees with that of most workers (Bowden and Church, 1962a; Baumgardt *et al*, 1962b; Yates, 1964; Tilley and Terry, 1963) but disagrees with those of Baumgardt and Oh (1964) who reported that day-to-day variation was slightly smaller than within trial variation. No clear reason for this day-to-day variation can be given. Possible effects might have been through a change in the microbial population caused by change of quality and quantity of feed and intake of water, particularly with grazing animal. No explanation can be offered for the smaller day-to-day variation with grass inoculum than with hay inoculum in *in vitro* O.M. digestibility of the standard grass.

The cause of variation within experiments is difficult to explain. Possible explanations include sampling of substrates, accuracy of weighing, and loss of some feed particles during the CO_2 flushing and filtering procedures. Another possible reason may be that, although no great variation was found among blank replicates within trials, the amount of rumen liquor residue contributed to fermentation bottles might not have been exactly the same and the amount of blank residue would change if a feed sample had been added. Loss of activity in the inoculum during the time required to carry out the inoculation appears to be a further possible factor likely to cause within trial variation.

8.2. Comparison of source of inoculum

8.2.1. Grass inoculum vs hay inoculum

There is a lack of agreement between workers as to the effect of type of feed on rumen inoculum. As Bezeau (1965) has shown, inocula from cows fed lucerne hays gave higher *in vitro* cellulose digestibility of forage substrates than those from grass hays. The lucerne hays were better quality feeds in terms of protein content but no reason was given as to whether the superior *in vitro* cellulose digestion was because of higher nitrogen content or because of other factors which were less easily explained.

The importance of nitrogen level in feed fed to donor animal is well demonstrated by Raymond and Terry (1966) who compared *in vitro* digestibilities of sweet grass of low nitrogen content (0.7%N) and S24 ryegrass of high protein content (4.1%N), using rumen inocula taken

from a sheep fed either hay (1.5%N) or straw (0.5%N). The further effect of adding 6 mg. of N as urea to each fermentation tube was also tested. With both grasses the in vitro D.M. digestibility measured with the hay inoculum was similar to that found in vivo: it was not increased by the addition of urea. With the straw inoculum the digestibility of both grasses was depressed but was increased to the in vivo level with the addition of 6 mg. urea.

The effect of source of inoculum whether the donor animal was grazed or fed hay has shown no significant difference in in vitro O.M. digestibility of the standard grass. The hay fed to the rumen liquor donor animal, as has been explained in the previous section, was the lowest quality among the three hays in terms of O.M. digestibility in vivo. However, the hay had reasonably high crude protein (2.3%N), which might have supplied adequate N source. Furthermore, the crude protein content of the standard grass used as a substrate was considerably high (3.3%N) and these combined effect probably did not have any insufficient nitrogen source to be required by rumen micro-organisms.

The reason is not clear that whether slightly higher in vitro O.M. digestibility of the standard grass with grass inoculum was because of higher content of crude protein of pasture or because of experimental error, since no measurement of crude protein content of pasture grazed by cow was made.

8.2.2. Comparison of cow difference

The results of the present experiment gave no significant difference in the in vitro O.M. digestibility of the standard grass, when inocula from 2 cows grazed on the same pasture were compared.

This agrees with results of several other workers, e.g., Van Dyne and Weir (1964a), Baumgardt and Oh (1964), and Drew (1966), who reported little differences between animals within species on the same diet. Furthermore, various workers reported only a negligible difference between sheep and cattle. Thus Le Fevre and Kamstra (1960) concluded that sheep and cattle could serve interchangeably as sources of inoculum if the rations were similar. In contrast to these reports, Yates (1964) reported a significant difference in the *in vitro* D.M. digestibility with inocula taken from two sheep fed on the same diet. However, it was not clear whether this difference was caused by different sources of inoculum or due to the method of collecting rumen liquor since it was collected via a stomach tube and it might be possible that rumen liquor was not collected from the same position of the rumen or was contaminated by large amounts of saliva.

8.2.3. Inoculum collected at different times after grazing

It is difficult to standardize or control the behaviour of grazing animals, particularly with the amount of water and feed eaten. With these limitations in mind, the present experiment was carried out to observe whether varying the time of collection of rumen liquor would affect the results of *in vitro* digestibility. Although the digestibility of the standard grass with inoculum collected 6 hours after grazing was slightly lower than those with inocula collected 2 and 12 hours after grazing, the differences were not significant. Drew (1966) also found no significant difference between the *in vitro* digestibilities of his standard feed samples with rumen inoculum collected at different times after feeding. However, the *in vitro* digestibility with inoculum

collected after a 15 hour fast gave the lowest digestibility.

The *in vitro* D.M. digestibility figures of Yates (1964) are lower than those of other workers such as Tilley and Terry (1963), Drew (1966) and Oh *et al* (1966). A possible reason was that the digestive power of the inocula used by Yates (*loc. cit.*) was weak since the inocula were collected after the donor animals had been starved for 48 hours. The delay in collection of inoculum seems hard to justify, as also suggested by Tilley and Terry (1963).

The amounts of undigested particles in the rumen liquor were significantly reduced from 2 hours to 6 hours and from 6 hours to 12 hours after grazing. Rumen liquor collected at 12 hours after grazing was easier to handle in the laboratory because of its lower rumen residue content.

8.3. Length of fermentation period

8.3.1. Comparison of different lengths of second stage (pepsin) fermentation period

In 1956 the Association of Official Agricultural Chemists (ADAC) set up a collaborative study to investigate pepsin digestibility of proteins as an index to protein quality, the length of fermentation time recommended was a duration of 16 hours. Drew (1966) used 24 hours for the second stage fermentation period replacing the 48 hours originated by Tilley *et al* (1960).

When 48 hours and 24 hours of pepsin digestion following the 48 hours rumen liquor digestion were compared in the present study, no significant difference was found between means of the two lengths of

fermentation period. However, variations (S.E. and C.V.) associated with 24 hour pepsin digestion were greater in all four feeds tested than 48 hours pepsin digestion.

Drew (1966) is the only worker who has shortened the second stage fermentation period of 48 hours to 24 hours. He suggested the use of a fermentation period of 72 hours with rumen liquor and 24-hour pepsin digestion. However, his results show that 48 hours with rumen liquor followed by 48 hours pepsin digestion has a smaller standard error of estimates than that of the former one.

8.3.2. Comparisons of different lengths of first stage fermentation period

Various workers have stated that the *in vitro* digestibility of D.M. or cellulose increases with longer fermentation periods. The important point is raised, then, as to what is the optimum length of the fermentation period which predicts *in vivo* digestibility with a small standard error of estimates? A fermentation period of 12 hours was considered sufficient to predict nutritive value indices by Johnson *et al* (1962), and Donefer, Crampton and Lloyd (1960), while other workers preferred a longer period, such as 24 hours (Baumgardt and Oh, 1964; Hershberger *et al*, 1959), and 72 hours (Walker, 1959; Drew, 1966). Most workers who have used the Hurley method have used 48 hours as the standard fermentation period.

When the results of the present study on the effect of different first stage fermentation periods were analysed, the 48 hours fermentation period gave the smallest variation between replicates within trials and the lowest standard error of estimate of regression

of *in vivo* O.M. digestibility on *in vitro* O.M. digestibility of three hays, A, B and C. This fact confirms the earlier reports by Baumgardt and Oh (1964) who reported that 48 hour fermentation gave the smallest variance when it was compared with 18 hours, 24 hours, and 36 hours fermentation periods, and also confirms the results of Yates (1964) who reported that 48 hours with rumen liquor and 48 hours with pepsin digestion gave the best regression equation with smaller standard error than those of shorter than 48 hours fermentation period. Similar results were reported by Bowden and Church (1962a).

An interesting point raised in the present experiment is that there was a significant correlation between *in vivo* and *in vitro* O.M. digestibility at the 24 hour fermentation, although the hays by fermentation period interaction was significant.

The rate of O.M. loss at different fermentation periods is also of some interest. The rate of O.M. losses of good quality feeds, i.e., hay A and standard grass were higher than poorer ones (hay B and C), as expected, but the O.M. loss of the poorest hay (hay B) was slightly higher than medium hay (hay C). The reason for this is not known.

When the *in vitro* fermentation period was extended from 48 hours to 72 hours, all *in vitro* O.M. digestibility of the three hays (A, B, C) were greater than the actual *in vivo* figures. This is a surprising result when compared to those of Yates (1964) and Drew (1966), who reported that even after 96 hours (Yates, *loc. cit.*) and 72 hours (Drew, *loc. cit.*), the *in vitro* digestibilities of feeds were less than their *in vivo* digestibility figures. Tilley and Terry (1963), however, showed that the *in vitro* digestibilities of their

feeds were almost identical after 48 hours fermentation period.

8.4. Fineness of grinding

The different degrees of grinding, i.e., 1.0 mm. and 2.0 mm. had no effect on *in vitro* O.M. digestibility with the hay samples used in this experiment.

One of the reasons may be because of the grinding method used in this experiment. As has been suggested by Tilley and Terry (1963) grinding is dependent not only on the type of mill and mill sieve used but also on the moisture content of the sample at the time of grinding. All the feed samples used in this experiment might have been finer than they should be since the grinding procedure was carried out immediately after drying.

Another possible reason is that the fermentation periods used were comparatively longer than those used by most workers such as 24 hours by Baumgardt and Oh (1964) and 36 and 48 hours by Dehority and Johnson (1961), since 48 hours and 72 hours of the first stage fermentation followed by 48 hours pepsin digestion period were employed. A third reason may be related to quality of feeds used. Minson (pers. comm.) found no significant difference due to fineness of grinding when using better quality hay (O.M. digestibility $>$ 50%) yet obtained significant differences in *in vitro* O.M. digestibility due to this cause with lower quality hay (O.M. digestibility $<$ 40%). Hays used in this experiment (O.M. digestibility $>$ 53%) showed no significant differences due to fineness of grinding. It seems possible that these hays are of quality high enough not to show these effects.

8.5. In vitro O.M. digestibility of hay A, B, and C and relationship between in vitro and in vivo digestibility

8.5.1. Within and between trial variability of in vitro O.M. digestibility

Bowden and Church (1962a), Baumgardt *et al* (1962b) and Baumgardt and Oh (1964) presented estimates of within and among trial variability and Barnes (1965) summarized other within and between trial variability estimates appearing in the literature. These values are of the same magnitude as those in this experiment.

The standard grass was included in each *in vitro* trial in order to adjust the digestibility estimates of the other hays in an attempt to reduce day-to-day variation due to inoculum differences. It appears that such an adjustment was not necessary in the present experiment, since covariance analysis, using digestibility of the standard grass as the independent variable and that of individual hays as the dependent variable, showed no significant trend of digestibility of the hays according to that of the standard grass. This result confirms the report by Baumgardt and Oh (1964) and disagrees with their earlier report (Baumgardt *et al*, 1962b) and the report by Tilley and Terry (1963), who suggested the importance of the inclusion of a control feed.

8.5.2. Relationship between in vitro and in vivo digestibility

Relationship between *in vitro* (cellulose, D.M. or O.M. digestibility) and *in vivo* digestibility appearing in literature are summarized in appendix 24 and 25. Considering only the results

obtained with the Hurley method, regression coefficients vary from 0.641 (Yates, 1964) to 1.15 (Armstrong et al, 1964) and standard errors of estimates vary from 0.74 (Drew, 1966) to 8.44 (Yates and Allden, 1966). When the results reported by Yates and Allden (loc. cit.) are excluded, the highest standard error of estimate is 2.96 (Oh et al, 1966).

The regression coefficient and the standard error of estimate of present experiment were 1.047 and 0.79 respectively.

The regression coefficient almost fits the line expressed by Y = X and the standard error of estimate is small compared with that of other workers. Main disadvantages of the recent study are that only 4 points (4 hays) were included and the range of O.M. digestibility of hays used was comparatively small (54 to 66%). It is, however, safe to say that 48 hours with rumen liquor and 48 hours with pepsin in vitro digestion gave a highly accurate prediction of in vivo O.M. digestibility of the four hays used in this experiment.

8.6. Relationship between chemical components and in vitro O.M. digestibility of hays and grasses

The magnitude of the correlation of in vitro O.M. digestibility with the crude protein and the crude fibre content of the grasses and hays indicates that chemical content of forages may be a good indicator of the digestibility of forages. However, as was shown in this experiment, the standard errors of estimate were too large (5.37 and 5.15) for the equation to be used in practice for accurate predictions. Moreover, when regressions of in vitro digestibility on chemical compositions were made separately, i.e., 4 hays and 4 grasses,

there were no significant correlation coefficients or significant regression coefficients.

Bowden and Church (1962b) found a significant correlation coefficient of 0.68 when *in vitro* D.M. digestibility and with crude protein content were analysed, however, they found no correlation between *in vitro* D.M. digestibility and crude fibre content of tall fescue used in their experiment.

The digestibility of hay B was the lowest among the hays used in the present experiment, although the crude protein and the crude fibre content were between hay A and C and its appearance was better than hay C.

The reasons for this are not clear but it might have been due the maturity of hay B compared with the other two hays or to the changing of quality during the storage as noted by Le Fevre and Kamstra (1960) and Clark and Mott (1960), since hay B had a longer storage period than the other two hays and pockets of mold were found during the chopping procedure. Hay B also largely consisted of cocksfoot whereas the other two hays consisted mainly of ryegrass and white clover for hay A or ryegrass for hay C. The lower digestibility coefficient of cocksfoot when compared to ryegrass at the same stage of maturity was reported by Tribe, Freer and Combe (1963).

CHAPTER 9. GENERAL DISCUSSION AND CONCLUSIONS

Digestibility is now recognised as the main determinant of nutritive value (Armstrong *et al*, 1964) and the net energy of a herbage ration can be predicted with considerable precision from the digestibility coefficient.

In contrast to net energy, which must be determined in complicated animal calorimeters, the digestibility of a feed can be determined in a relatively simple experiment in which the weights of feed eaten and faeces excreted are measured over a period of 7 to 14 days. Sheep are generally used in digestibility trials, for their digestive efficiencies are similar to those of cattle while their feed requirements are smaller and their handling easier.

To measure the digestibilities of all herbage feeds with animals would be quite impracticable, so laboratory methods have been sought and investigated. The development of reliable laboratory methods for estimating forage quality is one of the most challenging problems in agricultural research today. The use of *in vitro* rumen fermentation techniques for determining the digestibility has advanced since Marston (1948).

While the most effective *in vitro* systems have given close agreement for cellulose digestibility values determined *in vivo*, the D.M. and O.M. digestibility have been much lower in general than the *in vivo* values, particularly with grasses and legumes of high nitrogen content. A two stage fermentation technique, i.e., digestion with pepsin following the fermentation with rumen liquor has been extensively used by many workers since 1960 to overcome this problem.

The use of a filter aid and fibre-glass filter paper capable of withstanding ignition, and the determination of indigestible O.M. by loss on ignition of residue has the advantage that the filter aid need not be critically measured.

Minson *et al* (1964) point out the superiority of the digestible coefficient of O.M. as a parameter in making comparison of feeding value of forages over both the "D" value, i.e., the percentage digestible O.M. in the D.M., and the digestibility of D.M., in that the digestible D.M. is independent of ash content which may well be irrelevant to the comparisons being made.

The precision or reproducibility of the *in vitro* method is one of its greatest problems, and a standard forage is often employed in an attempt to measure some of the variability. Many possible factors which may affect the *in vitro* fermentation have been discussed by many workers and some are discussed in this thesis.

One of the greatest deterents to the accuracy of the *in vitro* methods is large variability inherent with the *in vivo* measurements, upon which the *in vitro* results must be based. The difference arises in part from the normal biological variation between experimental animals, but mainly because digestibility *in vivo* is in fact not constant. Thus, as the level of intake of a feed is increased, its digestibility tends to decrease. Clearly the *in vitro* method measures a basic attribute of a feed; it cannot take account of changes in digestibility in animals under different regimes of feeding. For this reason Tilley and Terry (1963) have preferred to state results as they stand, in terms of *in vitro* digestibility, rather than put them in the *in vivo* form.

In a recent American study, in which 17 laboratories used various *in vitro* fermentation techniques to study the digestibility of standard forages, a considerable variability occured within and between the techniques employed at the different laboratories. This report simply points out that, with the techniques presently in existence, it would not be possible for one laboratory to use the regression equations developed in another laboratory, even though identical techniques may be in use in both laboratories.

Once a technique has been standardized and regression equations have been determined, the *in vitro* rumen fermentation may prove highly valuable in studying forages such as for small samples of herbage collected in oesophageal fistulae and for plant breeding studies.

In vitro work by many laboratories has been carried out on samples previously subject to *in vivo* determinations at other experimental stations or often involving a period of storage. The adoption in the present work of an *in vivo* determination in conjunction with the *in vitro* work reduced possible errors resulting from different laboratory techniques or from storage. This, however, limited the number of feed samples which could be handled at one time to 4 hays and meant a total of only 4 points on the regression line.

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APPENDICES

Effect of length of preliminary and collection periods on O.M. digestibility of hay A, B and C

8. Analysis of Covariance

Digestibilities of hay A, B and C on the digestibility of the mixed hays

(a) 10 day collection period

(b) 7 day collection period

(c) 14 day collection period

(d) 7 day collection period

9. Analysis of Variance

Repeatability

(a) Rumen inoculum from a cow grazed on pasture

(b) Rumen inoculum from a cow fed hay

(c) Pooled

10. Analysis of Variance

Effect of source of inoculum

(a) Grass inoculum vs hay inoculum

(b) Cow differences

(c) Effect of time of rumen liquor collection

11. Analysis of Variance

Effect of rumen collection time on blank residue change

12. Analysis of Variance

Different lengths of second stage fermentation period

13. Analysis of Variance

Effect of fermentation period and substrates on pH change

14. Analysis of Variance

Different lengths of first stage fermentation period

15. Analysis of Regression of *in vivo* O.M. digestibility of hay A, B and C on *in vitro* O.M. digestibility

(a) 24 hours

(b) 36 hours

(c) 48 hours

16. Analysis of Variance

Effect of grinding

17. Analysis of Regression of *in vivo* O.M. digestibility of hay A, B and C on *in vitro* O.M. digestibility, using 72 hours and 48 hours

(a) Fineness of grinding of 1.0 mm.

(b) Fineness of grinding of 2.0 mm.

18. Analysis of Variance

In vitro O.M. digestibility of hay A, B and C, using 48 hours + 48 hours

(a) Hay A

(b) Hay B

(c) Hay C

(d) Pooled result

19. Analysis of Covariance

In vitro O.M. digestibility of the standard grass on *in vitro* O.M. digestibility of hay A, B and C

20. Analysis of Regression of hay A, B and C on *in vitro* O.M. digestibility of the standard grass

(a) Hay A

(b) Hay B

(c) Hay C

21. Analysis of Regression of *in vivo* O.M. digestibility on *in vitro* O.M. digestibility of hay A, B and C

22. Analysis of Variance

In vitro O.M. digestibility of three ryegrass samples

23. Analysis of Regression of Chemical Components on *in vitro* O.M. digestibility of hays and grasses

(a) Crude protein with hays

(b) Crude protein with grasses

(c) Crude fibre with hays

(d) Crude fibre with grasses

(e) Crude protein (pooled)

(f) Crude fibre (pooled)

24. Relationship between *in vitro* and *in vivo*, D.M. or O.M. digestibility as criteria in *in vitro*

25. Relationship between *in vitro* and *in vivo*, cellulose digestibility as criteria in *in vitro*

APPENDIX 1

Feed consumed during the ad lib. feeding period with mixed hays

a) For 2 weeks

ANALYSIS OF VARIANCE

Source	d.f.	S.S.	M.S.	F.	Result
Sheep	7	7156.15	1022.3	36.1	* * *
Days	13	1476.98	113.6	4.0	* * *
Error	91	2578.47	28.3		
Total	111	11211.60			

***P $<$ 0.01

b) For the last 7 days

ANALYSIS OF VARIANCE

Source	d.f.	S.S.	M.S.	F.	Result
Sheep	7	3886.79	555.3	268.3	* * *
Days	6	434.17	72.4	3.5	* * *
Error	42	867.65	20.7		
Total	55	5188.61			

***P $<$ 0.01

APPENDIX 2

Dry matter intake during the preliminary period with the mixed hays ($D.M.g/KgW^{.75}$)

ANALYSIS OF VARIANCE

Source	d.f.	S.S.	M.S.	F.	Result
Sheep	7	4073.45	581.9	135.1	* * *
Days	9	51.82	5.76	1.34	N.S.
Error	63	271.77	4.31		
Total	79	4397.04			

***$P < 0.01$
N.S. Not Significant

APPENDIX 3

Organic matter intake during the collection period with the mixed hays ($O.M.g/Kg W^{.75}$)

ANALYSIS OF VARIANCE

Source	d.f.	S.S.	M.S.	F.	Result
Sheep	7	3067.22	438.17	204.8	***
Days	9	12.62	1.40	0.65	N.S.
Error	63	134.52	2.14		
Total	79	3214.36			

***$P < 0.01$
N.S. Not Significant

APPENDIX 4

Mean daily dry matter intake during ad lib. feeding period with hay A, B and C

a) Between days and between sheep (D.M.g/day)

ANALYSIS OF VARIANCE

Source	d.f.	S.S.	M.S.	F.	Result
Days	6	45539	7589	7.38	* * *
Sheep	5	663698	132739	129.1	* * *
Error	31	31858	1028		
Total	42	740195			

b) Difference between hays on intake (D.M.g/$KgW^{.75}$)

ANALYSIS OF VARIANCE

Source	d.f.	S.S.	M.S.	F.	Result
Hays	2	210.69	105.35	1.93	N.S.
Error	3	163.63	54.54		
Total	5	374.32			

APPENDIX 5

Dry matter intake during preliminary period with hay A, B and C

a) **Daily dry matter intake**

ANALYSIS OF VARIANCE

Source	d.f.	S.S.	M.S.	F.	Result
Sheep	5	2243.37	448.67	364.7	* * *
Days	6	13.66	2.27	1.85	N.S.
Error	30	36.98	1.23		
Total	41	2294.01			

b) **Daily dry matter intake per metabolic body weight**

ANALYSIS OF VARIANCE

Source	d.f.	S.S.	M.S.	F.	Result
Hays	2	619.65	309.82	521	* * *
Days	6	6.79	1.13	1.9	N.S.
Error	12	7.14	0.595		
Total	20	633.54			

APPENDIX 6

O.M. intake during the collection periods with hay A, B and C

a) Collection period of 10 days (following 7-day preliminary)

ANALYSIS OF VARIANCE

Source	d.f.	S.S.	M.S.	F.	Result
Sheep	5	2649.84	529.97	552.1	* * *
Days	9	20.79	2.31	2.41	* *
Error	45	43.10	0.96		
Total	59	2713.73			

**P < 0.05

b) Collection period of 7 days (following 10-day preliminary)

ANALYSIS OF VARIANCE

Source	d.f.	S.S.	M.S.	F.	Result
Sheep	5	1921.51	384.3	362.5	* * *
Days	6	9.71	1.62	1.53	N.S.
Error	30	31.82	1.06		
Total	41	1963.04			

c) Collection period of 14 days (following 4-day preliminary)

ANALYSIS OF VARIANCE

Source	d.f.	S.S.	M.S.	F.	Result
Sheep	5	3567.25	713.5	631.4	* * *
Days	13	49.27	3.79	3.35	* * *
Error	65	73.21	1.13		
Total	83	3689.73			

d) Collection period of 7 days (following 7-day preliminary)

ANALYSIS OF VARIANCE

Source	d.f.	S.S.	M.S.	F.	Result
Sheep	5	1788.88	357.8	328.3	* * *
Days	6	11.30	1.88	1.72	N.S.
Error	30	32.66	1.09		
Total	41	1832.84			

APPENDIX 7

Effect of length of preliminary and collection periods on O.M. digestibility of hay A, B and C

ANALYSIS OF VARIANCE

Source	d.f.	S.S.	M.S.	F.	Result
Hays	2	278.89	139.45	1259.6	* * *
Lengths of period	3	0.06	0.02	0.2	N.S.
Error	7	0.76	0.11		
Total	12	279.72			

***P < 0.01
N.S. Not Significant

APPENDIX 8

Covariance Analysis, using digestibility of the mixed hays as Xs and those of individual hays as Ys

a) 7-day preliminary and 10-day collection period

Source	d.f.	SSX	SPXY	SSY	SSY'	d.f.'	M.S.	F.	Result
Total	5	0.4675	3.2967	147.8985					
Blocks	1	0.0002	0.0027	0.0502					
Hays	2	0.0796	2.4225	145.8015					
Error	2	0.3877	0.8715	2.0468	0.0878	1	0.0878		
Hay + Error	4	0.4673	3.2940	147.8483	124.6205	3			
Diffs for Hays					124.5327	2	62.2664	709	* * *

b) 10-day preliminary and 7-day collection period

Source	d.f.	SSX	SPXY	SSY	SSY'	d.f.'	M.S.	F.	Result
Total	5	0.4675	2.8094	162.8940					
Blocks	1	0.0002	-0.0033	0.0726					
Hays	2	0.0796	2.6362	162.5444					
Error	2	0.3877	0.1699	0.2770	0.2025	1	0.2025		
Hay + Error	4	0.4673	2.8061	162.8214	145.9710	3			
Diffs for Hays					145.7685	2	72.8842	359	* * *

c) 4-day preliminary and 14-day collection period

Source	d.f.	SSX	SPXY	SSY	SSY'	d.f.'	M.S.	F.	Result
Total	5	0.4675	3.4031	151.1037					
Blocks	1	0.0002	0.0003	0.0006					
Hays	2	0.0796	2.4416	148.5584					
Error	2	0.3877	0.9612	2.5447	0.1617	1	0.1617		
Hay + Error	4	0.4673	3.4028	151.1031	126.3245	3			
Diffs for Hays					126.1628	2	63.0814	315	* * *

d) 7-day preliminary and 7-day collection period

Source	d.f.	SSX	SPXY	SSY	SSY'	d.f.'	M.S.	F.	Result
Total	5	0.4675	3.5032	143.2997					
Blocks	1	0.0002	0.0008	0.0038					
Hays	2	0.0796	2.3928	139.5507					
Error	2	0.3877	1.1096	3.7452	0.5695	1	0.5695		
Hay + Error	4	0.4673	3.5024	143.2959	117.0456	3			
Diffs for Hays					116.4761	2	58.2381	102	* * *

APPENDIX 9

a) Repeatability - digestibility of standard grass in vitro with rumen inoculum from cow grazed on pasture

ANALYSIS OF VARIANCE

Source	d.f.	S.S.	M.S.	F.	Result
Days	3	24.7791	8.26	2.868	*
Error	34	97.8820	2.88		
Total	37	122.6611			

*Significant at 10% level ($P < 0.10$)

b) Analysis of variance of in vitro digestibility of standard grass using hay inoculum

Source	d.f.	S.S.	M.S.	F.	Result
Days	4	50.6738	12.67	9.53	* * *
Error	30	39.8654	1.33		
Total	34	90.5392			

***Significant at 1% level ($P < 0.01$)

c) Pooled analysis of variance

Source	d.f.	S.S.	M.S.	F.	Result
Inoculum	1	1.87	1.87	0.85	N.S.
Days	8	77.33	9.67	4.41	* * *
Error	62	135.87	2.19		
Total	71	215.07			

APPENDIX 10

Effect of source of inoculum on *in vitro* O.M. digestibility of standard grass

a) Grass inoculum vs hay inoculum (within a trial)

ANALYSIS OF VARIANCE

Source	d.f.	S.S.	M.S.	F.	Result
Inoculum	1	0.5864	0.5864	1.39	N.S.
Error	10	4.2184	0.4218		
Total	11	4.8048			

b) Cow differences

ANALYSIS OF VARIANCE

Source	d.f.	S.S.	M.S.	F.	Result
Cows	1	2.5849	2.5849	0.91	N.S.
Error	18	51.2956	2.8498		
Total	19	53.8805			

c) Effect of time of rumen liquor collection

ANALYSIS OF VARIANCE

Source	d.f.	S.S.	M.S.	F.	Result
Times	2	1.5774	0.7887	2.8	N.S.
Error	9	2.5308	0.2812		
Total	11	4.1082			

APPENDIX 11

Effect of time of rumen liquor collection in relation to grazing on Blank residual

ANALYSIS OF VARIANCE

Source	d.f.	S.S.	M.S.	F.	Result
Treatment	2	0.0005662	0.000283	21.6	* * *
Error	7	0.00000916	0.00000131		
Total	9	0.0005754			

APPENDIX 12

Comparison of different lengths of second stage fermentation period (24 vs 48 hrs)

ANALYSIS OF VARIANCE

Source	d.f.	S.S.	M.S.	F.	Result
Hays	3	4844.63	1614.88	1016	* * *
Fermentation Times	1	3.44	3.44	2.16	N.S.
F x T	3	5.78	1.93	0.21	N.S.
Error	52	82.53	1.59		
Total	59	4936.39			

APPENDIX 13

Effect of fermentation period and substrates on pH change

ANALYSIS OF VARIANCE

Source	d.f.	S.S.	M.S.	F.	Result
Substrates	3	0.0138	0.0046	9.2	* * *
Fermentation Periods	2	0.0528	0.0264	53.0	* * *
Error	30	0.0128	0.0005		
Total	35	0.0792			

APPENDIX 14

Comparison of different lengths of first stage fermentation period (24, 36 and 48 hrs)

ANALYSIS OF VARIANCE

Source	d.f.	S.S.	M.S.	F.	Result
Fermentation Times	2	747.78	373.89	56.91	* * *
Hays	3	4160.71	1386.90	211.1	* * *
H x F	6	39.43	6.57	8.42	* * *
Error	24	18.67	0.78		
Total	35	4966.59			

APPENDIX 15

Regression of <u>in vivo</u> O.M. digestibility (Y) on <u>in vitro</u> O.M. digestibility of hay A, B and C with 24 hrs (X_1), 36 hrs (X_2) and 48 hrs (X_3)

a) <u>24 hours</u> (X_1) (24 hrs (R.L) + 48 hrs (pepsin))

SSX = 102.31 SSY = 80.17 SPXY = 86.72

b = 0.848 Σdyx^2 = 6.67 $\bar{X}$ = 48.21 $\bar{Y}$ = 60.12

TEST FOR SIGNIFICANCE OF b

Source	d.f.	S.S.	M.S.	F.	Result
Lin. Reg.	1	73.50	73.5	11.02	N.S.
Error	1	6.67	6.67		
Total	2	80.17			

b) <u>36 hours</u>

SSX = 89.26 SSY = 80.17 SPXY = 83.29

b = 0.933 dyx^2 = 3.45 $\bar{X}$ = 55.78 $\bar{Y}$ = 60.12

TEST FOR SIGNIFICANCE OF b

Source	d.f.	S.S.	M.S.	F.	Result
Lin. Reg.	1	77.72	77.72	22.53	N.S.
Error	1	3.45	3.45		
Total	2	80.17			

c) 48 hours

SSX = 76.46 SSY = 80.17 SPXY = 78.16

b = 1.022 Σdyx^2 = 0.28 $\bar{X}$ = 60.63 $\bar{Y}$ = 60.12

TEST FOR SIGNIFICANCE OF b

Source	d.f.	S.S.	M.S.	F.	Result
Lin. Req.	1	79.89	79.89	285.32	* *
Error	1	0.28	0.28		
Total	2	80.17			

**P $<$ 0.05

APPENDIX 16

Effect of grinding and length of fermentation (2 different grindings and 2 different lengths)

ANALYSIS OF VARIANCE

Source	d.f.	S.S.	M.S.	F.	Result
Hays	2	1001.1274	500.56	98.60	* * *
Grindings	1	1.4844	1.48	1.59	N.S.
Fermentation times	1	79.2694	79.27	15.61	*
H x G	2	0.2945	0.29	1.04	N.S.
H x F	2	10.1561	5.08	18.14	* * *
G x F	1	0.9339	0.93	3.30	*
H x G x F	2	0.1285	0.06	0.023	N.S.
Error	24	6.7820	0.28		
Total	35	1100.1761			

APPENDIX 17

Regression of <u>in vivo</u> O.M. digestibility on <u>in vitro</u> O.M. digestibility of hay A, B and C, using 72 hrs + 48 hrs fermentation period

a) Fineness of grinding of 1.0 mm.

SSX = 96.55 SSY = 80.18 SPXY = 87.67

b = 0.908 Σdyx^2 = 79.61 $\bar{X}$ = 63.07 $\bar{Y}$ = 60.12

TEST FOR SIGNIFICANCE OF b

Source	d.f.	S.S.	M.S.	F.	Result
Lin. Req.	1	79.61	79.61	142.2	*
Error	1	0.57	0.57		
Total	2	80.17			

*P < 0.10

b) Fineness of grinding of 2.0 mm.

SSX = 92.38 SSY = 80.18 SPXY = 85.29

b = 0.923 Σdyx^2 = 78.74 $\bar{X}$ = 62.34 $\bar{Y}$ = 60.12

TEST FOR SIGNIFICANCE OF b

Source	d.f.	S.S.	M.S.	F.	Result
Lin. Req.	1	78.74	78.74	55.06	*
Error	1	1.43	1.43		
Total	2	80.17			

*P < 0.10

APPENDIX 18

IN VITRO O.M. digestibility of hay A, B and C

a) Hay A

ANALYSIS OF VARIANCE

Source		d.f.	S.S.	M.S.	F.	Result
Days		7	18.92	2.70	3.24	* * *
	adjusted		12.13	1.73	3.32	* * *
Error		32	26.68	0.83		
	adjusted		16.64	0.52		
Total		39	45.60			
	adjusted		28.77			

b) Hay B

ANALYSIS OF VARIANCE

Source		d.f.	S.S.	M.S.	F.	Result
Days		7	24.61	3.52	2.13	N.S.
	adjusted		11.34	1.62	0.96	N.S.
Error		32	52.84	1.65		
	adjusted		54.36	1.70		
Total		39	77.45			
	adjusted		65.70			

c) Hay C

ANALYSIS OF VARIANCE

Source		d.f.	S.S.	M.S.	F.	Result
Days		7	27.48	3.93	4.47	* * *
	adjusted		26.93	3.85	4.34	* * *
Error		32	28.16	0.88		
	adjusted		28.37	0.89		
Total		39	55.64			
	adjusted		55.30			

d) Pooled result

ANALYSIS OF VARIANCE

Source		d.f.	S.S.	M.S.	F.	Result
Hays		2	3046.72	1523.36	16926	* * *
	adjusted		2927.96	1463.98	1373	* * *
Days		7	50.96	7.28	80.9	* * *
	adjusted		29.52	4.22	3.9	* * *
Error		110	9.45	0.095		
	adjusted		117.24	1.066		
Total		119	3107.13			
	adjusted		3074.72			

APPENDIX 19

Covariance analysis, using *in vitro* O.M. digestibility of the standard grass as Xs and *in vitro* O.M. digestibility of hay A, B and C as Ys

Source	d.f.	SSX	SPXY	SSY	SSY'	d.f.'	M.S.	F.	Result
Total	23	17.52	8.69	602.4					
Days	7	17.52	8.69	10.05					
Hays	2	0	0	588.23					
Error	14	0	0	4.12	4.12	13	0.317		
H + E	16			592.35	592.35	15			
Diffs for Hays					588.23	2	294.12	927.8	* * *
D + E	21	17.52	8.69	14.17	9.86	20			
Diffs for Days					5.74	7	0.82	2.59	N.S.

APPENDIX 20

Regression of <u>in vitro</u> O.M. digestibility of hay A, B and C on <u>in vitro</u> O.M. digestibility of standard grass

a) Hay A

SSX = 5.84 SPXY = 2.58 SSY = 3.79

b = 0.442 Σdyx^2 = 2.65 Y = 0.442X + 30.15 ($\pm$0.67)

TEST OF SIGNIFICANCE FOR b

Source	d.f.	S.S.	M.S.	F.	Result
Lin. Req.	1	1.14	1.14	2.58	N.S.
Error	6	2.65	0.44		
Total	7	3.79			

b) Hay B

SSX = 5.84 SPXY = 3.77 SSY = 4.79

b = 0.646 Σdyx^2 = 2.36 Y = 0.646X + 1.81 ($\pm$ 0.62)

TEST OF SIGNIFICANCE FOR b

Source	d.f.	S.S.	M.S.	F.	Result
Lin. Req.	1	2.43	2.43	6.18	* *
Error	6	2.36	0.393		
Total	7	4.79			

**P 0.05

c) Hay C

SSX = 5.84 SPXY = 2.36 SSY = 5.60

b = 0.404 Σdyx^2 = 3.84 Y = 0.414X + 26.41 ($\pm$0.81)

TEST OF SIGNIFICANCE FOR b

Source	d.f.	S.S.	M.S.	F.	Result
Lin. Req.	1	1.76	1.76	2.75	N.S.
Error	6	3.84	0.64		
Total	7	5.60			

APPENDIX 21

Regression of <u>in vivo</u> O.M. digestibility on <u>in vitro</u> O.M. digestibility of hay A, B, C and mixed hays

SSX = 73.532 SPXY = 76.998 SSY = 81.877

b = 1.047 Σdyx^2 = 1.2491 Sb = 0.092

Y = 1.047X - 2.07 ($\pm$ 0.79)

TEST OF SIGNIFICANCE FOR b

Source	d.f.	S.S.	M.S.	F.	Result
Lin. Req.	1	80.6279	80.6279	129	* * *
Error	2	1.2491	0.6246		
Total	3	81.877			

APPENDIX 22

IN VITRO O.M. digestibility of three ryegrass samples (W.W., Per., and Italian)

ANALYSIS OF VARIANCE

Source	d.f.	S.S.	M.S.	F.	Result
Variety	2	12.82	6.41	14.91	* * *
Error	9	3.88	0.43		
Total	11	16.70			

MSD (treatments) to values for 9 d.f.

at 5% level 2.262 $\sqrt{0.43(2)/4}$ = 1.047

at 1% level 3.250 $\sqrt{0.43(2)/4}$ = 1.50

Comparisons

Means		W.W.	Per.	It.
W.W.	82.52	——	0.01	0.01
Per.	80.54	0.01	——	N.S.
It.	80.18	0.01	N.S.	——

APPENDIX 23

Analysis of regression of chemical components on <u>in vitro</u> O.M. digestibility of hays and grasses

a) **Crude protein with hays**

SSX = 11.47 SSY = 73.53 SPXY = 7.89

TEST FOR SIGNIFICANCE OF REGRESSION COEFFICIENT

Source	d.f.	S.S.	M.S.	F.	Result
Lin. Reg.	1	5.43	5.43	0.16	N.S.
Error	2	68.10	34.05		
Total	3	73.53			

b) **Crude protein with grasses**

SSX = 25.17 SSY = 4.34 SPXY = 3.98

TEST FOR SIGNIFICANCE OF REGRESSION COEFFICIENT

Source	d.f.	S.S.	M.S.	F.	Result
Lin. Reg.	1	0.63	0.63	0.34	N.S.
Error	2	3.72	1.86		
Total	3	4.35			

c) Crude fibre with hays

SSX = 25.37 SSY = 73.53 SPXY = -17.42

TEST FOR SIGNIFICANCE OF REGRESSION COEFFICIENT

Source	d.f.	S.S.	M.S.	F.	Result
Lin. Reg.	1	11.96	11.96	0.34	N.S.
Error	2	61.57	35.78		
Total	3	73.53			

d) Crude fibre with grasses

SSX = 37.59 SSY = 4.34 SPXY = -8.17

TEST FOR SIGNIFICANCE OF REGRESSION COEFFICIENT

Source	d.f.	S.S.	M.S.	F.	Result
Lin. Reg.	1	1.78	1.78	1.4	N.S.
Error	2	2.56	1.28		
Total	3	4.34			

e) Crude protein - <u>in vitro</u> O.M. digestibility of hays and grasses (pooled)

SSX = 341.44 SSY = 1022.26 SPXY = 538.38

b = 1.577 Σdyx^2 = 173.35 $\bar{X}$ = 20.67 $\bar{Y}$ = 69.90

Source	d.f.	S.S.	M.S.	F.	Result
Lin. Reg.	1	848.91	848.91	29.38	* * *
Error	6	173.35	28.89		
Total	7	1022.26			

f) Crude fibre - <u>in vitro</u> O.M. digestibility of hays and grasses (pooled)

SSX = 552.18 SSY = 1022.26 SPXY =-704.66

b = 1.276 Σdyx^2 = 159.23 $\bar{X}$ = 23.42 $\bar{Y}$ = 69.90

Source	d.f.	S.S.	M.S.	F.	Result
Lin. Reg.	1	863.03	863.03	32.52	* * *
Error	6	159.23	26.54		
Total	7	1022.26			

APPENDIX 24

Relationship between in vitro (D.M. or O.M. digestibility) and in vivo

REFERENCE	FERMENTATION TIME	RANGE OF IN VIVO DIGESTIBILITY OF SAMPLES	NO. OF SAMPLES	X (IN VITRO)	Y (IN VIVO)	REGRESSION EQUATION	SY.X	r
Pigden & Bell (1955)	48 hours		11	Carbohydrates	D.O.M.	Y = 0.90X + 10.6	2.76	-
Asplund et al (1958)	48 hours miniature A.R.	54 - 70	11	D.D.M.	D.D.M.	-	3.9	0.69
"	24 hours	"	11	"	"	-	3.5	0.75
"	"	58 - 77	6	"	"	-	-	0.94
Reid et al (1959)	24 hours		6	"	"	Y = 0.78X + 20.5	3.6	0.98
Walker (1959)	72 hours	40 - 55	7	"	"	Y = 0.913X + 5.33	0.81	0.994
Clark & Mott (1960)	24 hours miniature A.R.	54 - 69	11	"	"	Y = 0.2387X + 48.03	3.9	0.77
"	" spring	"	11	"	"	-	-	0.59
	autumn							0.49
Tilley, Deriaz & Terry (1960)	48 hours with R.L. alone	49 - 77	20	"	"	Y = 1.31X - 11.57	3.6	0.91
"	" "	"	20	"	"	Y = 1.21X - 4.17	4.4	0.87
"	48 hours with R.L. + 48 hours with pepsin	"	20	"	"	Y = 1.07X - 7.47	1.96	0.98
"	" "	"	20	"	"	Y = 0.98X + 2.22	2.0	0.99
Bowden & Church (1962)	48 hours	56 - 78	39	"	"	-	-	0.93
"	" (4 yr total)	"	39	"	"	-	-	0.73
Baumgardt, Cason & Taylor (1962)	24 hours	52 - 65	11	TDN from carbohydrate fermentation	T.D.N.	-	-	0.73
"	"	"	11		D.O.M.	-	-	0.79
"	"	"	11	"	D.D.M.	-	-	0.74
"	"	"	11	"	D.E.(%)	-	-	0.75
Harris (1963)	48 (R.L.) + 48 (PEPSIN) Fodder crops)	60 - 84	10	D.D.M.	D.D.M.	Y = 0.842X + 11.29	3.63	0.90
Tilley & Terry (1963)	48 (R.L.) + 48 (PEPSIN)	45 - 85	148	"	"	Y = 0.99X - 1.01	2.31	-

Reid, Clarke & Jung (1964)	36 hours (g.i.)	57 - 78	27	"	"	-	-	0.97
"	36 hours (h.i.)	"	27	"	"	-	-	0.91
Armstrong, Alexander & McGowan (1964)	48 R.L.) + 48 (PEPSIN) With addition of 0.02M ammonium sulphate to inoculum buffer mixture	55 - 85	12	D.O.M.	D.O.M. (at maintenance)	Y_1 = 0.92X + 12.48	-	0.986
"	"	"	12	"	" (at (2 x M))	Y_2 = 1.15X - 6.12	-	0.989
"	"	"	12	"	M.E.(Kcal/g.d.m.)	Y_{ME} = 0.037X + 0.122	0.083	Highly sig.
"	"	"	12	"	N.E.m (")	Y_{NEm} = 0.035X - 0.462	0.076	"
"	"	"	12	"	N.E.f (")	Y_{NEf} + 0.034X - 1.078	0.117	"
O'Shea & Wilson (1965)	48 (R.L.) + 48 (PEPSIN)	45 - 80	50	D.D.M.	D.D.M.	Y = 0.86X + 8.72	2.81	0.94
Oh et al (1966)	48 (R.L.) + 48 (PEPSIN)	44 - 77	56	"	"	Y = 0.74X + 16.7	2.96	0.88
"	" " (only with alfalfa & clovers)	53 - 72	18	"	"	Y = 0.85X + 8.37	1.42	0.99
Wilkins & Grimes (1966)	48 hours	38 - 80	27	D.O.M.	"	Y = 0.871X + 8.888	4.19	0.94
Yates & Allden (1966)	48 (R.L.) + 48 (PEPSIN) young herbage inoculum in nylon vessel	40 - 80	9	D.D.M.	"	Y = 0.787X + 17.3	3.99	0.97
"	48 (R.L.) + 48 (PEPSIN) chaff & pellets inoculum, nylon	"	6	"	"	Y = 0.641X + 35.5	4.39	0.966
"	48 (R.L.) + 48 (PEPSIN) hay inoculum, nylon	"	9	"	"	Y = 0.697X + 29.3	5.66	0.945
"	48 (R.L.) + 48 (PEPSIN) mature hay, nylon)	"	8	"	"	Y = 0.786X + 24.4	8.44	0.878
"	48 (R.L.) + 48 (PEPSIN) young grass, glass vessel	"	8	"	"	Y = 0.996X + 1.1	1.63	0.99
Drew (1966)	48 (R.L.) + 48 (PEPSIN) with grass inoculum	45 - 90	51	D.O.M.	D.O.M.	Y = 0.88X + H.O.	2.1	-
"	" with grass inoculum	62 - 82	20	"	"	Y = 0.86X + 12.01	0.74	-
"	" with hay inoculum	"	20	"	"	Y = 0.76X + 20.92	1.0	-
"	72 (R.L.) + 24 (PEPSIN) with grass inoculum	"	20	"	"	Y = 1.014X	1.06	-
"	" with hay inoculum	"	20	"	"	Y = 0.87X + 11.3	1.06	-
Alexander & McGowan (1966)	48 (R.L.) + 48 (PEPSIN)		43	"	"	Y = 0.97X + 5.05	2.33	0.96

*1,2 Original data was analysed by author
*3 Recalculation of original data gave different correlation coefficient

APPENDIX 25 Relationship between in vitro (cellulose digestibility) and in vivo (cellulose, D.M., DOM, TDN, DE, digestibility)

REFERENCE	FERMENTATION PERIOD AND METHOD USED	RANGE OF DIGESTIBILITY OF SAMPLE	NO. OF SAMPLES	X (IN VITRO)	Y (IN VIVO)	REGRESSION EQUATION $(Y - \bar{Y}) = b(X - \bar{X})$	SY.X	r
Barnett (1957)	48 hours	48 - 80	27	Cell. Dig.	Cru. f. Dig.	Y = X + 2.9		High
Hershberger et al (1959)	24 hours	55 - 90	35	"	D.E. (Cal/gdm)	Y = 29.8X + 1185	125	0.92
"	"	"	35	"	Cell. Dig.	Y = 0.77X + 30.7	2.0	0.97
Reid et al (1960)	24, 36, 48 hours		124	"	D.D.M. or D.E.	-		High
Le Fevre & Kamstra (1960)	48 hours (without long stored samples)	27 - 61	16	"	Cell. Dig.	-		0.84
"	" (including long stored samples)	26 - 61	22	"	"	-		0.40
Donefer, Crampton & Lloyd (1960)	24 hours	46 - 62	9	"	D.E. %	-	-	0.87
"	12 hours	34 - 71	9	"	N.V.I. %	Y = 1.314X - 7.8	-	0.91
"	12 hours	27 - 55	9	"	Relative intake	-	-	0.83
Bowden & Church (1962)	48 hours		8	"	D.D.M.	-	-	0.87
"	"		39	"	"	-	-	0.49
Baumgardt, Taylor & Cason (1962)	24 hours	51 - 75	31	"	D.E. %	Y = 0.71X + 29.3	3.3	0.78
"	"	2414 - 3010	31	"	D.E. (Cal/gdm)	Y = 33.2X + 1295	116	0.85
"	"	51 - 75	15	"	TON %	Y = 0.782X + 83.9	2.89	0.62
"	"	"	27	"	D.D.M. %	Y = 0.711X + 31.8	3.41	0.78
Baumgardt, Cason & Taylor (1962)	24 hours	52 - 65	11	"	TON %	-	-	0.67
"	"		11	"	D.O.M. %	-	-	0.84
"	"		11	"	D.D.M. %	-	-	0.81
"	"		11	"	D.E. %	-	-	0.80
Grimes et al (1966)	48 (R.L.) + 48 (PEPSIN)	70 - 80	28	"	D.O.M. %	Y = 0.691X + 25.8	0.43	
Wilkins & Grimes (1966)	48 (R.L. + 48 (PEPSIN)	40 - 80	27	"	D.D.M. %	Y = 0.704X + 21.576	3.83	0.95

Reid, Clark & Jung (1964)	36 hours (hay inoculum)	57 - 78	27	"	D.M. %	-	-	0.99
"	36 hours (grass inoculum)	"	27	"	"	-	-	0.99
"	36 hours (g.i.)	"	27	"	Cell. Dig. %	-	-	0.99
"	36 hours (h.i.)	"	27	"	"	-	-	0.99