EVALUATION OF FODDERS BY NYLON BAG AND IN VITRO TECHNIQUES AND THEIR COMPARISON WITH CONVENTIONAL VALUES

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CERTIFICATE

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IN

THE MEMORY OF MY FATHER
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April, 1975.

V.K. RAJPUT
## CONTENTS

<table>
<thead>
<tr>
<th>Chapter No.</th>
<th>Description</th>
<th>Page No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>II</td>
<td>REVIEW OF LITERATURE</td>
<td>7</td>
</tr>
<tr>
<td>III</td>
<td>EXPERIMENTAL TECHNIQUE AND METHODS OF CHEMICAL ANALYSIS</td>
<td>47</td>
</tr>
<tr>
<td>IV</td>
<td>PLAN OF EXPERIMENT</td>
<td>61</td>
</tr>
<tr>
<td>V</td>
<td>RESULTS AND DISCUSSION</td>
<td>66</td>
</tr>
<tr>
<td></td>
<td>A. Chemical composition and stage of maturity of forage plants</td>
<td>66</td>
</tr>
<tr>
<td></td>
<td>B. Dry matter digestibilities of 14 fodders as obtained with different techniques</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td>C. Comparison of 72 hours (nylon bag) dry matter digestibilities with the values obtained by 24 hr, 48 hr, and 96 hr (nylon bag) and in vitro techniques.</td>
<td>75</td>
</tr>
<tr>
<td></td>
<td>D. General discussion</td>
<td>77</td>
</tr>
<tr>
<td></td>
<td>E. Relationship between chemical constituents of forages and their dry matter digestibilities.</td>
<td>78</td>
</tr>
<tr>
<td>VI</td>
<td>SUMMARY</td>
<td>82</td>
</tr>
<tr>
<td></td>
<td>APPENDIX</td>
<td>88</td>
</tr>
<tr>
<td></td>
<td>BIBLIOGRAPHY</td>
<td>(i)-(xxviii)</td>
</tr>
</tbody>
</table>
CHAPTER I

INTRODUCTION

The country as a whole is short of basic foodstuffs and concentrated to a very considerable extent. More and more lands are being brought under the plough for the production of food crops for human consumption and this may be very risky for the future. Due to shortage of land and depauperation of natural resources resulting of causal ways, which will entail further cultivation, increase the output, and it is unlikely to the extent of being able to be spoken for. Further cultivation, only the best quality and highly valued improved varieties should be selected. Moreover, in order to gain the desired advantage in the qualitative and quantitative aspects of food production, should be understood.

The qualitative evaluation of different crops from the point of view of nutritional importance may be carried out by different standards of excellence for different nutritional states of animal body. For example, in the case of young cattle, this nutritional value of a given food might be measured in terms of gain of body weight. In case of growing sheep or any large animals, we of (and in particular, reproductive) animals, deos, we can measure growth in fatness.
INTRODUCTION

The country as a whole is short of both roughages and concentrates to a very considerable extent. More and more lands are being brought under the plough for the production of feed crops for human consumption and thus day by day very little is left for the dumb creatures. Due to shortage of land and on account of improved new varieties of cereal grains which are apparently more economic farmers in the country are very critical about fodder cultivation. Authoritative opinion, therefore, inclines to the view that if lands are to be assigned for fodder cultivation, only the best quality and high yielding improved varieties should be considered. Therefore, in order to achieve this objective researches on the qualitative and quantitative aspects of fodder production should be intensified.

The qualitative evaluation of different feeds from the point of view of nutritional importance may be carried out by different standards of excellency for different physiological states of animal body. For example in the case of young animals the nutritive value of a given feed might be expressed in terms of gain of body weight, for each unit of the food given and in case of breeding stock we may judge the adequacy of food by successful reproduction sustained throughout the normal breeding life span of that particular animal.
A feeding trial with the species in question is the most useful method of obtaining accurate nutritive value. It is, however, impracticable to carry out feeding trials with all the different feeds and their combinations which are used in making the practical rations. These trials which measure only the final effect of the feed in terms of the specified function under study, tell us nothing as to why one feed has proved better than the other, unless the poorer one either was so unpalatable as to be little consumed or caused absolute harm. There are other measures of nutritive value which give us more definite information as to why a particular result is obtained.

The actual and final value of a feed is dependent upon the ultimate use which the body is able to make of it. After ingestion of food the first and the major factor limiting its usefulness is the efficiency of digestion process, since undigested nutrients do not get into the body proper.

Thus in order to assess the nutritive value of fodders the most important tool in the hands of animal nutritionists so far is the conventional technique of digestibility trials. The conduction of digestibility trials, which is usually practised in most of the animal nutrition laboratories of the country is quite time consuming, pains taking and costly in the sense of using specially designed equipments and require relatively larger amounts of feed and lot of expenditure and
labour. Further it is practicable to measure the overall digestibility of a sward and not that of its individual botanical components. Consequently the plant breeder can not rely upon this method as a criterion during the initial selection of new varieties of fodders.

In fulfilling the requirements of our livestock with regard to the selection of nutritious and high yielding forages, forage-breeders need a quick and easy method for measuring forage quality, which requires only small quantity of forage material. By such types of methods it is also possible to estimate the comparative nutritional value of a large number of genotypes within a reasonably short period of time, so that the promising varieties may be sorted out for wide spread use.

Numerous such methods have been developed for quickly assessing the forage quality. One of such methods is based on the estimation of chemical composition, which is utilized to assess the quality of the feed with the help of already worked out regression equations. Such relationships have been reported between the digestibility, the proportion of TDN or the percentage of metabolizable energy of a given specimen of herbage and its contents of crude fibre, lignin, crude protein and methoxyl groups.

Some workers have established relationship between digestibility and chemical composition, based on accumulated
published data, but the collection in this way of data pertaining to different lands, seasons and experiments; results in loss of accuracy of prediction.

But it has been observed that the deviation from the regression calculated in this way are too great to permit accurate prediction of digestibility from chemical composition and can not be considered very satisfactory for practical application. Attention has, therefore, been turned to laboratory in vitro methods. Tilley and Terry (1962) advised a quite satisfactory method for quicker assessment of nutritive value of fodders by in vitro technique, in which feeding stuffs are digested by preparations of micro-organisms or of enzymes, which are similar in function to those present in the digestive tract of the ruminants. Efforts have also been made to simulate the complex digestion process in the laboratory with similar equipments under controlled conditions.

In order to judge the accuracy of the results obtained by in vitro technique attempts in the past have been made to co-relate them with those obtained by conventional method (in vivo).

In such studies a sample of the herbage is incubated with a mixed culture of organisms taken from the rumen, the percentage of herbage dry matter, or organic matter, or cellulose, which is digested during the time of incubation, being
measured. Thus *in vitro* digestibility results have been correlated with those obtained *in vivo* for a number of plant constituents including cellulose, dry matter, or organic matter and TDN or energy values.

However, the available present evidences suggest that the prediction of *in vivo* herbage digestibility from *in vitro* procedures is considerably more precise than by any of the purely chemical methods so far suggested.

Therefore, if we have to follow a quicker and accurate method for screening out the various genotypes evolved by the plant breeders for production of improved and high yielding varieties of fodders it is essential that the *in vitro* digestibility technique has to be followed. However, it may be noticed that if the above method is carried out properly, the conditions of temperature, gaseous environment, pH and frequent mixing similar to that existing inside the rumen along with the use of costly equipments becomes inevitable. Efforts were, therefore, made to find out the possibility of using rumen (in situ) of a living animal, by making a permanent fistula in it, for estimating the digestibility and thus the nutritive value of a feed. The basic idea behind this was based on the knowledge that the rumen is the main site for the break down of plant material in the gastro-intestinal tract of ruminants. Thus this technique has been used for the determination of digestibility of the different feeds taken for study in the present investigations.
Further, it has also been claimed by many animal nutritionists that by putting small quantity of fodder in the ventral sac of rumen through a nylon bag for 48 hours or more with proper control of the ration offered, sample size, type of nylon cloth, size of bag, the nylon bag evaluation about the digestibility agree favourably with those obtained by conventional digestibility trial methods.

Hence, attempts have also been made in the present investigations to work out the digestibility of all the feeds under study by the nylon bag technique by putting the feed samples in the ventral sac of the rumen for 24, 48, 72 and 96 hours. For undertaking this study on an extensive scale leguminous, non-leguminous and perennial fodders have been taken.

In the end in order to assess the accuracy of the results and to evolve a quicker and reliable method for determination of digestibility of forages, the different results obtained in the above investigations have been compared with each other and with those obtained by conventional technique. Necessary statistical analysis of the results has also been carried out where ever required in order to finalize the results. Further, in order to facilitate the practical working and for easy determination of digestibility of forages regression equation has also been worked out, which may prove of considerable help for screening out the different varieties evolved by the plant breeder.
CHAPTER II

REVIEW OF LITERATURE
REVIEW OF LITERATURE

The review of literature on the evaluation of fodders by different methods has been divided into four sub-heads in order to facilitate the understanding of the different aspects which have been taken up for investigation in the present programme.

1. Old traditional methods
2. Biological methods
3. Chemical methods
4. Quicker methods
   A. In vitro technique
   B. Nylon bag technique

1. Old traditional methods for evaluation of fodders

The review about the old traditional methods has been collected from the available literature and presented in the following paragraphs.

In 1810, many years before the nature of the organic nutrients in food was known, THAER developed his "hay values" as measures of relative nutritive value. This hay value consisted of the sum of the ingredients extractable with water, alcohol, dilute acid and dilute alkali.
In 1859, GROUVE, after the recognition of protein, fat and carbohydrate as the essential organic nutrients made the analysis for these nutrients to formulate the first feeding standard for farm animals.

In 1864 Wolff devised a method for assessing the nutritive value of the fodders on the basis of digestible protein, digestible ether extract and digestible carbohydrates derived from results obtained in feeding trials. He also calculated the ratio of digestible protein to digestible carbohydrate and called the albuminoid ratio, because he recognized that proportion of protein in the ration affects its digestibility.

In 1890 Atwater proposed a feeding standard for assessing the nutritive value of fodders on the basis of available fuel values of feeds. These values have been obtained by the use of Rubner's factor (4.1 kcal per gm for protein; 9.3 kcal per gm for fat and 4.1 kcal per gm for carbohydrates), applied to digestible nutrients. Since the Rubner factors for protein contained a deduction for urine losses of 1.25 kcal per gm of protein, so Atwater's values took account of both faecal and urine losses.

In 1898, Henry published the first edition of his book 'Feeds and Feeding', which contained tables showing the average composition of American feeds, digestion coefficients
for protein, crude fibre, nitrogen-free extract and ether extract. Table also included nutritive ratios calculated as follows:

\[
\text{Digestible protein} = \text{digestible carbohydrate} + \text{digestible ether extract} \times 2.4
\]

In 1914 Haecker made an accurate standard for dairy cows, showing that the nutritive requirements varied not only with the quantity of milk produced but also with its quality, especially its fat content.

2. Biological methods for evaluation of fodders

The review of literature in this regard has been presented below.

Cook et al. (1952) in evaluating rations of animals on the winter range where climate is cold, found that ME provided a more accurate measure of nutritive value than TDN or digestible energy.

Kane et al. (1953) compared various digestion trial techniques with dairy cattle and reported that the chromium and pigment ratio methods compared favourably with the standard 10 day period technique, but the lignin ratio method gave significantly lower digestion coefficients.
Raymond et al. (1953) observed that the voluntary intake is a better measure of the value of a feed than its digestibility determined under the usual condition of restricted constant daily intake.

Swift (1957) determined the DE and TDN from 312 digestion experiments and revealed that one pound of TDN is equivalent of 2000 calories of DE in roughages. He further reported in the same year that the adoption of ME as the measure of forages, though theoretically ideal, is impracticable. ME though considered satisfactory, requires estimations of energy in urine as methane. DE is simply estimated and more straightforward than that of total digestible nutrient where forage alone was given showed that DE was highly correlated with TDN (+0.97) and with ME (0.98).

Barth et al. (1959) conducted a survey of digestibility trials in which cattle and sheep were given roughages alone and reported that the conversion factor from TDN to DE was related to the percentage digestible protein by the equation per gm:

\[
TDN = 4.343 + 0.0199 \times \text{(Percentage digestible protein)}.
\]

Reid et al. (1959) examined a range of N. American first harvest forages and suggested that the percentage of dry matter, D, could be predicted from the equation.

\[
D = 85.0 - 0.48x
\]

where \( x \) is the number of days to harvest after April 30th.
Crampton et al. (1960) reported that the digestibility results should be reported in terms of DE. The energy content of faeces though not of intestinal gases can be readily determined in bomb calorimeter, DE values can be easily obtained from TDN data on the basis that 1 gm TDN = 4.4 kcal.

Glover et al. (1960) had shown that estimates of the average amounts of TDN and gross digestible energy in ruminant feeds can be derived from the knowledge of CP and CF content alone, ignoring class of feed and species of ruminants from the formula:–

$$\text{TDN} = 78.9 + 0.17 \times \text{CP} - 0.74 \times \text{CF}$$

They also indicated that when the CP level lies below 5 per cent on a DM basis there is likely to be a sharp fall in the total digestibility of the feed.

Blaxter et al. (1961) concluded that the voluntary intake of forages by ruminants was related to the digestibility and rate of passage of foods. The consumption being low on fodder of poor digestibility. Low food intake appears to be the result of the slower rate of digestion of such foods.

Broumer et al. (1961) reported that the requirement for metabolizable energy increased from 11270 kcal per day on an average, on an early cut hay to 12700 kcal on a mature hay from calorimetric experiments in which the energy requirement of the 500 kg cow for maintenance was measured. They concluded
that the metabolizable energy of all feeds was not in fact equally efficiently used for maintenance purposes. The efficiency tending to diminish as the feeds given are less digestible.

Moir (1961) has shown that there is almost a 1:1 relationship between the per cent apparently digestible energy (Y) and per cent dry matter digestibility (X) over a range of X from 30 to 75. The regression equation that he obtained Y = 1.006X - 2.013 accounted for 99.6 per cent of the variation in Y. For a large number of feeds of different types (but apparently excluding oil seed, which have high heats of combustion); of digestibilities (X) from 30 to 83 and given at various levels to both cattle and sheep, he found that 96.2 per cent of the variation in digestible energy kcal/gm DM (Y) was from 1.24 to 3.65 kcal/gm.

Minson and Milford (1966) reported similar and precise relationship for subtropical herbage.

Moir (1961) also considered that the digestible energy of herbage containing up to 18 per cent of CP, could be related to the digestibility of their dry matter 'D' by the following relationship:

\[
\text{Digestible energy (kcal/gm dry matter)} = 0.3462D - 0.158.
\]

Blaxter (1962) reported that in the absence of bomb calorimeter determination of per cent apparently digestible
Energy (ADE) or of dry matter digestibility can be made for the evaluation of feeds. In this system rations are devised to predict animal performance on the basis of the amount and concentration of metabolizable energy (ME) in the feeds to be supplied. The determination of ME requires the measurements of methane production as well as of energy losses in faeces and urine but only small errors are noticed if ME is assumed to be 80 per cent of apparent digestible energy, reported by lift (1957), Armstrong (1964) and Blaxter (1965).

Cooper et al. (1962) reported considerable differences in the percentage digestibility of the dry matter (individual genotypes of rye grass and cooks foot) between the individual genotypes. Even within the single rye grass (S.23) a range of 63–84 per cent was recorded; the cooks foot digestibility ranged from 53 to 68 per cent. The lower digestibility levels of the cooks foot may have been due in part to inherent differences in digestibility and in part to the different dates of harvestings. They adopted Tilley and Terry in vitro method for estimating the digestibility.

Conard et al. (1963) reported that the regression of digestibility on stage of growth of the forages could be expressed by the formula \( Y = 71.4 - 0.286X \), where \( Y \) is percentage digestibility and \( X \) is the number of days after 30 April. When the regression of digestibility of DM on its intake was computed
and then combined with the foregoing formula obtained was -
Y = 25.4 - 0.212X, where Y is digestible dry matter and X the
number of days to cutting after 20 April.

Armstrong (1964), Graham (1964) both found that the
metabolizable energy content of herbage at the maintenance level
of feeding was about 81 per cent of the digestible energy.
Apparent digestibility (D) of the energy dry matter or organic
matter of a feed, times its voluntary intake (I) gives total
digestible intake (DI). Voluntary intake and apparent diges-
tibility are correlated.

Armstrong et al. (1964) reported that the metaboliz-
able energy and net energy for fattening and for maintenance
of the organic matter of sixteen dried grasses have been related
to their apparent digestibilities and to their chemical compo-
sitions. It was found that ME could be predicted with residual
standard deviations of ±1.6 per cent of the mean value from
determinations of apparent digestibility and with residual
standard deviation of ±4.7 per cent from determinations of the
lignin or CF per cent of the herbage. It is concluded that for
the routine estimation of the nutritive value of dried herbages
from their chemical composition, lignin determination or possibly
protein determination provide an accurate guide.

Raymond (1966) reported that, if during the course of
digestibility trials, one assumes a constant rate of loss of
methane, and in addition, determines the losses of energy in the corresponding urine, one should be able to obtain quite accurate estimates of the metabolizable energies of the rations employed and thus evaluate the foods more precisely than in terms of simple digestible energy.

Ovezero (1967) reported that digestible energy was positively related to digestibility of organic matter and negatively to lignin content. He observed that digestible energy was from 56 to 63 per cent of gross digestible energy.

**Chemical methods for evaluation of fodders**

During the execution of many experiments *in vivo* on the digestibility of herbage, associated chemical analyses of the fodder have been carried out, with the main objective to develop mathematical relationships between digestibility and chemical composition. A review of the literature in this connection is given in the following pages.

Morrison (1956) reveals that the relationship between the protein content of feed on the dry basis and the apparent digestibility of its protein is not a rectilinear one, but a hyperbolic one. For roughages, the relationship for feeds containing 5 per cent or more of protein on dry basis is represented by the following equation:
D = 42.64 (P-5)^0.215

in which D is the apparent digestibility of the protein and P is the protein content on the dry basis.

Axelson (1936) demonstrated a close negative correlation between the crude fibre content of the dry matter of feeds and the digestibility of the organic substances by cattle. The regression proved to be rectilinear and represented by the equation \( Y = 90.1 - 0.383X \), in which Y is the digestibility of organic substance of the feed and X is the crude fibre content on dry basis.

Jarl (1938) demonstrated a high negative correlation between digestibility and crude fibre content.

Schneider (1947) calculated relationships between digestibility and chemical composition, based on accumulated published data.

Forbes and Carrings (1948) studied the digestibility of pasture forages by steers and wethers with an application of the lignin ratio technique and reported that dry matter digestibility and TDN content of the forages were found to vary inversely with the lignin content of the forage.

Forbs (1950) presented a method of calculating DM digestibility of forages from the protein content and predicted protein digestibility. The average digestibilities calculated
in the various ways were, in general, similar, but the slopes of the regression of DM digestibility on lignin content are generally less when data are obtained by the 'protein digestibility' method.

Huni (1956) reported that digestibility of CP \((V_{KE})\) rose for CP contents between 5 and 35 per cent.

\[ V_{KE} = 57.0 + 0.94E, \quad r = 0.764 \]

Digestibility of fat depended on the fat content \((F)\) and the regression equation was

\[ V_{KE} = 12.4 + 25.2F - 2.64F^2 + 0.09F^3 \]

Digestibility of NFE satisfied the regression equation

\[ V_{KNFR} = 91.8 RF_E \quad r = 0.688 \]

Lofgreen and Meyer (1956) suggested that when the digestibility coefficient of organic matter is known the TDN can be determined by the formula

\[ F = M(0.01 + 0.000125E), \]

where \(M\) is the per cent organic matter in the dry matter of the feed and \(E\) is the ether extract as a percentage or organic matter.

Kamstra et al. (1958) determined the in vitro digestibilities of cellulose within various whole plant materials and correlated with the digestion of cellulose. They observed that maturity of plant material has an effect upon cellulose digestion — the young plant cellulose being the more digestible.
Richards et al. (1958) observed no consistent relation between digestibility of DM and lignin concentration in feed or faeces. CF was no better as an indicator of digestibility than methoxyl. The digestibility of DM from all forages gave correlation coefficients of -0.724 and -0.725 with concentration of methoxyl and -0.218 and 0.528 with concentration of CP in food and feed respectively.

Kivimae (1959) observed that timothy grass showed a more rapid decrease in digestibility as its content of CF increased than did red clover. Where Y denotes the percentage digestibility of the organic matter and X is the percentage of CF in the herbage, the regression equations for timothy grass and red clover were:

\[ Y \text{ (Timothy)} = 117.0 - 1.72 X \]
\[ Y \text{ (Red clover)} = 94.3 - 1.01 X \]

Richards et al. (1959) noted that as the ash content of the forage increased there was an increasingly large difference between apparent digestibility obtained by the faecal chromogens method and that from either the conventional method or the chromic oxide method.

Bickel (1960) related the SE in 100 kg organic matter to proximate composition by the formulae:

\[ SE \text{ (OM)} = 90.4210 - 1.2468 \text{ CF (OM)} \]
\[ SE \text{ (OM)} = 53.7450 - 1.2036 \text{ CF (OM)} + 0.4256 \text{ KCP}. \]
Where \( KCP \) = Coefficient of solubility of CP in pepsin and HCl. Standard deviation on an 86 per cent DM basis was \( \pm 1.9 \) by the simple regression and \( \pm 1.4 \) by the compound.

For DCP the formulae were:

\[
\begin{align*}
\text{DCP (DM)} &= -2.5271 + 0.8051 \text{ CP (OM)} \\
\text{DCP (OM)} &= -2.1247 + 1.0434 \text{ SCP}
\end{align*}
\]

where SCP = soluble crude protein. Standard deviation were \( \pm 0.36 \) and \( \pm 0.37 \) respectively.

Kivimae (1961) estimated the digestibility by difference in 18 trials and observed that digestibility of OM was inversely related to content of cellulose, lignin and methoxyl and directly to protein. Methoxyl group and lignin were most closely related to digestibility.

Zukov (1961) has observed a close relation between the digestibility of OM of feeds and the percentage solubility of their DM in chlorophenol reagent and reported that the total nutritive value can be calculated from DM content, fibre content and digestibility of organic matter.

Sullivan (1962) suggested that regressions for evaluation of forages from their proximate composition should be treated with caution.

Burdick and Sullivan (1963) reported that the case of solubilization/ or hydrolysis of the hemicellulose with dilute \( \text{H}_2\text{SO}_4 \) was positively correlated with the digestion coefficient.
of dry matter.

Stafijcuk (1964) presented formulae for calculation of nutritive value of silage and hays in terms of the new feed energy unit, 2500 kcal metabolizable energy, from their CF contents.

Arroyo et al. (1965) calculated a regression equation to predict digestible protein (Y) from crude protein (X) as percentages of DM. The equation was \( Y = 0.813X - 2.05 \). There was a highly significant correlation, \( r = +0.90 \) between the values.

Van Soest and Moore (1965) have found high correlations of the in vivo digestibility of the cell contents (neutral detergent solubles or NDS) of the cell wall (N.D. Fibre) and of the lignin with the in vitro data. Their formula for predicting the true digestibility of forage feed is:

\[
(1) \quad 0.98 \text{ NDS} + 147.3 \text{ NDF} - 78.9 \text{ (log lignin)}
\]

where NDS, NDF and lignin are expressed as percentages of a unit weight of feed. A general formula for predicting the in vivo apparent digestibility of cattle feed (as dry matter) recently proposed by them is:

\[
(2) \quad 0.98 \text{ NDS} + W (147.3 - 78.9 \text{ log lignin}) + 12.9 \text{ per cent}
\]

in which 12.9 per cent is the constant percentage of the weight of the feed due to metabolic faecal DM and the other factors are as in formula (1).
Duinum and Van-Soest (1963) estimated the digestibility of 106 samples by regression from cell contents and cell wall constituents estimated chemically and observed that values were not as closely related to digestibility in vivo by sheep as were values obtained by digestion with rumen fluid in vitro.

Tilley and Terry (1963) discussed the relationship between the amount extracted by acid pepsin or of water and the crude or true protein and soluble carbohydrate contents, especially in relation to herbage digestibility. It is suggested that these simple extracts may be more useful than conventional chemical techniques for assessments of comparative nutritive value of herbage.

Allinson and Osbourn (1970) reported that changes in digestibility of DM with maturity inversely related to lignin content of fodder and difference in digestibility between varieties was closely associated with lignin.

Smith et al. (1972) during their digestibility experiments observed that the rate of digestion of cell walls was more closely related to content of soluble DM than to lignin, ratio of lignin to cellulose or its log or indigestibility of cells wall in vitro for 72 hours.

Kirchgesner and Roth (1972) reported that the percentage of DCP (Y) could be found by the formula:-
$Y = -2.16 + 0.93X$, where $X = \text{CP in the DM}$. Starch equivalent in g/kg DM could be calculated by a multiple regression formula:

$$Y = 297.6 + 1.71X_1 + 26.0X_2 - 9.61X_3 + 6.78X_4$$

where $X_1 = \text{percentage CP}$, $X_2 = \text{crude fat}$, $X_3 = \text{CF}$ and $X_4 = \text{NFE}$.

Parra et al. (1972) reported a highly significant negative correlation between the indigestible lignin $X100$/acid detergent fibre and the digestibility of fibrous component, except for hemicellulose in leguminous plants.

Further relationships have been reported between the digestibility, the proportion of total digestible nutrients, or the percentage of metabolizable energy of a given specimen of herbage and its contents of CF, lignin, CP and methoxyl groups (Kimivae, 1959), of lignin and nitrogen (Soulskai and Patterson, 1961) of nitrogen alone (Mison and Kemp, 1961), of fibre fractions (Gaillard, 1962) of acid detergent fibre (Van Soest, 1963) or, finally, of lignin alone (Blaxter, 1964; Armstrong and co-workers, 1964).

The digestibilities and starch equivalents of some tropical grasses have been related to their CF contents by Dijkstra and Dirven (1962). French (1961) and Butterworth (1963) have shown that the relationships between digestibility and CF content for tropical forages differ markedly from those found for corresponding foods of temperate zones, so that data derived in temperate regions should not be applied to tropical conditions.
4. Quicker methods for evaluation of fodders

The review regarding quicker methods for evaluation of fodders has been done under the following two sub-heads:-

A. In vitro technique
B. Nylon bag technique

A. In vitro technique

Burroughs et al. (1950) reported that cellulose in the good quality roughages was digested efficiently without supplementation of minerals and autoclaved manure extract; conversely, the cellulose in the poor quality roughages failed to be digested efficiently without supplementation using an artificial rumen.

Burroughs et al. (1950) reported that dried distillers solubles, soyabean oil meal and linseed oil meal influence rumen microorganisms favourably in cellulose digestion, while meat scraps, fishmeal, oats showed little or no favourable influence using an artificial rumen.

Pigden and Bell (1955) used anthrone carbohydrate digestion in vitro and estimated TDN and DCP, which agreed closely with those obtained from conventional digestion trials with sheep.
Barnett (1957) reported that in vitro digestibility results of cellulose (Y) were reproducible and showed good agreement with values for digestibility of CF (X) obtained in trials with sheep. They were related by the equation:

\[ X = Y + 2.9 \]

Meiske et al. (1958) studied the effect of starvation and subsequent refeeding on activities of rumen micro-organisms in vitro and reported that the ability of rumen fluid to digest cellulose in vitro decreased greatly when the steer was starved for 3 days. The ability to digest cellulose in vitro was normal in 3 to 4 days after feeding was resumed following starvation. They further stated that no difference could be attributed to the type of hay the steers received before or after the starvation period.

Hershberger et al. (1953) observed high correlation \( r = 0.97 \) between digestibility coefficient of cellulose estimated in vitro and with wethers in vivo. In vitro digestibility of cellulose was found to be highly correlated with DE of the feed.

\[ r = 0.92 \]

Quicke et al. (1959) reported that digestibility coefficients of the cellulose in hulls and flakes were lower, 54 and 59 per cent respectively, than the values obtained in vitro in sheep.
Quicke et al. (1953) reported that the in vitro digestibility of cellulose of grasses by the rumen micro-organisms from a steer fed on alfalfa-hay agreed well with results obtained in digestion trials with sheep. The in vitro digestibility of cellulose from legumes was some times less and some times more than in vivo; no significant difference was observed between results obtained with grass hays.

Drori and Loosli (1953) performed digestibility trials with 3 intact steers and 3 with a rumen fistula and observed no significant difference in digestibility of the components of the ration between steers with and without fistula.

Clark and Mott (1960) reported a decrease in the correlation between in vivo and in vitro digestibilities with trials run in the fall with the same forage as in trials the previous spring. They suggested changes during storage as an explanation.

Donefer et al. (1960) reported that the values of 24 hour in vitro break down of cellulose in milled samples of 3 forages was highly correlated with digestibility of energy in vivo by sheep.

Leferve and Kamstra (1960) reported that in vitro digestion of cellulose by sheep and steers was similar and incubation period of 48 hours in vitro gave cellulose digestion coefficients similar to those obtained in the digestion trials.
Reid et al. (1960) reported that the relationship between in vitro and in vivo digestibility varied with the diet fed to the animal providing the inocula for in vitro digestion.

Dehority and Johnson (1961) reported that in vitro cellulose digestion increased with the time of ball-milling up to 72 hours, and this increase became larger with advancing maturity and lignification of the forages.

Boden and Church (1962) observed a positive correlation between the digestibility of DM in vitro and with sheep.

Doneffer et al. (1962) reported that chopped forages, 9 legumes 17 grasses and ground forages which had been studied in vivo with sheep were digested in vitro and nutritive value indices (IVI) estimated in vivo were studied in relation to digestibility during 12 hours in vitro. Nutritive value index (NVI) differed with the form in which the forage was given and regression equation for predicting the index (Y) from digestibility in vivo (X), were: \( Y = -3.5 + 1.23X \) and \( Y = 7.4 + 1.23X \) for chopped and ground forages respectively. The corresponding correlation between the digestibility in vitro were \( r = 0.91 \) and 0.87. Grinding increased the NVI by 10.9 units and the second equation could be expressed as \( Y = -3.5 + 1.23X + 10.9. \)

Baumgardt et al. (1962) reported that forage cellulose digestion in the artificial rumen was closely related to the
in vivo digestibility, being significantly correlated with TDN as well as digestion coefficients for DM, OM and energy.

Baumgarett et al. (1962) modified the usual artificial rumen procedure and reported that the per cent forage cellulose digested was significantly correlated with TDN, DDM, DE and the digestion coefficient of energy determined in conventional animal digestibility trials.

Johnson et al. (1962a) compared the digestion of cellulose in vitro in finely cut 5 grasses at different stages of maturity with digestibility in vivo of DM, cellulose and energy components obtained with sheep. They observed good correlation between the digestibility of DM, cellulose and energy components in vivo and cellulose fermentation in vitro after 12 to 48 hours incubation.

Johnson et al. (1962b) estimated the digestibility of DM of the herbage by cows and reported that digestibility in vitro of the cellulose in the two samples was similar when fermentation continued for more than 12 hr, at 12 hr values were about 7 per cent higher for the frozen samples. The correlation at this time with digestibility of DM in vivo was 0.359 while with the other samples the correlation was 0.711.

Arroyo-Aguilu et al. (1963) reported that nutritive values of cock foot (Pectypis glomerata) brome grass (Broomus energias) and timothy (Phleum pretense) were estimated with
cattle in vitro. Regression equation to predict biological total digestible nutrients calculated from digestion in vivo for 24 hr (TDN), \((Y_1)\) and digestible energy \((Y_2)\) from 24 hr digestion of cellulose in vitro \((X)\) were \(Y_1 = 12.2 + 1.19X\) and \(Y_2 = 75 + 0.5X\). Correlation coefficients between digestion in vivo and in vitro for 24 hr. were +0.98, +0.99 for TDN and digestible energy respectively.

Naga and EL-Shazly (1963) reported considerable divergencies between digestibilities determined in vitro and those measured in vivo and found particular different results for grasses and legumes.

Tilley and Terry (1963) described a two stage technique for the in vitro digestion of forage crops and observed a close correlation \((r = 0.97)\) between digestibilities measure in vivo and by this two stage in vitro technique for a wide range of forages.

Frederiksen (1964) reported that digestibility of finely ground clover and grass estimated in vitro agreed well with values estimated with cows.

Fried et al. (1964) reported that correlations between digestibilities in vivo and in vitro were highly significant with sheep.
Reid et al. (1964) showed a positive correlation between the apparent digestibilities in vivo and in vitro.

Tisserand and Zelter (1965) described an apparatus for estimation of digestibility of cellulose in vitro. They incubated the substrate for 24 hours at 39°C in 20 ml of each artificial saliva and rumen fluid from sheep and reported that the results are within 5 per cent of true values.

O'Shea and Wilson (1965) found significant correlation $r = 0.94$, with the regression equation $Y = 0.86X + 8.72$, where $Y$ and $X$ are percentage digestibility in vivo and in vitro respectively, between the dry matter digestibilities in vivo and in vitro.

Alexander and Mc Gown (1966) reported that the addition of N as ($\text{NH}_4)_2 \text{SO}_4$ increased the in vitro digestibility of organic matter in 21 out of 23 samples with crude protein content of 6.10 to 25.05 and in vivo digestibility of 52.2 to 80.5 per cent with 13 grasses and 25 hays the regression $Y = 0.97X + 5.05$ was derived where $Y$ is digestibility in vivo and $X$ is digestibility in vitro.

Chalupa and Lee (1966) reported that there was a difference between in vitro cellulose digestion between grasses up to 18 hours but incubation for 24 hours or more gave a consistent measure of total digestion.
Hi Kon Oh et al. (1966) studied samples of 24 legumes and 32 grass hays of which the digestibility of DM in vivo by cattle or sheep was known and reported that correlation between digestibility in vivo and in vitro were significant. The correlation within the species were better.

Mahammed (1966) reported that digestibility in vivo was predicted well from digestion of cellulose in vitro in 24 hours with cattle.

Troelsen et al. (1966) reported that in the 2 stage method of digestion in vitro with fermentation by rumen microorganisms followed by acid pepsin digestion the non-cellulose organic matter of lucerne was digestible by acid pepsin unlike of wheat straw. Cellulose of lucern was digested within 48 hr of incubation, digestibility of cellulose in wheat straw increased up to 96 hours.

Wilkins (1966) observed that the digestibility of cellulose and organic matter of grasses in vitro studied were closely correlated. \( r = + 0.95 \). There was a significant negative correlation between cellulose content and digestibility of organic matter for all materials \( r = -0.74 \) for grasses and \(-0.86\) for non-grasses.

Ademosum (1967) studied the digestibility of sudan grass and a hybrid sorghum and sudan grass with sheep and reported significant simple correlation between values estimated in vivo and in vitro.
Arroyo-Aguilu (1967) reported that the values in vivo cellulose digestibility were most closely related to digestibility in vitro after 36 hours.

Barth and Mohammed (1967) reported that the digestibility of cellulose in vitro for 24 hours with suspension of washed cell or whole rumen liquor was not highly correlated with digestibility of DM, or organic matter or contents of TDN or DE estimated in vivo than the whole rumen liquor. Correlation coefficient of digestibility after 24 hr in cell suspension was 0.73 with TDN was 0.83.

Den Braver and Eriksson (1967) reported best correlations of digestibility in vivo with the estimated digestibility in vitro.

Engles et al. (1967) found that the digestibility of some South African forages in vitro by the method of Tilley and Terry differed significantly when the same sample was digested in rumen liquor from sheep on lucerne hay or on oat hay. Further he found that the digestibility of organic matter in vivo was significantly correlated with digestibility in vitro.

Karn et al. (1967) studied the digestion of cellulose and rate of disappearance of DM in vitro and in vivo for 65 forages and reported highest correlation coefficient with digestibility of DM in vivo was 0.30.
Long (1967) reported that the digestibility values **in vivo** and **in vitro** of 5 Ugandan grasses, either green or hay, were closely related \( r = 0.87 \) by cattle. The regression \( Y = 0.871X + 16.8 \), where \( Y \) and \( X \) are percentage **in vivo** and **in vitro** digestibility was derived.

Troelsen (1967) reported that quantitative relations differed among species ranging from 0.72 to 1.05 percentage unit in digestibility **in vitro** for each unit change of digestibility **in vivo**.

Kumeno *et al.* (1968) and Dehory *et al.* (1968) studied **in vitro** and **in vivo** digestibility of forages and reported high correlations between measurements.

Park (1968) found that the **in vivo** digestibilities of dry matter in forages could be estimated from **in vitro** digestibilities by the regression equation \( Y = 0.606X - 11.92 \). There was no significance influence of inoculum from 3 sheep donors.

Troelsen *et al.* (1968) studied the digestibility **in vitro** was of hays after fermentation 24 hr, 48 hr, 96 hr and reported significant correlations between digestibilities **in vivo** and **in vitro** at each of these time. Correlation was highest for most hays at 48 or 96 hours.
Alexander (1969) reported a correlation coefficient of 0.89 with \textit{in vivo} and \textit{in vitro} digestibility.

Burzy and Paladines (1969) estimated \textit{in vitro} digestibility by the method of Tilley and Terry and found significantly correlated values \textit{in vivo} in sheep.

Troelsen and Bell (1969) during their \textit{in vitro} digestibility studies observed that fine grinding did not increase the digestibility of immature hay but with advancing maturity the fine substrate was progressively more digestible than coarse.

Den Braver (1969) reported that the digestibility of hays \textit{in vivo} (Y) was related to that \textit{in vitro} (X) by the equation \( Y = 8.3 + 0.313X \) with standard deviation 2.02 units.

Dijkstra (1969) estimated organic matter digestibility in 2 stage \textit{in vitro} of green feed, hay and silage of which digestibility by sheep was known and reported high correlation between values by the two methods and low standard deviation.

McLeod and Minson (1969) considered the method \textit{in vitro} is suitable for estimation of feeding value in relation to digestibility \textit{in vivo}.

McLeod and Minson (1969) observed that the digestibility \textit{in vitro} was affected by fineness of grinding size of sample, pH of original rumen fluid and amount of rumen fluid
inoculated. There were predicted difference of 3.5 per cent units between results in vivo and in vitro.

Cottyn et al. (1970) estimated the digestibility of 33 forages with wethers and in vitro and found a positive correlation between values of both methods for grass hay, pelleted roughages and mixture of roughages. The regression calculated was $Y = 0.69X + 19.52$ where $Y$ = digestibility of organic matter in vivo and $X$ = digestibility of organic matter in vitro.

Engles et al. (1970) reported high correlation between digestibility in vivo and in vitro with sheep and cattle.

Mellenberger et al. (1970) described a modified in vitro technique and the method was used for 7 grasses and 5 samples of lucern and reported that the values for digestibility of DM were closely correlated with values estimated in vivo.

Mc Cullough and Swart (1970) estimated the digestibility of pelleted diet and reported that disappearance of cellulose in vitro was similar for all forms of the feed and results compared well with those in vivo in sheep.

Chenost (1970) reported the close correlations between digestibility of 68 fresh forages in 8 hours or for 57 hays in 24 hours during in vitro digestion for prediction of feeding value of forages.
Guedas et al. (1970) estimated the digestibility \textit{in vivo} with sheep and \textit{in vitro} by the two stage method of Tilley and Terry in 4 hays and reported that for 2 of the hays the values of digestibility \textit{in vitro} were similar to those \textit{in vivo}, but for one the values \textit{in vitro} were lower and for one the same was higher.

Singh et al. (1970) reported that cellulose digestibility \textit{in vitro} was significantly related to digestibility of DM and energy \textit{in vivo} by cattle and to that of cellulose by buffaloes. In both animals nutritive value index were related to digestion of cellulose \textit{in vitro} 12 hr. The regression equation relating to NVI (Y) to digestion of cellulose was for buffalo. \( Y = 12.62 + 1.3X \) \((r = 0.92)\).

Troelsen (1970) estimated the digestible energy of 102 hays with sheep and \textit{in vitro} and found significant correlation.

Troelsen and Beacon (1970) observed that the OM digestibility \textit{in vitro} and DE \textit{in vivo} was similar.

Wilkins and Minson (1970) found that the digestibility of cellulose and organic matter \textit{in vitro} with each duration of incubation and with any degree of grinding were significantly correlated with digestibility estimated \textit{in vivo} with sheep.

Zark and Chomyszyn (1970) compared the digestibility
of dry matter of Meadow hay and found similar results i.e., digestibility of dry matter was 64.3 per cent in vivo and 64.5 per cent in vitro.

Brown and Radcliffe (1971) found that the digestibilities of dry matter and organic matter in vivo and in vitro were closely correlated but that of energy was not. A 48 hour rumen liquor digestion time gave the most accurate prediction of in vivo dry matter (r = 0.88) organic matter (r = 0.86).

Horii et al. (1971) reported a high correlation between values of in vitro and in vivo digestibilities of roughages.

Koolen et al. (1971) reported that average digestion coefficient of organic matter in vitro was often a little lower than the values in vivo but for ground and pelleted roughages the values in vitro was 3.8 units higher than that obtained in vivo.

Ludri et al. (1971) observed no significant differences between the rumen inocula of buffalo and zebu in their effect on DM and cellulose digestibilities of wheat straw or in the production of total volatile fatty acids using in vitro evaluation of forage nutritive value.

Piotrowski (1971) used the artificial liquor in the estimation of digestibility in vitro by the two stage method.
of Tilley and Terry. The substrates were ground leaves or stems of lucern. He observed that the values for digestibility of DM were less with artificial liquor than with true rumen liquor, generally by about 2.5 per cent units.

Meyer et al. (1971) compared the in vivo artificial rumen method of Fina et al. (1962) with the Taylor and Cason (1962), Tilley and Terry (1963) two stage and the van Soest and Wine (1966) digested neutral detergent fibre (NDF) methods of estimating forage digestibility. Dry matter disappearance (DMD) was determined after in vitro fermentation with fluid inoculum for the Wisconsin (Taylor and Cason, 1962) procedure and after a subsequent pepsin or neutral detergent treatment in the Tilley and Terry (1963) and van Soest and Wine (1966) digested NDF methods respectively. Forage DMD was determined by the Fina et al. (1962) procedure. Of the four methods, the two stage techniques (Tilley and Terry and NDF) were reported superior than the other methods.

Nehring (1971) reported that the digestibility value of organic constituents of green feeds agreed well with the values in vivo but feeds with high digestibility gave higher values and those with low digestibility lower values in vitro.

Abe et al. (1972) used the 2 step method for estimation of dry matter digestibility of roughage and after the pre-treatment with 5 per cent Na₂CO₃ total dry matter digestibility in vitro agreed well with values in vivo.
Joshi (1972) studied the digestibility of DM *in vivo* and *in vitro* technique in 32 forages and found highest correlation between *in vitro* digestibility and digestibility by sheep for hay, artificial dried grass and silage.

Love lace *et al.* (1972) observed a significant difference in dry matter digestibility of 7 lines of buffel grass with *in vivo* and *in vitro* technique with sheep.

McLeod (1972) estimated the *in vitro* digestibility and found that when the sample size was reduced from the normal 0.5 gm to 0.1 gm the residual standard deviation of the regression relating *in vitro* and *in vivo* digestibilities was increased from \( \pm 2.5 \) to \( \pm 3.4 \) percentage units. It was further reported that grinding of the samples more finely than through 1.0 mm screen did not improve accuracy.

Minson and McLeod (1972) introduced some modifications in the method of Tilley and Terry (1963). By comparing with samples of known digestibility *in vivo*, values of 6 grasses *in vivo* were calculated from values *in vitro* and reported a decline in digestibility by 0.2 per cent unit per day in summer growth and 0.1 unit in autumn regrowth.

Naidenov and Dimitrova (1972) estimated the digestibility of the Sorghum X Sudan grass hybrid Sordan by the *in vitro* method of Tilley and Terry (1963) and reported a better reproducibility of results than a *in vivo* method.
The same authors (1972) estimated the digestibility of DM of concentrates, dried pulp and hay of different cut by collection of faeces and by in vitro method of Tilley and Terry (1963). Values of the two methods were not significantly correlated. Values in vivo were 4.08 and 4.18 units higher than those in vitro for the second cut hays, with the first cut hay the method in vitro gave higher values by 0.64 and 1.30 units.

O'Hara and Sugihashi (1972) studied the patterns of gas production (GPR) in artificial rumen and their specificity by substrate and reported that coefficient of variation of GPR in ml/H from different fermentors with same substrate was small regardless of difference in incubation time, substrate and rumen juice used.

O'shea et al. (1972) estimated the digestibilities of DM, organic matter in vivo in 31 silages, with the digestibility in vitro and chemical constituents in the same silage. The best relation was content of organic matter digestible in vivo = 0.31 content of organic matter digestible in vitro + 15.5.

r = 0.83

Hacker and Minson (1972) grown six Setaria introductions at 3 sites differing in soil type and location and reported 6.6 per cent unit difference in in vitro DM digestibility between varieties. They observed higher digestibility in winter
B. Nylon bag technique

The review of literature regarding the use of nylon bag technique for the determination of digestibility of forages is given below.

Balch and Johnson (1950) reported that the rate of break down of the DM of hay suspended in the rumen in silk bags was faster in the ventral sack than in the dorsal sac of the rumen.

Erwin and Elliston (1953) placed the nylon (4"x 3") bags in the anterior dorsal sac of the rumen through a fistula and studied the digestibility of the feed stuffs kept in the bags and reported that the position of the bag on a chain or between chains within a steer did not influence digestibility. As the amount of feed was increased from 10 to 24 gm digestibility was linearly decreased 4 - 5.5 per cent. Fineness of grind of feed stuff had less effect on digestibility as time of incubation was increased.

Lusk et al. (1962) compared dry matter disappearance value obtained by nylon bag technique with those obtained from conventional trials of several roughages, found that the 72 hours dry matter disappearance value were more closely associated with those obtained in conventional digestion trials with any of the other time period studied.
Archibard et al. (1962) estimated the digestibility of alfalfa hay and canary grass from total collection of faeces and with dacron bags. The bag technique over estimated digestibility of crude protein, nitrogen-free extract, ether extract, energy and lignin and under estimated that of crude fibre.

Van Dyne (1962) observed with the bag technique that as the amount of test sample was increased, dry matter disappearance value decreases. Samples of test material from 1 gm to 2.850 kg have been recorded.

Van Keuren and Heinemann (1962) observed an increase in per cent DM digestibility of orchard grass and sudan grass with each 24 hour increase in length of time that the material remain suspended in the rumen in nylon bags. Generally, larger sample size resulted in lower digestibility for comparable periods of time in the rumen. The dietary regimen of the fistulated animal appeared to influence digestibility of the forage sample.

Hipson et al. (1963) evaluated the dacron bag technique as a method for measuring cellulose digestibility and rate of forage digestion. Cellulose digestibility results with dacron bags were unrelated to coefficients of digestibility by balance trial.

Ferrando et al. (1965) estimated the digestibility of hay from collection of excreta during 20 days and values
were compared with those estimated with nylon bags suspended in the rumen for 24 hours to 60 hours. Nylon bag’s method was considered unsatisfactory for estimation of digestibility of hay.

O’Donovan (1966) reported that the digestibility of dry matter estimated in vivo was similar to that estimated with nylon bag suspended for 48 hours in the rumen of a bullock with a fistula. He, further, told that there was no more increase in digestibility after 48 hours.

Omori et al. (1966) estimated the digestibility of dry matter of hays by suspending samples in the silk bags in the rumen of a goat and reported that the digestibility noted by this method was related to the digestibility estimated in vivo.

Van Dyne and Weir (1967) estimated the digestibility of dry matter and of cellulose in vivo and with nylon bags suspended for 48 hours in the rumen of bullock and wethers and reported no significant difference in digestibility of cellulose or of dry matter.

Lusk (1967) estimated the dry matter digestibility of different hays, silages and green feeds from suspension of nylon bags in the rumen and also by the conventional method. He reported that correlations between values by the two methods
varied widely, close correlations were obtained usually when similar feeds were given.

Klett (1967) compared the digestibility of dry matter estimated from total collection of excreta, with nylon bags suspended in the rumen for 24, 48 or 72 hr and in vitro with incubation for 12 or 24 hours and reported similar results with collection and nylon bags suspended for 48 hours and with incubation for 48 hours in vitro.

Rodriguez (1968) reported that neither fineness of mash nor of meal of lucern affected digestibility significantly in bullocks with bag technique.

Demarquilly et al. (1969) reported that digestion of lucern was almost complete with suspension of nylon bag in the rumen for 24 hours, but for grasses 48 hours was necessary. Further he stated that the digestibility of hays in vivo could be predicted from 48 hour digestion.

Monson et al. (1969) studied the dry matter digestibility of 55 grass samples with the help of nylon bags suspended in the rumen of bullock for 72 hours and by digestibility in vitro rumen fluid, then acid pepsin each for 48 hours. In all samples values estimated by each method were correlated, range of values for r was 0.56 to 0.9 and average for all values was 0.81.
Neathery (1969) reported a similar percentage disappearance of dry matter of lucerne and Coastal Bermuda grass with nylon bag and conventional digestibility trial in bullock. There was no increase in percentage disappearance after 72 hours on either diet.

Galvez Morros and Agar Soto (1971) studied the dry matter digestibility of lucerne by the method of Tilley and Terry (1963) and nylon bag suspended in the rumen of a cow with a fistula for periods ranging from 1 to 33 hours and then digested in vitro with pepsin and HCl to record the further loss of dry matter. After 36 hours the difference was small and did not diminish significantly after this. They recommended 48 hours time for the bag in the rumen.

Figroid et al. (1972) reported that disappearance of dry matter agreed favourably with nylon bag and total collection trial methods in bullock.

Mc Manus et al. (1972) reported that values obtained with terylene bag technique were of acceptable repeatability to that estimated from feed and faeces method.

Neathery (1972) compared the digestibility of Midland Bermuda grass (Cynodon dactylon) by faeces collection from wethers and by 48 and 72 hours nylon bag method with a bullock and gave a conclusion that after 72 hours suspension
of bag, value was slightly less than that of faecal collection method.

Plane et al. (1972) assessed the magnitude of the errors of estimation for DM digestion and reported that of the seeds of stylosanthes humilis contained in terylene bags in the bovine rumen 77 per cent of DM was digested after 48 hours, largely due to almost complete digestion of cell contents and of the pod of stylosanthes humilis 14 per cent was digested after 48 hours.
CHAPTER III

EXPERIMENTAL TECHNIQUES AND METHODS OF CHEMICAL ANALYSIS
EXPERIMENTAL TECHNIQUES AND METHODS OF CHEMICAL ANALYSIS

In the present chapter details about the experimental techniques and methods of chemical analysis have been outlined.

Collection of forage samples

Different forage samples were collected at random (including the stem as well as leaves) from the campus of Veterinary College, Mathura and nearby village Mukund Pura for study. Samples were chosen in three groups i.e., legumes, non-legumes and perennial grasses.

A representative portion weighing about 2 kg from each of the forage sample was taken and was cheffed, oven dried at 100°C, grounded and stored for future use.

Details about the selected forages are being given in Table-1.
Table 1 - Details of forages selected for study

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<thead>
<tr>
<th>Sl. No.</th>
<th>Name of the fodder</th>
<th>Botanical name</th>
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<th>Place of collection</th>
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<tbody>
<tr>
<td>1</td>
<td>Cowpea</td>
<td>Kigna catiang</td>
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<td>2</td>
<td>Guar</td>
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<td>Sanai</td>
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<td>Mukund Pura</td>
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<tr>
<td>4</td>
<td>Dhencha</td>
<td>Sesbania aculeata</td>
<td>30.8.74</td>
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LEGUMES

NON-LEGUMES

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<th>Place of collection</th>
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<tr>
<td>2</td>
<td>Jowar</td>
<td>Sorghum vulgareis</td>
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<tr>
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<td>Sawan</td>
<td>Elionurus hirsutus</td>
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<td>Maize</td>
<td>Zea mays</td>
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PERENNIALS

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<th>Place of collection</th>
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Table-1 contd.

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<tr>
<td></td>
<td></td>
<td>P. typoides</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Rumen fistulation

Two Murrah bull calves about one year of age were fistulated in the Department of Surgery in the college. The calves were starved for 24 hours before actual operation and then rumanotomy was performed by the help of clamps. About 10 days later the clamped fold of the rumen was sloughed off leaving the rumen opening in which ice cap was fitted. The ice cap was supported by a ring of rubber tubing joined by a 2" long and 1 cm thick glass rod. Later on the intact layer of the ice cap was cut in a 1½" diameter circle in the centre. The cut edges were reflected inside and upward on the plastic mouth of the ice cap and tied away by steel wire. Now a direct passage from outside to rumen was formed which could be closed by a plastic cap. The ice cap ready for insertion in the rumen, the fistulated animal and the ice cap fitted in rumen fistula have been shown in Figs. 1, 2 and 3 respectively.
Fig. 1: An ice cap improvised to work as an rumen cannula
Fig. 2: An experimental animal with rumen fistule

Fig. 3: An experimental animal having improvised cannula in the rumen fistule
Post operative treatment

As a preventive measure one ampule of A.T.S. (1500 I.U.) was given to each calf. Combiotic (Half gram) was injected intra-muscular to each of the animals for three successive days. Antiseptic dressing of the wound with tincture of iodine was done daily till healing was complete. After sloughing of the rumen fold the dressing was done with boric acid ointment. After the operation calves were maintained upon green pasture only.

Method of chemical analysis

The methods of chemical analysis of the various forages in present investigation were those recommended by A.O.A.C. (1970). These methods are briefly outlined below:

For analysis of dried and powdered materials, the samples were first spread over in the trays for about an hour to absorb moisture so that no difficulty may be felt in weighing. Three weights were taken simultaneously one after the other namely, for dry matter, crude protein and ashing.

Dry matter

For dry matter estimation a gm sample was accurately weighed in a dried and previously weighed moisture cup, which was kept over night in the electric oven at 100°C. Next morning
the cup was cooled in a desiccator and again weighed. The difference in the two weights gave the amount of dry matter in 2 gm sample from which percentage dry matter in sample was worked out.

**Crude protein**

The estimation of crude protein was done by estimating the nitrogen content by usual Kjeldahl's method and the value is multiplied by the factor 6.25 to get the crude protein content.

For nitrogen estimation by this method a suitable quantity of the powdered sample was digested with reagent quality sulphuric acid, in the presence of 5 to 10 gm of potassium sulphate and 1 gm of copper sulphate. The completely digested material was cooled and transferred quantitatively through repeated washings into a two litre distillation flask, the contents made alkaline with 50 per cent sodium hydroxide solution and distilled. The liberated ammonia was trapped in a known amount of 3N/7 standard sulphuric acid solution and the excess of acid was titrated back by N/7 NaOH. A blank determination of sulphuric acid used was also done simultaneously to estimate its nitrogen content. The value for the blank was deducted from the value of the sample. Thus the amount of nitrogen in the sample was calculated using the formula that one ml of N/7 standard sulphuric acid corresponds to 0.002 gm
nitrogen and finally the crude protein was calculated by multiplying it with 6.25.

**Ether extract**

The two gm sample taken in the moisture cup for dry matter estimation after drying was transferred quantitatively into a 18.5 cm. Whatman No.1 filter paper thimble. The dried material was extracted with analytically pure petroleum ether having a boiling point of 40° - 60° C in a Soxhlet’s ether extractor for 8 to 10 hours with a speed of about 20 extraction per hour. The excess of ether was recovered and the extract in previously weighed oil flask dried in the hot air oven at 100°C for about half an hour, cooled in desiccator and then weighed. The difference in weights gave the amount of ether extract in 2 gm and there after the percentage was calculated.

**Crude fibre**

The residue of the ether extraction was transferred to a 800 ml spoutless beaker and digested for 30 minutes with 200 ml of 1.25 per cent sulphuric acid. Actually 25 c.c. of 10 per cent sulphuric acid was taken and diluted to 200 ml, which gave the strength of sulphuric acid 1.25 per cent. The material was then filtered and washed with neutral water till acid free on filter linen attached to a suction pump. The washed residue was again digested for 30 minutes in the same
beaker with 200 ml 1.25 per cent sodium hydroxide solution (25 ml of 10 per cent NaOH diluted to 200 ml). The contents were filtered and thoroughly washed with neutral water till free from alkali. The residue was quantitatively transferred to a small silica basin and dried in an electric hot air oven at 100°C over night. It was then weighed and ashed. From the loss of weight on ignition crude fibre percentage was calculated.

**Ash**

In a clean dried silica basin exactly 10 gm of the powdered sample was weighed and ignited to a temperature below 600°C by adjusting the burner so as to avoid the volatilization of the alkali metals and phosphorus. The greyish ash left over was cooled in a desiccator and weighed. The process of heating and cooling was done till a constant weight was obtained. From the difference in the initial and final weights of the silica basin the ash percentage was calculated.

**Nitrogen-free extract**

The sum of the percentages of crude protein, ether extract, crude fibre and ash is deducted from 100 to get the percentage of nitrogen free extract.

**Preparation of nylon bags**

A circular piece of nylon cloth (15 cm diameter) having 180 threads per cm was used for the preparation of the
nylon bag. It was tested for permeability of the powdered feed sample and it was observed that the powdered feed as such could not pass outside through the cloth even after keeping the bag for sufficiently long duration in the water. However, a large number of rumen protozoa were seen under microscope in the rumen fluid after it passed through this nylon cloth.

Twentyfour nylon bags were prepared having purse string nylon line which were sewn in a circle all round near the edge. Care was taken in sewing the bags to assure a smooth interior with no folds or pockets and no exposed edges to fray. A fishing nylon cord was used to close the bag (Van Keuren and Heinemann, 1962).

Method of use of the nylon bag

Two grams of powdered sample of test material was placed in the centre of the nylon bag and it was closed by pulling the nylon cord (Mc Manus et al., 1972). Bags were tied securely with nylon cord to avoid the chances of spilling over the material. An iron chain having five rings to which 9 to 12 nylon bags containing the sample could be attached was used to suspend the bags in the rumen of fistulated animal. In this regard it may be mentioned that it has already been reported by Erwin and Ellibson (1959) that the position of bag on a chain with in an animal did not influence digestibility.
Three nylon bags were tied with each ring of the iron chain and the last ring was connected with a nylon cord to the exterior of the cap of cannula. Bags were allowed to suspend in the rumen for 24, 48, 72 and 96 hours.

One end (distal) of the iron chain was inserted through the cannula, the cord attached to the proximal end was then held tightly with the left hand while the remainder of the chain was pushed through with the right hand and with the help of a smooth wooden rod. The purpose of using the rod was to break open the thick layer of solid forage mass floating over the rumen fluid. The iron chain with nylon bags was pressed firmly so as to reach the ventral sac of the rumen; otherwise dry matter disappearance may be variable, because the rumen liquor may not come in contact with the nylon bags (Balch and Johnson, 1950).

The nylon bags remained in the rumen for a predetermined length of time. These were removed from the rumen by pulling one end of the iron chain connected to the cannula plug with a short length of nylon cord. Immediately upon removal the bags were cleaned of adhering ingesta by dipping in water and then soaked in 75 per cent ethyl alcohol to stop fermentation. The bags from each animal were washed with constant agitation under running tap water for 3 minutes according to McManus et al. (1972). The bags with identifying marks were dried for 48 hours at 65°C. The material in the bags was
crushed by hand to facilitate drying as suggested by Keuren and Heinemann (1962) during the drying period. The bags were weighed and dry matter digestibility was determined by weight difference. A typical nylon bag containing sample has been shown in Fig. 4. The pattern of fixing nylon bags to the iron chain for insertion in the rumen and the procedure of putting in and taking out from rumen (through rumen cannula) of these bags alongwith iron chain have been depicted photographically vide Figs. 5 and 6 respectively.

**In vitro** technique

For the determination of digestibility of forages by **in vitro** technique the following four methods have been used by the different workers from time to time in the past. The name of these methods are given below:

(1) Two stage dry matter disappearance method of Tilley and Terry (1962).


(3) One stage **in vitro** fermentation method described by Baumgardt, Taylor and Cason (1962) and for convenience referred to as Wisconsin method; and

(4) **In vitro** artificial rumen method of Fina et al. (1962).
Fig. 4: A typical nylon bag containing sample for suspension in the rumen along with iron chain

Fig. 5: The pattern of fixing various nylon bags to the iron chain
Fig. 6: Procedure of taking out of the nylon bags from the rumen
Out of the above four methods the two stage technique (Tilley and Terry, 1963) has been followed in the present investigations as it has gained widespread recognition (Meyer et al., 1971). The details of this method are given below.

**Two stage dry matter disappearance method of Tilley and Terry**

A method for the in vitro determination of the dry matter digestibility of small (0.5 g) samples of dry forages as described which has been followed in these experiments. It involves the incubation of the test feed with rumen liquor in controlled environment followed by acid pepsin treatment. The details are given below:

**Principle of method**

The method has two main stages in the first a small sample of dried forage is digested anaerobically with rumen microorganisms at 38°C in the dark. It has been found that the process of fibre digestion is complete by the end of 48 hours, the conversion of herbage protein into soluble 'digested' products is not. A second stage of pepsin digestion has been introduced to remove this indigested protein, Tilley, Deriaz and Terry (1960).

**Details of method**

A representative sample of each forage was dried for hours at 100°C and ground to pass the 0.8 mm laboratory grinding mill.
Rumen liquor was removed through a permanent fistula of the Murrah bull calf maintained on a diet of Bholai leaves and pasture. For taking out the strained rumen liquor, a plastic tube was taken and so many perforations were made at one end so that the liquor may pass in the tube. A terylene cloth was sewn in circular form in order to wrap the plastic tube. One end of the plastic tube was closed with the help of rubber cork and a rubber tube of 3 mm inner diameter was inserted in the plastic tube. Now the wrapped plastic tube was inserted in the rumen through the cannula; strained liquor was taken out with the help of syringe as shown in Fig. 7.

The buffer solution was made up according to the formula for "Synthetic saliva" of Mc Dougall (1948) adding the calcium chloride last. The solution was thoroughly saturated with CO₂ at 28°C until it became clear. pH was adjusted at 6.7 to 6.9 by passing CO₂ through the solution, then kept in refrigerator for future use.

**Composition of Mc Dougall's 'Synthetic saliva'**

*(made with distilled water in gm per litre)*

- Sodium bicarbonate (NaHCO₃) ... ... ... 9.8
- Disodium hydrogen phosphate (Na₂HPO₄·2H₂O) ... 4.626
- Potassium chloride (KCl) ... ... ... 0.570
- Sodium chloride (NaCl) ... ... ... 0.470
- Calcium chloride (CaCl₂) ... ... ... 0.040
- Magnesium sulphate (MgSO₄·7H₂O) ... ... 0.120
Fig. 7: Rumen liquor collecting assembly
CO₂ is bubbled vigorously into this solution for about 10 minutes or until the pH is 6.6 to 7.0.

Pepsin solution was made up by dissolving 2.0 gm of pepsin (1200 E/gm made in Germany) in 850 ml demineralised water (double distilled water). 100 ml of normal hydrochloric acid (N HCl) was added and the solution was made up to one litre with double glass distilled water.

First stage rumen liquor digestion

0.5 gm sample of oven dried forage was weighed into 3 glass centrifuge tubes having 100 ml capacity. This capacity allowed space for the formation of foam and prevented losses during shaking. The tubes were then stored at 38°C until required.

To each tube 40 ml of the buffer solution were added, followed by 10 ml of the strained rumen liquor. The mixture was stirred, gassed with CO₂ for three minutes and the tube was then sealed with a rubber cork fitted with a Bunsen gas release valve. The 4 mm slit in the rubber tube on the valve was cut with a sharp knife, the slit normally remained closed, opening only to release gas from inside the tube. After sealing, the tubes, as shown in Fig. 8, were incubated at 38°C in the dark for 48 hours, being shaken slowly 2 or 4 times a day by hand.
Fig. 8: Incubation tube sealed with a cork and fitted with a Bunsen gas release valve
Second stage pepsin digestion

At the end of the first incubation period, bacterial activity was checked by the addition of 1 ml of 5 per cent Hg cl₂ (Mercuric chloride). 2 ml of N Na₂ CO₃ were also added to improve sedimentation.

The tubes were centrifuged immediately for 15 minutes at 2700 R.P.M. After discarding the supernatant, 50 ml of freshly-made pepsin solution were added to the residue in each tube. The tubes were then incubated at 38°C for 48 hours with occasional shaking. Anaerobic conditions were not necessary during this stage. At the end of incubation, the tubes were centrifuged for 15 minutes at 2700 R.P.M. and the supernatants were discarded and the insoluble residues washed with double distilled water and again centrifuged for 15 minutes. Tubes containing the residues were dried at 100°C to constant weight. The dry weight of residue was calculated. From this was subtracted the weight of residue found in the 'blank' tubes (which represented undigested food particles and micro-organisms derived from the rumen liquor) and thus the weight of undigested residue from each 0.5 gm of forage was obtained. From this residual sample digestibility was calculated as the weight of digested material in each 100 gm of forage dry matter.
CHAPTER IV

PLAN OF EXPERIMENT
PLAN OF EXPERIMENT

Two young Murrah bull calves were selected and procured from the District Dairy Demonstration Farm, Mathura for the present investigations. The particulars of these animals have been given in Table 2.

Table 2 - Particulars of experimental animals at the start of the experiment

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Brand No.</th>
<th>Age (Years</th>
<th>Months</th>
<th>Body weight in kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>15</td>
<td>10</td>
<td></td>
<td>75</td>
</tr>
<tr>
<td>2.</td>
<td>7</td>
<td>1</td>
<td></td>
<td>118</td>
</tr>
</tbody>
</table>

After recording the body weights of these animals they were sent out for grazing to natural pasture in the vicinity of the college.

The calves were castrated by Burdezo castrator on 22nd August, 1974.

The faecal samples of both the animals were subjected to intensive parasitological examination. Every precaution was taken to keep the calves free from any kind of parasitic infestation.
Housing and management

During the entire experimental period, the animals were kept under strict hygienic conditions in the shed having cemented floor. A close and continuous watch was kept for their proper health.

Feeding schedule

The calves were continuously kept on ad lib. feeding of Bhimal leaves (Gremia oppositifolia Roxb.) and pasture grazing during the experimental period. The chemical composition of these two fodders as determined by the author is given below in Table-3.

Table 3 - Chemical composition of fodders on DM basis

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Name of the fodder</th>
<th>C.P. (%)</th>
<th>E.E. (%)</th>
<th>Ash (%)</th>
<th>C.F. (%)</th>
<th>N.F.E. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.</td>
<td>Pasture (Mixed grasses)</td>
<td>9.51</td>
<td>1.02</td>
<td>10.22</td>
<td>32.19</td>
<td>47.06</td>
</tr>
</tbody>
</table>

Data in the table indicates that the fodders seem to provide a good maintenance ration when consumed in sufficient amounts.

The body weights of calves were recorded fortnightly throughout the course of these investigations which is shown
in the appendix at serial No.1. The record of body weights of these calves clearly indicate that these fodders were able to maintain the animal properly.

Both animals were entirely kept on the above feeds throughout the experimental period so as to avoid any unnecessary variable factor as was recommended by many workers (Baurroughs et al., 1950; Reid et al., 1960; McCullough and Swart, 1970; Nehring, 1971; Van Kauren and Heinemann, 1962).

**Selection of the fodders for the present study**

It was planned to evaluate all the fodders which were available in the college campus and in the near about villages. Thus a total of fourteen green fodders were taken for determination of digestibility by nylon bag and *in vivo* techniques (Tilley and Terry, 1963) and their comparison with conventional values. The particulars about all these fodders have already been mentioned earlier in Chapter III. During the selection of these fodders special care was taken to see that they represent legumes, non-legumes and the different types of perennial fodders commonly used by the animal husbandry men for feeding to their farm animals.

The samples of these fodders were analysed for their chemical composition and dry matter digestibility by using two different techniques namely nylon bag and two stage *in vitro* techniques. Though the recommended time interval for nylon bag
technique is forty eight hours (Demarquilly and Chenost, 1969; Galvez and Agar, 1971; Prasad, 1974) but in order to make a careful study about the digestibility pattern of the different feeds four different time intervals were followed during the present investigations (Neathery, 1963). This was done particularly to follow the extent of digestion of complex plant material in rumen. It is established that the cell walls of plants contain large amounts of lignocellulose deposited which interferes with the digestion of cell contents. Therefore, it may be presumed that if the disintegration of crude fibre is completed, most of the cell contents will be digested. The break down of fibre is likely to increase if it is exposed for microbial action in the rumen for a longer period. With this view in mind the duration of keeping the feed samples in rumen was not limited to 48 hours but it lasted upto 96 hours in four successive stages.

No matter how complete digestion is achieved by improved techniques, the results of conventional digestibility trials are the ideal standards, because they represent the normal behaviour of livestock towards their ration (Archibard et al., 1962). To get such standard values no digestibility trials were conducted by the author but the results of such trials, which have already been conducted in this country and most of them in the same institution have been taken from the available literature. While selecting such results comparable chemical composition and stage of maturity of forage plants was specially kept in mind.
The main idea behind all these investigations was to explore the possibility of assessing the nutritive value of fodders available in quantities insufficient for conducting digestibility trial particularly for quickly screening out the various genotypes which are being evolved by the plant breeders during the course of their investigations as it saves lot of time and expenditure on labour, management and technical skill.
CHAPTER - V

RESULTS AND DISCUSSION
RESULTS AND DISCUSSION

The results of the research work carried out during the course of these investigations have been discussed under the following heads:

A. Chemical composition and stage of maturity of forage plants.

B. Dry matter digestibilities of 14 fodders as obtained with different techniques.

C. Comparison of 72 hr (Nylon bag) D.M. digestibilities with the values obtained by 24 hr, 48 hr and 36 hr (Nylon bag) and in vitro techniques.

D. General discussion.

E. Relationship between chemical constituents of forages and their D.M. digestibilities.

A. Chemical composition and stage of maturity of forage plants

The proximate analysis of the fourteen selected fodder samples has been done and the results are given in Table-4 on dry matter basis along with the stage of maturity and other specific point, if any about them.
<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Name of the fodder</th>
<th>Botanical name</th>
<th>G.P. %</th>
<th>E.E. %</th>
<th>Ash %</th>
<th>G.F. %</th>
<th>N.F.E. %</th>
<th>Stage of growth</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Cow pea</td>
<td>Vigna catjang</td>
<td>18.50</td>
<td>2.67</td>
<td>10.59</td>
<td>25.02</td>
<td>43.22</td>
<td>Preflowering</td>
</tr>
<tr>
<td>2.</td>
<td>Guar</td>
<td>Cyanopsis pascalcides</td>
<td>16.30</td>
<td>1.91</td>
<td>10.06</td>
<td>29.01</td>
<td>42.72</td>
<td>Flowering with few pods</td>
</tr>
<tr>
<td>3.</td>
<td>Sesai</td>
<td>Crotalaria juncea</td>
<td>17.18</td>
<td>2.61</td>
<td>9.51</td>
<td>33.19</td>
<td>37.51</td>
<td>Preflowering</td>
</tr>
<tr>
<td>4.</td>
<td>Dhencha</td>
<td>Sesbania aculeata</td>
<td>20.16</td>
<td>3.23</td>
<td>11.52</td>
<td>28.21</td>
<td>36.88</td>
<td>---do---</td>
</tr>
<tr>
<td>5.</td>
<td>Gahabati</td>
<td>Ipomoea pestidrigida</td>
<td>22.00</td>
<td>2.75</td>
<td>13.90</td>
<td>19.97</td>
<td>41.38</td>
<td>---do---</td>
</tr>
<tr>
<td>6.</td>
<td>Jowar</td>
<td>Sorghum vulgaria</td>
<td>6.30</td>
<td>2.12</td>
<td>10.03</td>
<td>35.66</td>
<td>45.99</td>
<td>---do---</td>
</tr>
<tr>
<td>7.</td>
<td>Sawan</td>
<td>Eichnurus hirsutus</td>
<td>10.40</td>
<td>2.27</td>
<td>17.23</td>
<td>29.20</td>
<td>46.90</td>
<td>Milk</td>
</tr>
<tr>
<td>8.</td>
<td>Maize</td>
<td>Zea mays</td>
<td>7.56</td>
<td>1.86</td>
<td>7.72</td>
<td>21.68</td>
<td>61.18</td>
<td>Small cobs</td>
</tr>
<tr>
<td>9.</td>
<td>Blue Panic</td>
<td>Panicum antidotale</td>
<td>8.90</td>
<td>1.53</td>
<td>8.50</td>
<td>36.51</td>
<td>44.56</td>
<td>Flowering</td>
</tr>
<tr>
<td>10.</td>
<td>Madhu vine</td>
<td>Fueraria thumberghana</td>
<td>14.82</td>
<td>2.29</td>
<td>11.04</td>
<td>32.15</td>
<td>39.90</td>
<td>Preflowering</td>
</tr>
<tr>
<td>11.</td>
<td>Buffel</td>
<td>Cenchrus ciliaris</td>
<td>8.60</td>
<td>1.90</td>
<td>11.20</td>
<td>32.69</td>
<td>45.61</td>
<td>Flowering</td>
</tr>
<tr>
<td>12.</td>
<td>Doob</td>
<td>Gynoden dactylon</td>
<td>12.80</td>
<td>2.04</td>
<td>10.36</td>
<td>26.90</td>
<td>47.90</td>
<td>Preflowering</td>
</tr>
<tr>
<td>13.</td>
<td>Setaria</td>
<td>Setaria sphacelata</td>
<td>12.0</td>
<td>3.72</td>
<td>10.50</td>
<td>24.67</td>
<td>48.91</td>
<td>19'-24' height</td>
</tr>
<tr>
<td>14.</td>
<td>P.G. Napier</td>
<td>Pennisetum perpereus X P. typoides</td>
<td>10.30</td>
<td>3.29</td>
<td>15.20</td>
<td>24.97</td>
<td>46.24</td>
<td>15'-20' height</td>
</tr>
</tbody>
</table>
In order to compare the results of nylon bag and *in vitro* digestibility with those obtained by conventional method the results of the proximate analysis of these fodders as available in the literature and as worked out by the animal nutrition workers in the past while conducting the digestibility trials by the conventional method has been presented in Table-5. The results for chemical composition and the stage of maturity of the fodder plants at which the digestion trials were conducted have been collected from the available literature for all these 14 fodders. During the collection of all these data efforts were made to adopt the results of these digestibility trials, which resembled to our investigations as far as the chemical composition of the fodders was concerned.

However, no dry matter digestibility could be obtained from the literature for one fodder i.e., Blue Panic which was selected for the present investigations.

From the perusal of the above two tables it is clear that the chemical composition of most of the fodders is comparable particularly as far as the contents of CP and CF are concerned; except in case of Kudzu vine, Dhencha and P.G. Napiar, where CF is slightly more in the samples used in the present study. On the other hand Ghiabati contained
<table>
<thead>
<tr>
<th>No.</th>
<th>Name of the fodder</th>
<th>Botanical name</th>
<th>C.F.</th>
<th>R.E.</th>
<th>Ash</th>
<th>C.F.</th>
<th>N.F.E.</th>
<th>Stage of growth</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cowpea</td>
<td>Vigna catjang</td>
<td>18.18</td>
<td>1.40</td>
<td>10.85</td>
<td>24.56</td>
<td>45.02</td>
<td>Green</td>
<td>Manohar (1965)</td>
</tr>
<tr>
<td>2</td>
<td>Guar</td>
<td>Cynacopsis porsuloides</td>
<td>16.75</td>
<td>1.86</td>
<td>9.45</td>
<td>31.72</td>
<td>40.22</td>
<td>Pod attained edible stage</td>
<td>P.F.R.D. (1972-73)</td>
</tr>
<tr>
<td>4</td>
<td>Pancha</td>
<td>Sesbania aculeata</td>
<td>27.50</td>
<td>3.80</td>
<td>9.70</td>
<td>16.00</td>
<td>43.00</td>
<td>Green pref.</td>
<td>P.F.R.D. (1968-69)</td>
</tr>
<tr>
<td>5</td>
<td>Chivat</td>
<td>Ipomoea pestigridis</td>
<td>12.27</td>
<td>3.00</td>
<td>10.29</td>
<td>26.23</td>
<td>48.21</td>
<td>Sowing mature &amp; immature seeds.</td>
<td>Sainaul (1958)</td>
</tr>
<tr>
<td>6</td>
<td>Jowar</td>
<td>Sorghum vulgaris</td>
<td>6.64</td>
<td>2.23</td>
<td>10.64</td>
<td>31.31</td>
<td>49.10</td>
<td>Preflowering</td>
<td>Sen (1964)</td>
</tr>
<tr>
<td>7</td>
<td>Peshan</td>
<td>Kicnarius hirsatus</td>
<td>3.06</td>
<td>3.98</td>
<td>18.24</td>
<td>26.05</td>
<td>45.66</td>
<td>Flowering</td>
<td>P.F.R.D. (1965-66)</td>
</tr>
<tr>
<td>8</td>
<td>Maize</td>
<td>Zea mays</td>
<td>7.25</td>
<td>1.94</td>
<td>6.42</td>
<td>19.52</td>
<td>65.47</td>
<td>Cob stage</td>
<td>Kalashratak et al. (1972)</td>
</tr>
<tr>
<td>9</td>
<td>Blue Panic</td>
<td>Panicum antidotale</td>
<td>10.42</td>
<td>2.74</td>
<td>12.23</td>
<td>33.54</td>
<td>40.94</td>
<td>II cut green</td>
<td>Sen (1964)</td>
</tr>
<tr>
<td>10</td>
<td>Kudzu vine</td>
<td>Pueraria thambegiana</td>
<td>17.60</td>
<td>0.55</td>
<td>34.10</td>
<td>28.40</td>
<td>24.25</td>
<td>Preflowering</td>
<td>Kehar (1946)</td>
</tr>
<tr>
<td>11</td>
<td>Buffel</td>
<td>Cenochus ciliaris</td>
<td>6.68</td>
<td>2.02</td>
<td>15.25</td>
<td>33.19</td>
<td>42.96</td>
<td>Preflowering</td>
<td>Joshi &amp; Ludri (1967)</td>
</tr>
<tr>
<td>12</td>
<td>Deob</td>
<td>Cynodon dactylon</td>
<td>11.63</td>
<td>1.64</td>
<td>13.03</td>
<td>30.21</td>
<td>43.49</td>
<td>Green</td>
<td>Ogumbuyade (1961)</td>
</tr>
<tr>
<td>13</td>
<td>Setaria</td>
<td>Setaria aphaseata</td>
<td>10.50</td>
<td>2.14</td>
<td>12.35</td>
<td>26.35</td>
<td>48.66</td>
<td>3' height</td>
<td>Sharma et al. (1979)</td>
</tr>
</tbody>
</table>
a higher percentage of protein and Dhencha contained less protein than the collected data from the literature.

B. **Dry matter digestibilities of 14 fodders as obtained with different techniques**

As the data of the chemical composition obtained from the actual chemical analysis and that obtained from the literature were comparable to a great extent, it was expected that the digestibilities of these fodders should also be quite comparable. With these expectations in view the results of digestibility trials (which have been conducted by the usual conventional method) as available from the literature have been taken as the standard and have been compared with the results of nylon bag and *in vitro* digestibilities determined during the present investigations. All this data has been presented in Table-6 for giving a clear picture about the methods used and their comparison.

From a careful perusal of the Table-6, it may be observed that it is very necessary at this stage to test the deviation of each of these figures from the standard figure of digestibility i.e., digestibility determined by conventional method. Therefore, such comparison has been given in Table-7.
Table 6 - Dry matter digestibility coefficients of fodders by various techniques

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Name of the fodder</th>
<th>Digestibility coefficient by nylon bag technique 24 hours</th>
<th>Digestibility coefficient by nylon bag technique 48 hours</th>
<th>Digestibility coefficient by nylon bag technique 72 hours</th>
<th>Digestibility coefficient by nylon bag technique 96 hours</th>
<th>Digestibility coefficient by in vitro technique</th>
<th>Digestibility coefficient by conventional technique</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Cow pea</td>
<td>41.99 ± 0.12</td>
<td>66.20 ± 0.10</td>
<td>69.57 ± 0.07</td>
<td>71.85 ± 0.11</td>
<td>61.26 ± 0.11</td>
<td>63.44</td>
</tr>
<tr>
<td>2.</td>
<td>Guar</td>
<td>58.72 ± 0.12</td>
<td>69.12 ± 0.05</td>
<td>70.18 ± 0.35</td>
<td>70.99 ± 0.19</td>
<td>51.06 ± 0.11</td>
<td>58.40</td>
</tr>
<tr>
<td>3.</td>
<td>Senai</td>
<td>40.80 ± 0.13</td>
<td>52.66 ± 0.05</td>
<td>57.53 ± 0.04</td>
<td>61.19 ± 0.04</td>
<td>56.62 ± 0.26</td>
<td>56.89</td>
</tr>
<tr>
<td>4.</td>
<td>Dhuncha</td>
<td>42.27 ± 0.06</td>
<td>48.65 ± 0.31</td>
<td>53.98 ± 0.04</td>
<td>59.62 ± 0.05</td>
<td>48.59 ± 0.11</td>
<td>62.10</td>
</tr>
<tr>
<td>5.</td>
<td>Ghinabati</td>
<td>46.00 ± 0.47</td>
<td>57.96 ± 0.48</td>
<td>62.08 ± 0.23</td>
<td>66.37 ± 0.05</td>
<td>59.24 ± 0.19</td>
<td>66.80</td>
</tr>
<tr>
<td>6.</td>
<td>Jowar</td>
<td>36.95 ± 0.05</td>
<td>47.37 ± 0.37</td>
<td>57.65 ± 0.23</td>
<td>60.80 ± 0.07</td>
<td>56.56 ± 0.11</td>
<td>56.52</td>
</tr>
<tr>
<td>7.</td>
<td>Savan</td>
<td>33.29 ± 0.43</td>
<td>47.23 ± 0.15</td>
<td>60.71 ± 1.14</td>
<td>63.44 ± 0.86</td>
<td>62.76 ± 0.05</td>
<td>62.52</td>
</tr>
<tr>
<td>8.</td>
<td>Maize</td>
<td>32.99 ± 0.17</td>
<td>49.86 ± 0.54</td>
<td>66.43 ± 0.15</td>
<td>67.61 ± 0.24</td>
<td>61.13 ± 0.14</td>
<td>67.22 ± 2.06</td>
</tr>
<tr>
<td>9.</td>
<td>Blue Panic</td>
<td>24.22 ± 0.04</td>
<td>36.73 ± 0.33</td>
<td>41.93 ± 0.25</td>
<td>43.07 ± 0.18</td>
<td>35.86 ± 0.06</td>
<td>-</td>
</tr>
<tr>
<td>10.</td>
<td>Endou vine</td>
<td>29.26 ± 0.53</td>
<td>41.87 ± 0.35</td>
<td>47.39 ± 0.29</td>
<td>48.42 ± 0.06</td>
<td>46.79 ± 0.07</td>
<td>51.72</td>
</tr>
<tr>
<td>11.</td>
<td>Buffel</td>
<td>25.56 ± 0.06</td>
<td>46.86 ± 0.12</td>
<td>53.42 ± 0.22</td>
<td>60.42 ± 0.05</td>
<td>42.96 ± 0.08</td>
<td>63.72 ± 0.95</td>
</tr>
<tr>
<td>12.</td>
<td>Setaria</td>
<td>35.49 ± 0.28</td>
<td>51.16 ± 0.13</td>
<td>59.67 ± 0.06</td>
<td>62.35 ± 0.07</td>
<td>58.67 ± 0.02</td>
<td>59.80 ± 0.90</td>
</tr>
<tr>
<td>13.</td>
<td>Doab</td>
<td>28.39 ± 0.49</td>
<td>36.25 ± 0.52</td>
<td>44.66 ± 0.14</td>
<td>52.94 ± 0.08</td>
<td>44.57 ± 0.14</td>
<td>49.25</td>
</tr>
<tr>
<td>14.</td>
<td>P.C. Napiar</td>
<td>37.27 ± 1.17</td>
<td>51.54 ± 0.37</td>
<td>64.39 ± 0.10</td>
<td>68.51 ± 0.04</td>
<td>53.21 ± 0.19</td>
<td>59.60</td>
</tr>
</tbody>
</table>

*Note:* (1) Each figure of digestibility under nylon bag and in vitro technique is the average of six readings, out of which three readings were obtained with one buffalo calf and remaining three with the second buffalo calf.
Table 7 - Percentage difference in dry matter digestibility coefficients of different fodders when determined by nylon bag and *in vitro* techniques in comparison to the standard conventional method

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Name of the fodder</th>
<th>Nylon bag values</th>
<th>In vitro values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>24 hours</td>
<td>48 hours</td>
</tr>
<tr>
<td>1.</td>
<td>Cow pea</td>
<td>-22.80</td>
<td>+2.80</td>
</tr>
<tr>
<td>2.</td>
<td>Guar</td>
<td>-17.00</td>
<td>-0.40</td>
</tr>
<tr>
<td>3.</td>
<td>Sanai</td>
<td>-23.50</td>
<td>-7.40</td>
</tr>
<tr>
<td>5.</td>
<td>Ghiabati</td>
<td>-61.10</td>
<td>-13.80</td>
</tr>
<tr>
<td>6.</td>
<td>Jowar</td>
<td>-34.70</td>
<td>-15.90</td>
</tr>
<tr>
<td>7.</td>
<td>Sawan</td>
<td>-46.70</td>
<td>-24.20</td>
</tr>
<tr>
<td>8.</td>
<td>Maize</td>
<td>-50.90</td>
<td>-25.80</td>
</tr>
<tr>
<td>9.</td>
<td>Kudzu vine</td>
<td>-34.00</td>
<td>-19.00</td>
</tr>
<tr>
<td>12.</td>
<td>Setaria</td>
<td>-40.60</td>
<td>-14.40</td>
</tr>
</tbody>
</table>

Total:  -479.00 -295.00 -33.10 +46.50 -134.20  
Average: -36.84 -15.76 -2.54 +5.87 -10.32 

Note: (i) (−) denotes the amount of lower percentage in comparison to the standard conventional values.  
(ii) (+) denotes the amount of higher percentage in comparison to the standard conventional values.
It may be seen from Table-7 that the trend of the average figures of deviation in the above table indicates that the digestion with nylon bag technique at 24 hours duration is incomplete and the results on an average are lower to the extent of about 37 per cent of that observed by conventional method. These results are in line with the thinking of the previous workers (Demarquilly and Chenost, 1963; Galvez and Agar, 1971 and Prasad, 1974) who advocated 48 hours as the time required for the completion of the digestion process in the rumen. The results obtained by nylon bag technique, when it was kept for 48 hours in the rumen, were lower by 15.76 per cent on an average than the values obtained by the conventional method. This difference of lower results in case of fodder samples kept in the rumen for 72 hours was reduced on an average to only 2.54 per cent. Thus by increasing the duration of time of keeping the sample in rumen, sufficient improvement in digestibility has been noticed. This trend, however, could not be maintained by further increasing the duration of keeping the fodder sample in rumen up to 96 hours, because now the difference instead of reduction increased and results were higher by 3.57 per cent. This difference may be due to the possible existence of a state of maximum rumen digestion somewhere in between 72 and 96 hours duration. By keeping a fodder sample longer than this period of maximum digestion may be of negative
utility, because excessive detention of food in rumen after microbial degradation and resynthesis may result in destructive decomposition.

As such it appears that the suspension of a fodder sample in the rumen for a period of 72 hours is likely to give more accurate results which are quite close to the digestibility coefficients obtained by conducting actual digestibility trials by the conventional method. (Lusk et al., 1962).

The figures obtained by two stage in vitro technique of Tilley and Terry (1962) on an average are lower than the conventional values by 10.32 per cent. Though in this technique the provision has been made for peptic digestion followed by ruminal digestion in addition to other similarities of environment and temperature as found in the normal process of in vivo digestion, the results obtained varied significantly. It is well recognised that any in vitro technique with the provision of all the similarities can not accurately duplicate a physiological function.

Under the above circumstances and as evident from the results of the dry matter digestibilities obtained with nylon bag technique, it appears that the results obtained by keeping the fodder sample in the rumen for 72 hours gives the best results in the shortest possible time, which are
quite comparable with the results obtained by conducting the standard conventional trials. Hence these values may be considered quite accurate and reliable for screening out the different genotypes or for any other quick evaluation of forage, whenever required.

C. Comparison of 72 hr (Nylon bag) D.M. digestibilities with the values obtained by 24 hr, 48 hr and 96 hr (Nylon bag) and in vitro techniques

Calculations were made to see how far the average values of D.M. digestibility of 24, 48 and 96 hours (Nylon bag technique) and in vitro technique differed from the values of 72 hours Nylon bag technique. Such calculations have been presented in Table-8.

It may be observed from Table-8 that the digestibility coefficients were lower by 36.3 and 15.5 per cent respectively with the 24 and 48 hours nylon bag technique while the same were higher by 6.29 and 6.03 per cent respectively when compared to the 96 hours nylon bag technique and in vitro technique.
Table 8 - Percentage difference in dry matter digestibility coefficients of different fodders as determined by 24, 48 and 96 hours Nylon bag and in vitro techniques in comparison to the 72 hours Nylon bag technique

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Name of the fodder</th>
<th>Nylon bag values</th>
<th>In vitro values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>24 hours</td>
<td>48 hours</td>
</tr>
<tr>
<td>2.</td>
<td>Guar</td>
<td>-19.10</td>
<td>-2.90</td>
</tr>
<tr>
<td>3.</td>
<td>Senai</td>
<td>-29.00</td>
<td>-8.40</td>
</tr>
<tr>
<td>4.</td>
<td>Dhencha</td>
<td>-21.50</td>
<td>-9.60</td>
</tr>
<tr>
<td>5.</td>
<td>Ghiabati</td>
<td>-25.30</td>
<td>-6.70</td>
</tr>
<tr>
<td>6.</td>
<td>Jowar</td>
<td>-25.30</td>
<td>-17.40</td>
</tr>
<tr>
<td>7.</td>
<td>Sawan</td>
<td>-45.10</td>
<td>-22.00</td>
</tr>
<tr>
<td>8.</td>
<td>Maize</td>
<td>-50.80</td>
<td>-24.30</td>
</tr>
<tr>
<td>9.</td>
<td>Kudzu vine</td>
<td>-38.20</td>
<td>-11.60</td>
</tr>
<tr>
<td>11.</td>
<td>Doob</td>
<td>-24.20</td>
<td>-18.80</td>
</tr>
<tr>
<td>12.</td>
<td>Setaria</td>
<td>-40.50</td>
<td>-14.20</td>
</tr>
<tr>
<td>13.</td>
<td>P.G. Napier</td>
<td>-42.10</td>
<td>-19.90</td>
</tr>
</tbody>
</table>

Total:-  
-471.30   -175.80   +81.80   +78.50

Average:-  
-36.30   -13.52   +6.29   +6.08

Note: (i) (-) denotes the amount of lower percentage in comparison to the 72 hr Nylon bag values.

(ii) (+) denotes the amount of higher percentage in comparison to the 72 hr Nylon bag values.
From the above observations it appears quite safe to select the values of Nylon bag technique with 72 hours duration as the basis for comparing and selecting a large number of genotypes for their nutritive value (Monson et al., 1969). It may be mentioned over here that although such figures are not very accurate to know the nutritive values of the fodders, but it will definitely prove helpful in making demarcation between the fodders in a qualitative basis. This may also serve the need of the plant breeders for a method which will evaluate a large number of genotypes within a very short period and with quite small quantities of the plant material.

Attempts were also made to co-relate the 72 hr Nylon bag D.M. digestibility with that of *in vitro* values and a regression equation was evolved, which is being given below:

\[ Y = 11.34 + 0.7082 \times X \]

where \( Y \) denotes the *in vitro* D.M. digestibility and \( X \) stands for 72 hr Nylon bag D.M. digestibility. Thus by inserting the value of \( X \) in the equation the value of \( Y \) can be obtained.

**D. General Discussion**

There is an obvious dissimilarity between the digestible residues from *in vitro* and Nylon bag digestion and animal faeces which contains metabolic products in addition to
undigested food residues. Apart from this, the failure to predict exactly \textit{in vivo} digestibilities from nylon bag and \textit{in vitro} results reflects not only the analytical errors in the three techniques, but also the fact that \textit{in vivo} digestibility is not a constant characteristic of a herbage.

The results of the digestibility coefficients as determined by the conventional method may vary according to whether species of animals used in the trials, the age and general health status of the animal and the level of food intake.

Hence, such type of minor differences in their values does not appear to be of any great importance and the values obtained by the above mentioned quicker techniques may be considered quite good and reliable for practical purposes.

\textbf{E. Relationship between chemical constituents of forages and their dry matter digestibilities}

Adoption of 72 hour (Nylon bags technique) dry matter digestibility is no doubt an advancement over the time consuming and costly digestibility trial for the comparative evaluation of fodder samples. However, it requires animals, controlled temperature ovens and application of skilled surgical techniques; efforts were, therefore, made to make
At first stage this relationship was searched for crude fibre content. The relevant data is given in the Table-9.

Table 9 - Correlation between crude fibre content of fodders and their 72 hours nylon bag digestibility

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Name of the fodder</th>
<th>C.F. content (X)</th>
<th>72 hr Nylon bag digestibility (Y)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Cow pea</td>
<td>25.02</td>
<td>63.97</td>
</tr>
<tr>
<td>2.</td>
<td>Guar</td>
<td>23.01</td>
<td>70.13</td>
</tr>
<tr>
<td>3.</td>
<td>Sansi</td>
<td>23.19</td>
<td>67.53</td>
</tr>
<tr>
<td>4.</td>
<td>Dhencha</td>
<td>26.21</td>
<td>53.86</td>
</tr>
<tr>
<td>5.</td>
<td>Chiabati</td>
<td>23.97</td>
<td>62.88</td>
</tr>
<tr>
<td>6.</td>
<td>Jowar</td>
<td>25.63</td>
<td>57.65</td>
</tr>
<tr>
<td>7.</td>
<td>Sawan</td>
<td>23.20</td>
<td>60.71</td>
</tr>
<tr>
<td>8.</td>
<td>Maize</td>
<td>21.62</td>
<td>66.43</td>
</tr>
<tr>
<td>9.</td>
<td>Blue Penic</td>
<td>36.51</td>
<td>41.99</td>
</tr>
<tr>
<td>10.</td>
<td>Kudzu vine</td>
<td>32.15</td>
<td>47.29</td>
</tr>
<tr>
<td>11.</td>
<td>Buffel</td>
<td>32.69</td>
<td>53.42</td>
</tr>
<tr>
<td>12.</td>
<td>Doob</td>
<td>26.90</td>
<td>44.68</td>
</tr>
<tr>
<td>13.</td>
<td>Setaria</td>
<td>24.37</td>
<td>59.67</td>
</tr>
<tr>
<td>14.</td>
<td>P.G. Napiar</td>
<td>24.97</td>
<td>64.39</td>
</tr>
</tbody>
</table>

r = 0.5666

From Table-9 it may be observed that the content of CF in the experimental fodders had significant correlation with their 72 hr digestibility; \( r = 0.5666 \). Attempts were, therefore, made to derive equation so that from the value of CF content of a fodder its digestibility could be readily calculated.
The equation was, therefore, worked out and is given below:

\[ Y = 84.83 - 0.9586 \times X \]

Here \( Y \) denotes the digestibility of DM of a fodder and \( X \) denotes its CF content in per cent on DM basis. Thus by inserting the value of \( X \) in the equation the value of \( Y \) can be easily obtained.

While attempting for getting correlation between the CP content of the fodders and their digestibility no success could be achieved. As such no regression equation could be derived. It appears that the effect of protein on the digestibility of a fodder may not be an independent one and might be governed by a number of miscellaneous factors.

Hence, it appears that probably the correlation is not very clear and may be hidden by some unknown factors and interactions.
CHAPTER VI

SUMMARY
SUMMARY

India as a whole at present is in short supply of fodders for her live stock, hence combined efforts at all levels are being made to increase the production of fodder, qualitatively as well as quantitatively by forage breeders and animal nutrition scientists. Therefore, in this regard it becomes necessary to explore a suitable technique for quick evaluation of fodders, available in insufficient quantities for conducting digestibility trials, particularly for quickly screening out the various genotypes, which are being evolved by the forage breeders during the course of their investigations. It is a well established fact that such a technique will save a lot of time, expenditure on labour, management and technical skill. Therefore, in order to achieve this objective nylon bag, in vitro and chemical composition techniques for fodder evaluation were taken up for the present investigations and a careful and thorough review of the available literature on these aspects have been made.

The different techniques of the methods applied and the methods of chemical analysis used during the course of these experiments have been described. The various techniques followed in the present investigations for evaluating the fodders were, the different durations of the nylon bag
technique, *in vitro* technique of Tilley and Terry (1963), crude fibre and crude protein of the fodders in question.

The plan of the different experiments conducted during the course of these investigations have been described in detail. The present investigations were started with two Murrah bull calves, which were surgically operated to make a fistula in the rumen. These calves were continuously kept on *ad lib* feeding of Bimal leaves (*Grewia oppositifolia* Roxb.) and pasture grazing during the experimental period and were continuously used during the course of all these experiments for determination of digestibility coefficients by the nylon bag and *in vitro* techniques.

All the available fourteen green fodders in the Veterinary College campus and nearby villages during the Kharif season were taken up for the present investigations. The samples of these fodders were analysed for their chemical composition and dry matter digestibility by using two different techniques namely, nylon bag and two stage *in vitro* techniques. In order to make a careful study about the digestibility pattern of the different fodders by Nylon bag technique, four different time intervals, that is, 24 hr, 48 hr, 72 hr and 96 hr were taken up.

In order to compare the results of digestibility of all these fodders as obtained by Nylon bag and *in vitro*
techniques the results of conventional trials on these fodders which had already been conducted in the past and most of which was available in the literature were taken. It may also be mentioned here that most of this work in the past was conducted in our institution and as such it gave a very good comparison under more or less identical conditions. While selecting such results comparable chemical composition and stage of maturity of the forage plants was specially kept in mind.

It was observed from the above data that the chemical composition of most of the fodders under the above investigations was comparable particularly as far as the contents of crude protein and crude fibre were concerned, except in case of Kudzu vine, Dhencha (Sesbania aculeata) and P.G. Napier, where crude fibre was slightly more in the samples used in the present study. On the other hand Ghisbati (Ipomoea Pestigridis) contained a higher percentage of protein and Dhencha contained less protein than the available data of the same feeds from the literature.

As the figures of chemical composition obtained from the actual chemical analysis and that obtained from the literature were comparable to a great extent, it was expected that the digestibilities of these fodders should also be quite comparable. Keeping this in view the results of
digestibility trials available from the literature have been taken as the standard and have been compared with the results of nylon bag and *in vitro* digestibilities determined during the present investigations.

It was observed from the average figures of six samples, three from each fistulated animal, that the dry matter digestion after 24 hours duration with Nylon bag technique was found to be about 37 per cent lower than that of the conventional values. In the case of 48 hr Nylon bag values the results were lower by 15.76 per cent. This difference in case of 72 hr duration was reduced on an average to only 2.54 per cent. This trend, however, could not be maintained by further increasing the duration of keeping the sample in rumen up to 96 hours. During this period it was observed that instead of reduction the values were 3.57 per cent higher.

The figures obtained by two stage *in vitro* technique of Tilley and Terry (1962) on all the 14 fodders were on an average lower than the conventional values by 10.32 per cent.

On the basis of the above observations it was concluded to use the values of dry matter digestibilities obtained with 72 hr nylon bag technique as the standard for further investigations. A comparison from these values with such
values of 24 hr, 48 hr and 96 hr (Nylon bag technique) and in vitro technique was, therefore, made and it was noticed that the dry matter digestibilities, when compared to 72 hr results were lower by 26.3 and 13.52 per cent for 24 hours and 48 hours respectively while the same were higher by 6.29 and 6.0 per cent for 96 hours Nylon bag and in vitro techniques respectively.

From all these observations of Nylon bag and in vitro techniques it was concluded that the results of 72 hours dry matter digestibility of Nylon bag technique are quite promising; and this technique can be satisfactorily used for quick evaluation of fodders by animal nutrition workers.

Further, in order to predict the in vitro dry matter digestibility of fodders by the results of 72 hour Nylon bag dry matter digestibility the following regression equation was evolved:

\[
Y = 11.84 + 0.7082 X
\]

where \(Y = \text{in vitro D.M. digestibility and}
\]

\(X = \text{72 hour Nylon bag D.M. digestibility.}
\]

A relationship between crude fibre and crude protein content of forages and their dry matter digestibilities was also tried. It was noticed that the content of crude fibre in the experimental fodders has got significant correlation
with their 72 hr D.M. digestibility of Nylon bag technique
\((r = 0.5666)\) and hence correlation equation was derived to
calculate the D.M. digestibility of fodders from their C.F.
contents, which is given below:

\[ Y = 84.83 - 0.3586 \times X \]

where

- \(Y\) = dry matter digestibility of fodder and
- \(X\) = crude fibre content in per cent on D.M. basis.

However, in the case of crude protein no such correlation
or regression equation could be derived with the present
results.
# APPENDIX-1

## Body weights of experimental Murrah calves

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>No. of fortnights</th>
<th>Calf No.1</th>
<th>Calf No.2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Fortnight starting from 1.9.1974</td>
<td>82.0</td>
<td>128.0</td>
</tr>
<tr>
<td>2.</td>
<td>II</td>
<td>82.0</td>
<td>124.0</td>
</tr>
<tr>
<td>3.</td>
<td>III</td>
<td>86.0</td>
<td>127.0</td>
</tr>
<tr>
<td>4.</td>
<td>IV</td>
<td>90.0</td>
<td>131.0</td>
</tr>
<tr>
<td>5.</td>
<td>V</td>
<td>94.0</td>
<td>135.0</td>
</tr>
<tr>
<td>6.</td>
<td>VI</td>
<td>95.0</td>
<td>140.0</td>
</tr>
<tr>
<td>7.</td>
<td>VII</td>
<td>96.0</td>
<td>142.0</td>
</tr>
<tr>
<td>8.</td>
<td>VIII</td>
<td>97.0</td>
<td>145.0</td>
</tr>
<tr>
<td>9.</td>
<td>IX</td>
<td>98.0</td>
<td>147.0</td>
</tr>
<tr>
<td>10.</td>
<td>X</td>
<td>99.0</td>
<td>149.0</td>
</tr>
</tbody>
</table>

Note: On account of the surgical operation for rumen fistula the body weight of calf No.1 remained constant and of calf No.2 decreased during the second quarter of October, 1974.
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R.D. (1965-66) ------do------
R.D. (1966-67) ------do------
R.D. (1968-69) ------do------
R.D. (1972-73) ------do------


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