FORAGE EVALUATION USING VARIOUS LABORATORY TECHNIQUES

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ABSTRACT

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By

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Forages from tropical and temperate regions were evaluated by several laboratory methods. Samples were analyzed for crude protein, cell walls, acid-detergent fiber, lignin, cellulose, silica, and other chemical components by standard AOAC methods and by the Van Soest system of feed analysis. Various *in vitro* fermentations, and extents of solubility by enzymes (cellulase, pepsin, amylase) and acidic buffers were determined on these forages. Simple and multiple correlations and regressions among various laboratory estimates and their relationships to *in vivo* measurements were conducted to determine and select the most precise prediction equations for *in vivo* parameters.

Temperate grasses and legumes had higher levels of crude protein and *in vitro* dry matter disappearance than did tropical forages but tropical forages had higher levels of cell walls, acid-detergent fiber, cellulose and ash than

did temperate forages. The rates of decline in crude protein and *in vitro* dry matter disappearance with advancing maturity for temperate forages were much greater than those for tropical forages and conversely the rate of increase in cell walls of temperate forages was greater than that for tropical forages. Generally, crude protein in forages was positively correlated with ash but negatively correlated with cell walls, acid-detergent fiber, cellulose, lignin and silica and all fibrous fractions were positively and mutually correlated.

For temperate forages *in vivo* dry matter digestibility, total digestible nutrients, digestible energy, dry matter intake, and digestible dry matter intake had positive correlation coefficients ($r = 0.07$ to 0.92) with crude protein, ash, *in vitro* fermentations, or enzymatic incubations but negative correlations ($r = -.07$ to $-.82$) with cell walls, acid-detergent fiber, cellulose, hemicellulose and lignin.

Water-soluble carbohydrates, total nonstructural carbohydrates and total available carbohydrates after cellulase plus amylase incubations had low correlations with *in vivo* parameters and these chemical components as well as total ash could not be used as single predictors of any *in vivo* parameters. Acid-detergent fiber and lignin could predict *in vivo* dry matter digestibility of forages with moderate to high accuracy ($r = -.70$ to $-.93$, SEE = 2.9 to 5.6). Enzymatic incubation values, cellulase,

amylase, pepsin or a sequential hydrolysis by two enzymes predicted digestible dry matter with an accuracy similar to that for the chemical components. With some forage species these enzymes predicted dry matter digestibility with useable accuracy having standard errors of 2.3 to 6.1 and correlation coefficients of 0.52 to 0.93. Two-stage *in vitro* fermentation (IVDMD or IVOMD) was the method of choice for predicting *in vivo* dry matter digestibility of both grasses and legumes with small standard errors of estimate (SEE = 1.8 to 4.4).

Dry matter intake of forages could be predicted more precisely from cellulase, amylase or cellulase plus amylase than from chemical components or the two-stage *in vitro* fermentation. Total digestible nutrient content could be predicted from acid-detergent fiber, cellulase plus pepsin, cellulase or the two-stage *in vitro* fermentation with standard errors of 1.8 to 6.5. Digestible energy content might be predicted from cellulase, cellulase plus pepsin or the two-state *in vitro* fermentation with more accuracy than that from chemical components.

The best predictors of *in vivo* parameters for various types of forages were not the same and the prediction equations using the same predictor were different for each forage species.

Multiple correlation and regression technique using combinations of chemical components did not significantly improve the precision of prediction for digestible dry

matter and intake. However, combinations of 36-hour or two-stage *in vitro* fermentations with these chemical components significantly improved the precision of prediction for digestibility and intake of legumes or grasses. Combinations of the two-stage *in vitro* fermentation plus crude protein and ash accurately predicted total digestible nutrients of legumes whereas 36-hour *in vitro* fermentation plus acid-detergent fiber accurately predicted total digestible nutrients of grasses. The combination of ether extract plus nitrogen-free extract or crude protein plus crude fiber plus ether extract accurately predicted total digestible nutrients of silages.

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INTRODUCTION

Forages are an important source of feeds for ruminants and other animals. Horses, cattle, sheep, goats, deer, rabbits, termites, or their associated intestinal microflora are able to digest cellulose to a varying degree. Cellulose and hemicellulose in forage crops can be broken down by the rumen microflora and provide the host animals with a source of energy. Fifty percent or more of the potentially useful energy of forages can be obtained from cellulose and hemicellulose fractions. Cellulose is the most abundant carbohydrate in the plant kingdom and can become the cheapest source of energy for ruminant animals under some conditions.

In many geographical areas, the economic development of a livestock industry parallels the development of grassland farming or a good forage production program. There are about 10,000 species of grasses (Gramineae) in the world and only 40 species are used to any large extent in the development of the cultivated pastures (73). In many areas, grass when farmed at its highest potential, yields more energy and protein equivalent per acre than any other crop. In humid temperate regions, grass yields over 11,000 kilograms dry matter per hectare per year

whereas in the tropics it may yield 22,400 kilograms dry matter per hectare per year.

Tropical forages are important to future world food supplies. In the tropics, there are probably 10,000 million acres of land which provide grazing, food and shelter to animals. The overall contribution of these tropical grasslands is to sustain about half the domestic animals of the world and to produce one-third of the meat and one-fifth of the milk products produced globally (32). Besides, more than half of the cattle of the world are raised in the tropics with their plane of nutrition being very low.

Forages may not supply sufficient energy but do provide sufficient crude protein for most ruminant animal enterprises. The greater part of energy provided by forages comes from the carbohydrates and its value depends on the quantity and digestibility of these carbohydrate fractions. The nutritive value of forages varies with species, cultivars, age, stage of cutting, environmental factors, fertilization, cultural practices, etc. Proper evaluation of forage nutritive value is useful to animal feeders, forage producers, and researchers such as animal nutritionists, forage breeders, and forage management specialists.

The best method to determine quality and nutritive value of any forage is to feed that forage to animals. However, a digestion trial is time-consuming and needs

considerable quantities of material and equipment. Recently, many laboratory procedures have been developed for estimating forage nutritive value. The objectives of the present study were:

1. To further evaluate and verify some new laboratory methods with additional samples of grasses and legumes from both temperate and tropical regions;
2. To develop and evaluate some new techniques for forage evaluation using cellulase, amylase, pepsin and a combination of these enzymes;
3. To determine correlations among laboratory estimates and *in vivo* data and to develop prediction equations for various parameters.

REVIEW OF LITERATURE

TERMINOLOGY OF FORAGE EVALUATION

As in many disciplines, terms and definitions used are important in communicating ideas. However, many terms are peculiar to some disciplines and readily understood only by those familiar with the subject matter. Several of the terms and abbreviations used throughout this presentation are given in Appendix Table 1.

SYSTEMS AND METHODS OF FORAGE EVALUATION

I. PROXIMATE ANALYSIS

The Weende system of proximate analysis which was developed over 100 years ago is universally used by many laboratories. Moisture, crude protein, crude fiber, ether extract, nitrogen-free extract and ash in feedingstuffs are determined and from these values an evaluation of the feed can be made.

a. Variables Used

1. Nitrogen (N). Nitrogen is a basic and characteristic constituent of all proteins and many other compounds and total nitrogen is determined by the Kjeldahl method. On the average, crude protein (CP) in common

feedingstuffs contains about 16% N. Therefore, a factor of 6.25 (100/16) is generally used to convert % N in feeds to % CP (4,130). In fact, CP also contains non-protein nitrogen, amides, amines and amino acids. The term true protein is used in certain situations.

2. Crude Fiber (CF). As originally proposed, CF represents an indigestible, fibrous fraction of the feed. It contains 50 to 80% of cellulose (C), 15 to 25% of hemicellulose (HC), 10 to 50% of lignin (L) and some insoluble substances (42,123). Crude fiber is determined by alternately boiling the sample with dilute H_2SO_4 and dilute NaOH (4,130).

3. Ether Extract (EE). Ether extract represents crude fat which contains both triglycerides, fatty acids and many non-triglyceride components such as chlorophyll, sterols, anthocyanin, waxes, etc. Ether extract is obtained by percolating ether over the sample for 8-16 hours and evaporating ether to obtain ether extract (4,130).

4. Ash. This fraction represents the mineral residue of feeds. It is obtained by igniting the sample at 600 C and contains various major and trace elements and/or other oxides (4,130).

5. Nitrogen-free Extract (NFE). This fraction represents the highly digestible carbohydrates of the feed. It contains starch, sugars, pentosans, fructosans, hexosans and some impurities as well as errors resulting from previous determinations. Nitrogen-free extract is obtained

by subtracting percentages of moisture, CP, CF, EE and ash from 100 (4,130).

6. Moisture. The water content of feeds can be determined by drying the sample at 100 to 103 C for 4 to 12 hours depending on type of sample, etc. (4,130).

b. Usefulness of Proximate Analysis

The system of proximate analysis has been used in human, nonruminant and ruminant nutrition studies for more than a century. The determinations of chemical constituents in this system are simple and less time consuming than methods that more precisely identify nutrients. The measurement of food energy expressed as total digestible nutrients (TDN) is derived from this proximate analysis. The use of TDN in feeds and feeding is internationally accepted and many TDN values exist so that this system will likely continue to be used for many years. Data using TDN and proximate analysis values have been used in the development of feeding standards (71,83). In addition, many scientists have used chemical constituents from this system to predict *in vivo* performances. Bredon *et al.* (19) used CP, CF and NFE to predict *in vivo* dry matter digestibility and TDN of tropical forages whereas Adams *et al.* (1) used CP, CF to predict TDN values of many temperate forages. Finally, many prediction equations for digestible protein (DP) have been developed using CP (19,23,59).

c. Shortcomings of Proximate Analysis

The proximate analysis does not properly separate plant carbohydrates into discrete chemical entities based on their biological availability (121,122). The CF residue does not include all HC, L and acid-insoluble ash (42,123). Nitrogen-free extract which is supposed to contain soluble carbohydrates, contains variable amounts of HC, L, C, and acid-insoluble ash. The method of determining NFE by difference, therefore, accumulates errors from previous determinations. Ether extract or crude fat does not recover protein-bound lipids and contains many non-nutritive impurities as discussed earlier (42). The TDN system resulting from these methods is therefore based on inaccurate assumptions.

Another drawback of the system is related to nutrient digestibility. In many cases, CF is more digestible than NFE because the latter contains L, HC and some C. Butterworth (22) reported that the digestion coefficients of CF for Para grass, Bermuda, Guinea and Speargrass were 57, 66, 72 and 74% whereas the coefficients of NFE for the same grasses were only 51, 59, 67 and 57%, respectively. One final drawback of the proximate analysis system is that the chemical estimates are poorly related to the *in vivo* data. They are poor predictors of forage quality and no useable prediction of digestible energy could be made from these chemical constituents (21,80,81).

II. VAN SOEST SYSTEM

In the early sixties Van Soest proposed a new system of feed partitioning which overcomes some of the shortcomings of the TDN and proximate analysis system (115, 116,119,120,126), and uses detergents to differentially solubilize forages and partition forage dry matter into high digestible and low digestible components. With this system, feed dry matter is partitioned into 2 parts, namely, cell wall constituents and cell contents and the details of which are discussed below. Diagrammatic representation of major feed compounds from chemical analysis is shown in Figure 1.

a. Variables Used

1. Cell Wall Constituent (CWC or CW). This fraction represents the total fiber of forages. It is composed of HC, C, L, attached protein, lignified nitrogenous compounds, heat-damaged protein, keratin and silica. This fraction is determined by boiling forages with neutral detergent (sodium lauryl sulphate) solution for 1 hour (43). The components of CW can only be digested by the microorganisms. The digestibility of CW fraction is variable but could be calculated from the extent of lignification of the acid-detergent fiber (ligno-cellulose) fraction.

2. Acid-Detergent Fiber (ADF). This fibrous fraction is composed of C, L, acid-insoluble ash (silica)

and some cutin. Acid-detergent fiber is determined by boiling forages for 1 hour with acid-detergent solution (43).

3. Lignin (L). This component is a polymer of phenyl propane units found in plants and forages. Basically, L in feeds is indigestible and has no nutritional value to the animals. Lignin is determined by either solubilizing C in ADF with 72% H_2SO_4 or by oxidizing L in ADF by permanganate solution (43).

4. Cellulose (C). This is a β-D-Glucose polymer. Cellulose in this system is determined by the difference between the ADF and L content (43) or as the residue after boiling in an acetic-nitric acid mixture.

5. Hemicelluloses (HC). They are amorphous mixed polysaccharides found in plant cell walls. A simple determination used for HC is the difference between CW and ADF (43).

6. Silica (Si) and Cutin. Plant Si has no nutritional value to animals. Silica builds up in plant tissues through plant metabolism or from soil contamination. Silica in this system is obtained by oxidizing acid-insoluble ash with hydrobromic acid (43).

Cutin is an aliphatic compound composed of fatty acids, hydrocarbons, alcohol and aldehydes. The amount is obtained by treating permanganate C with 72% H_2SO_4 and calculating cutin after ashing (43).

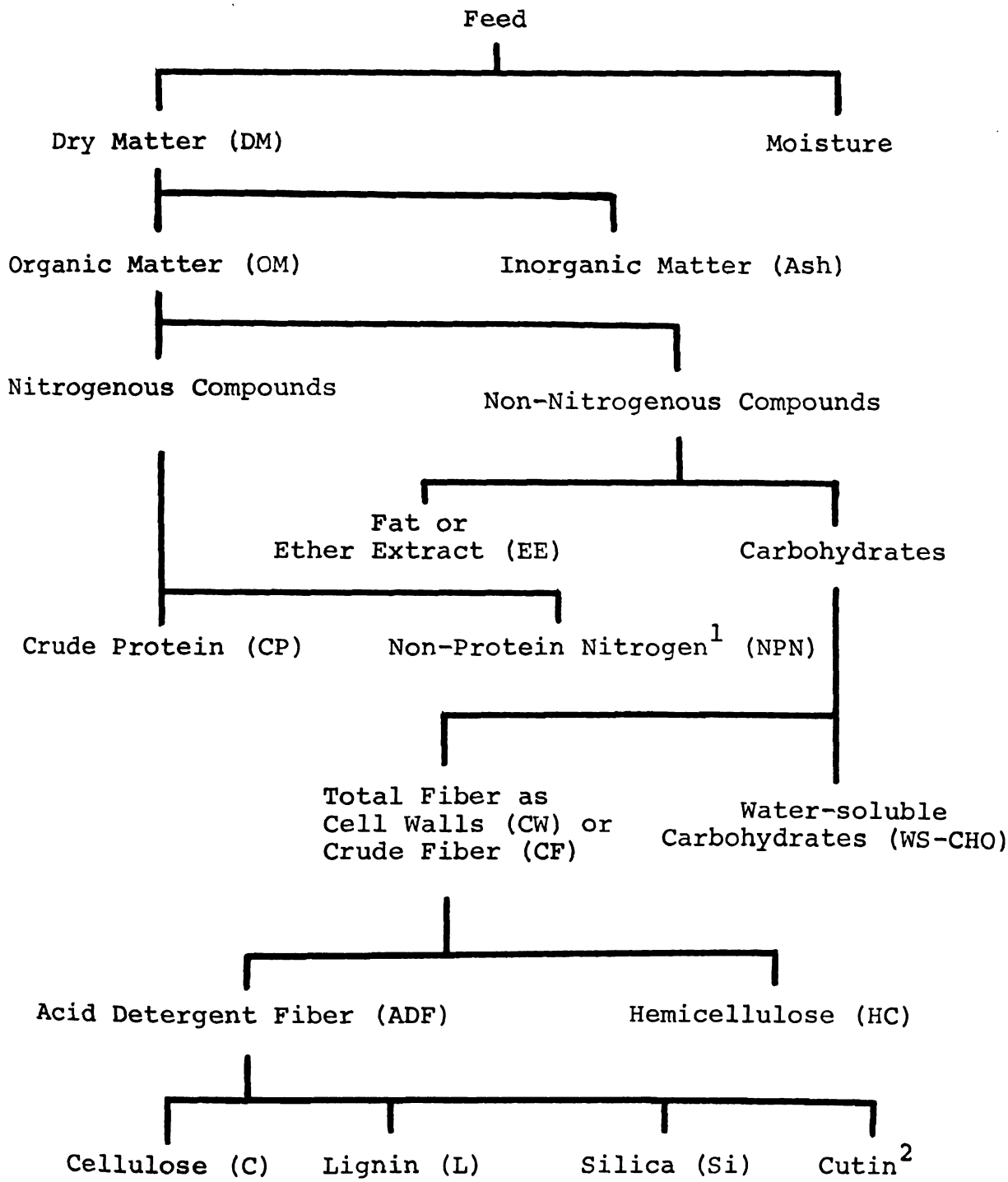

Figure 1. Diagrammatic representation of major feed compounds from chemical analysis.

[1,2]Not determined in this study.

7. Cell Contents (CC). This fraction representing the highly digestible portion of plant cells is composed of lipids, protein, amino acids, sugars, starch, organic acids, non-protein nitrogen (NPN), pectin and other water-soluble material. They are assumed to be completely digestible by animals without the aid of microbial fermentation. The digestibility of CC is assumed to be 98% (120). Cell contents are equal to 100-CWC (43).

b. Advantages of Van Soest System

Feed partitioning and analysis by the Van Soest system has been accepted by scientists all over the world because it classifies feeds and forages according to nutritional functions by monogastric and ruminant animals (43). The results of chemical analysis have been satisfactorily correlated with in vivo data and accurate prediction equations have been developed. For example, the ratio between L and ADF or log L/ADF is found to be highly correlated with CW digestibility (118,120). Another advantage of this system is a recent development reported by Van Soest and Robertson (125) who developed techniques to recover some analytical reagents from the, otherwise, discarded detergent solutions.

c. Shortcomings of Van Soest System

Even though Van Soest's system is one of the best procedures, criticisms have been made by others (36,42). Firstly, about 30% of the total protein remains in the CW

fraction and most of the silica is extracted into CC (42). Secondly, the acid-detergent solution dissolves a considerable amount of lignin and ADF also contains some nitrogen. Thirdly, hemicelluloses and C are determined by differences so they may contain errors from previous determinations. Fourthly, Van Soest's procedures with high energy cereal feeds, protein supplements and mixed diets filter very slowly and the resulting CWC values are erroneously high. Finally, many scientists found that Van Soest's system (summative equation) used to estimate forage digestibility did not agree with *in vivo* digestibility (36) or dry matter disappearance (DMD) by the Tilley and Terry method (64).

At present, scientists are attempting to develop, refine or alter this system in order to improve its relationship to *in vivo* data.

III. FONNESBECK AND HARRIS SYSTEM

A modification of the Van Soest's system has been proposed by Fonnesbeck and Harris (42) and this is outlined as follows.

a. Variables Used

The chemical constituents and major partitioning are similar to those of the Van Soest system. However, the methods for determining some constituents are different and the system requires quantitative determination of more

constituents than does the Van Soest or proximate analysis system.

1. Cell Wall Constituents (CWC). These fractions are further partitioned into HC, C, L, acid-insoluble ash (Si). Cellulose and HC are partially nutritive matters which can be digested only by microbial enzymes. Lignin and acid-insoluble ash (mostly Si) are non-nutritive residue.

2. Cell Contents (CC). These fractions are further partitioned into soluble carbohydrates, protein, solvent extract, and soluble ash. The solvent extract is further separated into fats plus fatty acids and non-fat extract. The cell contents include nutritive matters that are digestible by enzymes secreted in the GI tract or are otherwise soluble enough for absorption. The non-nutritive solvent extract is the non-saponified fraction composed mostly of chlorophyll, sterols, carotenoids, waxes, etc.

This new system of feed partitioning requires analyses for DM, CWC, HC, L, acid-insoluble ash (Si), N, ash, solvent extract and nutritive solvent extract. Other fractions can be calculated as follows:

1. CC = 100 - CWC
2. Soluble carbohydrate = CC - (protein + solvent extract + soluble ash)
3. Soluble ash = Total ash - Acid insoluble ash
4. Cellulose = CWC - (HC + L + Acid insoluble ash)
5. Non-nutritive solvent extract = Solvent extract - nutritive solvent extract

b. Advantages of the System

In this system, CWC are free from CP due to the use of pepsin and the CWC value is higher than that of Van Soest's because of increased recovery of HC and Si. There is no necessity to determine ADF. Cell wall constituents, HC, L and acid insoluble ash are determined directly and C is obtained by difference as indicated earlier. Lignin can be determined using the CWC residue and there is no need to add asbestos to the crucible when determining lignin. These new methods provide for the determination of solvent extract, nutritive and non-nutritive solvent extract, soluble ash and soluble carbohydrates.

IV. OTHER METHODS USED IN FORAGE EVALUATION

a. Digestion Trial

Results of a digestion trial are usually considered one of the best methods for evaluation of different forages. It is also used in studying nutrient requirements, digestibility, intake, energy metabolism, mineral utilization, body weight gain and toxic substances with many animal species. The techniques and procedures in digestion trials have been fully described in Bulletin 45, Commonwealth Agricultural Bureau (28), and by Lindahl (68), Maynard and Loosli (71). Although most investigators use a 7-day collection period, a 5-day trial was found sufficient for tropical forages (46). Comparative digestibilities for various forages by sheep, rabbits and heifers have been discussed by Ingalls et al. (52,53).

b. In Vitro Fermentation

1. Types of In Vitro Systems

There are many types of systems and procedures for performing in vitro rumen fermentations. These measure the disappearance of any of the following constituents, dry matter, cellulose, cell walls, other carbohydrates or the production of acids or gas (55). Generally speaking, short-time fermentations are superior in predicting dry matter intake (DMI), digestible dry matter intake (DDMI), nutritive value index (NVI) and weight gain (24,51) whereas long-time or two-stage fermentations are superior for predicting apparent digestibility (11,55,57,73). Techniques and procedures for one- or two-stage rumen fermentations can be found in the papers by Tilley and Terry (112), Barnes (10,11), Johnson (55,57), Troelsen (113), Goering and Van Soest (43), Mellenberger et al. (76) and Minson and McLeod (80) and those for in vitro true dry matter digestibility (IVTDMD) or in vitro cell wall digestibility (IVCWD) by Van Soest et al. (126) and Goering and Van Soest (43).

Many scientists presently use a two-stage in vitro rumen fermentation (48-hour with buffer plus rumen fluid plus a 24 or 48-hour pepsin digestion) as the most practical technique to predict forage digestibility with a small residual standard deviation. This method is also satisfactory for evaluating tropical forages even though the mean dry matter in vitro digestion coefficient of tropical

grasses was 2.6 percentage units lower than *in vivo* value (98). However, this method may not be satisfactory for silage samples (90).

From this two-stage *in vitro* technique, *in vitro* organic matter digestibility (IVOMD) or *in vitro* digestible organic matter (IVDOM) can be obtained after ashing the residue. *In vitro* OMD is preferred to IVDMD because it can eliminate the variation in IVDMD when the samples or feces are contaminated with dirt and sand (81).

2. Factors Affecting In Vitro Fermentation

A detailed discussion on factors affecting *in vitro* rumen fermentations is found in a paper by Johnson (57). However, some important points will be discussed here.

2.1 Inocula

Bezeau (14) reported that the activity of inocula from an Ayrshire cow was significantly higher than that from a Holstein cow when fed alike. Troelsen (113) indicated that there were no differences due to use of inoculum from either sheep, cattle or goats. In general, the IVCD using inocula from an alfalfa-fed cow is greater than that when using inocula from the one fed grass (14). Robertson and Van Soest (100) found the inocula from a forage-fed donor (timothy hay) to digest greater amounts of CW from forage and concentrate substrates than did inocula from a concentrate-fed animal.

2.2 Forage Species and Cuttings

There are significant differences in *in vitro* DMD between genera and species of grasses and legumes (11,98). Forages from a 4-cutting system have higher IVDMD values than those of a 3-cutting, 2-cutting and 1-cutting system (3).

2.3 Effects of Drying and Temperature Treatment

There are no significant differences in rate and extent of IVDMD, CWD for samples which have been freeze-dried or oven-dried at 100 C. However, heating and drying at 100 C for over 4 days will decrease the IVDMD of forages (63,112). Johnson *et al.* (59) reported that undried samples of forages had higher digestibility values and correlation coefficients between IVCD and DDM than did dried samples. Forages grown under a high temperature regime have lower IVDMD than those exposed to a cool temperature (106).

2.4 Effects of Grinding

The particle size does not affect dry matter disappearance because finely ground or coarsely ground samples from the same herbage have identical values (112). For mature forages, grinding slightly increased IVDMD. Grinding through a 1 mm screen is recommended (113) because grinding more finely does not improve prediction accuracy (74).

2.5 Effects of Sample Size

McLeod (74) reported that the use of 0.1 gm sample for fermentation will increase the residual standard error of prediction to 3.4% units as compared to 2.5 units when using 0.5 gm sample. Others found that increasing the size of the sample led to a decrease in the *in vitro* digestibility value and, therefore, 0.5 gm sample has been recommended (80,113).

2.6 Minerals and Other Substances

The *in vitro* fermentation values have increased variation and differences when urea and glucose are omitted from the media and especially when the inocula are from different donors. With ample urea and glucose, differences in IVDMD due to inocula are decreased (87). Many trace minerals stimulate IVCD when used at low concentrations. These are Co, I, Fe, Mn, Mo, Rb, Zn, Cd, Cr, Sr. Minerals that depress IVCD are Ba, B, Cd, Cr, Co, Cu, F, Fe, Mn, Ni, Se, Sr, V, and Zn (70). Other substances that reduce IVDMD values are Si, L, high level of fat, tannin, alkaloids and other plant inhibitors.

c. Solubility and Turbidity Tests

The extent of solubility of forages in dilute acids and other chemicals can be used as a rapid and inexpensive method of forage evaluation. Dry matter solubility of forages in 1 N H_2SO_4 and cellulose solubility in 1 M cupriethylene diamine have been determined and the

results are highly correlated with many in vivo parameters (34,35,60).

A simple procedure of measuring turbidity of a forage extract was found to correlate well with some chemical components (5,13).

d. Forage Evaluation using Enzymes

Hydrolysis of forages by enzymes was proposed over 10 years ago by Donefer et al. (39) as an evaluation technique. They used the amount of hydrolysis by cellulase, pepsin and a mixture of these enzymes to estimate forage DDM. Tilley and Terry (112) used pepsin after fermentation with rumen fluid. Smith (105,106) and Grotelueschen and Smith (47) used takadiastase (amylase) to determine total available carbohydrates (TAC) and total nonstructural carbohydrates (TNC) in plant tissues. Later, Jarrige et al. (54) used cellulase hydrolysis to estimate OMD and DOM. Guggolz et al. (48) proposed cellulase plus pronase to evalute forages and crop or woody residues, while Moore et al. (82) proposed another cellulase preparation (Onozuka) for this purpose. Recently, McQueen and Van Soest (75) used a cellulase from Trichoderma viride (fungi) to evaluate forages.

1. Cellulases

Available cellulase preparations are crude enzymes. The cellulase complex contains C_1 enzyme, β-1-4 Glucanases (= C_x) and β-Glucosidases (66). Cellulase can hydrolyze

HC, C, some starch and other nitrogenous compounds and may yield 30% glucose from cellulose digestion (48,54,103).

The Onozuka product is obtained from fungi Trichoderma viride and contains hemicellulase, lactose, galactose, glucose and arabinose. It solubilizes pure cellulose to the extent of 10 and 46% for Solka-floc (48, 82). This preparation was the most active preparation investigated.

Cellulase used by French investigators contains 19% CP and 60% of the powder is water soluble. It contains other enzymes which attack HC, CP, starch and some nitrogenous compounds (54).

Jarrige et al, (54) reported that the residue remaining after cellulase digestion was highly correlated ($r = -.921^{**}$) with in vivo OMD using 100 samples of hays and herbages and the standard error of prediction was 3.22%. The correlation coefficient between total solubles after cellulase (TSAC) and digestible organic matter (DOM) was highly significant ($r = 0.922^{**}$) and the standard error of prediction was only 2.82%. They concluded that cellulase digestion appears to be a better predictor of in vivo digestibility than ADF and the Tilley-Terry method as far as time, equipment and manipulations are concerned.

In addition, the correlation coefficient between cellulase residue and DMI was highly significant ($r = -.70$) and that between cellulase residue and DOM intake was -.81 ($P < .01$). The prediction of DMI and DOM intake from

cellulase residue was not very precise but satisfactory with standard errors of 8.93 and 6.32 g/kg 0.75, respectively (54).

Guggolz et al. (48) reported that the correlation coefficient between total solubles after enzymes (TSAE) and in vivo DMD was 0.900 ($P < .01$) and that between TSAE and TDN was also highly significant. Results from these two groups of investigators clearly indicate that the new cellulase technique can predict in vivo DDM with sufficient precision thus eliminating any necessity for rumen fluid from a donor animal.

2. Pepsin

Forages have been incubated with pepsin alone or pepsin has been used as a second-stage incubation after cellulase or rumen fluid fermentation (39,40,65,123). The addition of pepsin or pronase as a second-stage incubation will increase DMD by 4 to 5% units (48,82). This type of second incubation may not be necessary for samples low in protein. Moore and Mott (81) reported the DMD values of tropical grasses (Panicum spp.) by pepsin digestion to have a low correlation with in vivo DDM and DMI with standard errors of 3% and 8 $g/kg^{0.75}$, respectively.

On the contrary, Donefer et al. (39,40) found highly significant correlations between pepsin DMD and in vivo data for different forages as shown below:

	r	Samples
DMD (pepsin) vs. NVI	0.95**	All forages
DMD (pepsin) vs. RI	0.87** to 0.94**	"
DMD (pepsin) vs. ED	0.68** to 0.73**	"

(** P < .01)

Furthermore, Wilkins and Minson (129) reported that OM solubility in pepsin was significantly correlated with *in vivo* OMD or *in vivo* CD with standard errors of prediction of 5 and 6%, respectively.

3. Takadiastase

Takadiastase is a crude amylase which also contains some sucrase, maltase, oligo-1, 6-glucosidase and traces of hemicellulase or cellulase and is used to determine TAC in plants (47). There is meager evidence concerning the use of takadiastase to estimate forage digestibility and DMI by farm animals.

e. Soluble and Nonstructural Carbohydrates

Some scientists use water-soluble carbohydrates (WS-CHO) and nonstructural carbohydrates (TAC, TNC) in their forage evaluation programs (37,47,61,105,106). The significance of WS-CHO in ruminant nutrition is unclear because Ingalls (51) found negative and non-significant correlations between soluble carbohydrate and certain *in vivo* measurements. On the other hand, TAC has been used to predict *in vivo* OMD and cellulose digestibility satisfactorily (129).

f. Energy System

1. Gross Energy

Most feedingstuffs, forages and even feces contain approximately 4.40 KCal/g of dry matter and thus gross energy values are not useful in evaluating feeds or forages.

2. Digestible Energy (DE)

Apparent digestible energy represents energy intake minus fecal energy and is a measure of the portion of food energy that can be used by the animals (71).

3. Metabolizable Energy (ME)

Metabolizable energy represents actual energy absorbed and utilized by the animal and is obtained by subtracting urinary and gaseous energy from DE (71).

4. Net Energy (NE)

During energy metabolism a portion of energy is used for metabolic processes and called heat increment. Net energy is therefore obtained by subtracting heat increment from ME and represents energy used for maintenance, growth, production of meat, milk, eggs, etc. (71).

5. Total Digestible Nutrients (TDN)

Total digestible nutrients represent an expression of the energy content of feedingstuffs. Chemical components measured in the proximate analysis system were used to

formulate the TDN system by Henry and Morrison in 1910 (42) and TDN represents the sum of digestible CP, digestible CF, digestible NFE, digestible EE (2.25) expressed as a percent.

g. Nutritive Value Index (NVI)

Nutritive value index (NVI) is the mathematical product of forage digestibility and intake (31). However, these two factors may not be closely related. Nutritive value index is relatively useless in practical ration formulation (57) but may be useful in evaluating forages. Nutritive value index of alfalfa is higher than that of bromegrass which is higher than that of reed canary grass (51). For tropical forages, NVI is highest at 4-5 weeks of regrowth indicating that grazing or feeding should be done at this stage of growth and the high correlation ($r = 0.91$, $p < .01$) between NVI and body weight gain supports this idea (46).

V. CONSIDERATIONS IN FORAGE EVALUATION

Forage evaluation is truly an interdisciplinary science involving groups of investigators such as the livestock feeders, nutritionists, forage producers, and plant breeders, etc. Emphasis by each group may be different but many considerations should be recognized by all so that proper forage evaluation can be successfully accomplished.

a. Goals and Precautions

The objectives and systems used in forage evaluation may vary among groups. For example, livestock feeders may be interested only in particular constituents (i.e., CP, TDN, etc.) in forages whereas the nutritionist may be interested in interrelationships among chemical components, mineral contents, vitamins, digestibility and nutrient metabolism. Therefore, each discipline should clearly outline its goals of forage evaluation so that the results and interpretation will be clear to all concerned (56).

A feeding trial is expensive in terms of animals, feeds, labor, time and equipment. Therefore, investigators attempt to replace the feeding trial with laboratory or chemical methods. The major goal of forage evaluation is to develop and utilize laboratory methods for determining forage quality that is related to animal performance. A useful laboratory procedure for routine evaluation of forages should have these characteristics:

1. Require a small sample of the forage under study;
2. Simple enough to permit rapid evaluation with minimum equipment and reagents;
3. Must produce repeatable results with high degree of accuracy in predicting forage nutritive value.

The most economical approach would be to analyze a series of forages having known animal data and then to correlate animal with laboratory data followed by establishment of prediction equations (90,123).

Many animal and laboratory variables may influence the results and relationship obtained in one location so that a somewhat different relationship may be obtained under different conditions. Without similar techniques, standardization and other precautions, prediction equations should be used with caution.

b. Sampling Techniques

The best laboratory estimates may be useless if the sample obtained for analysis is not representative of the entire lot of material. Also, the results of a forage analysis will be reliable and useful only if the sample taken is representative of what the animal consumes (102). Larsen (67) describes sampling techniques for baled hay, loose hay, haylage, grass silage, corn silage, etc. Grier (45) described the preparation of plant materials for chemical analysis. Troelsen (113) suggested sampling techniques for forages including the methods of collection, morphological fractionation, drying, grinding and weighing. Goering and Van Soest (43) describe some excellent sampling techniques for dry and wet feeds. Other details on sampling are presented in Bulletin 45, Commonwealth Agricultural Bureau (28).

c. Data Collection and Source Form

Some standardization for reporting forage data would facilitate data collection, tabulation and use by investigators. Harris et al. (49) have developed an excellent

computerized source form for reporting data that should be considered by all feed and forage investigators.

VI. FACTORS INFLUENCING NUTRITIVE VALUE OF FORAGES

The nutritive value of forages usually refers to chemical compositon of feeds, their digestibility, animal intake and the nature of the digested products (85).

a. Chemical Composition

Chemical composition is the most simple and the generally accepted criterion used in feed evaluation and is influenced by a number of factors, some of which are briefly discussed below.

1. Species and Cultivars

All chemical fractions of tropical grasses differed between species (30,64). Generally, legumes contain more CP and less CW than do grasses. Legume CW contains less HC and is more lignified than grass CW (107). A cultivar of alfalfa named Vernal had only 17% CP whereas Du Puits alfalfa contained 22% CP when cut at the same age. Rohweder and Henderson (101) also reported that different cultivars of oats had different chemical composition. Cultivars of Goodfield, Portal, Rodney varieties contained 22, 17, 14% CP, respectively.

2. Age or Stage of Maturity

As forages get older, CP, DP, ash, EE, TDN, soluble carbohydrates, P, K and carotene contents decrease whereas

CW, ADF, CF, L, C, methoxyl, pentosan, and hexosan contents increase while NFE and Ca contents may remain unchanged depending on the species (3,15,16,19,37,64,72,73,92,101, 104,114,120,128).

3. Leaf-Stem Ratio

Forage legumes have a different leaf/stem ratio from that of grasses and usually leaves contain more nutrients than stems. McIlroy (73) found the CP content in leaves of several forages was higher than that in stems.

4. Nitrogen Fertilization

Nitrogen fertilization increases CP content of grasses while maintaining CWC, C, L, DE at the same level (16,73,122). With corn silage, 179 Kg N/ha increased CP from 6 to 9% and TDN from 65 to 66% whereas ADF decreased from 34 to 26% (101).

5. Climatic Conditions

Important environmental factors that cause changes in forage nutritive value are light, temperature and fertility level. An increase in light intensity will increase WS-CHO and DM but decrease CP, ash, CWC, C and L without materially altering digestibility. Van Soest (122) further reported that an increase in temperature will cause an increase in CWC, C, L, DM with a decrease in CP and WS-CHO. Therefore, the increase in both light and temperature will lower the nutritive value of the forages. The above statement may be true when the temperature

increases beyond 32 C. On the contrary, alfalfa grown in chambers maintained under cool (18 C) or warm (32 C) temperatures, showed an increase in CP but a decrease in WS-CHO and DM at 32 C. Crude fiber was relatively unchanged (106,107).

b. Voluntary Intake

Animal performance is more related to voluntary intake than to digestibility (10,51) and feed intake varies much more than does the latter (81). McIlroy (73) reported that intake of legumes is greater than that of grasses. This is in agreement with Ingalls (51) who showed that the DMI of 4 forages were in the following order: birdsfoot trefoil $\geq$ alfalfa > bromegrass > reed canary grass. In addition, lactating cows consume much more feed in relation to body size than do other animals. Therefore, some standardization is necessary when comparing intake data.

1. Expression of Feed Intake

Since voluntary intake is much influenced by forage species and body size, Crampton et al. (31) have developed a method to eliminate the variation in forage intake due to different sizes. They found that the coefficient of variation (CV) in expressing feed intake per unit of metabolic size was only 13% but CV was 20% when based on consumption per animal per day. They arbitrarily suggested that DMI of a standard forage by sheep was 80 $g/BW_{Kg}^{0.75}$

and further suggested that the metabolic size and relative intake terms be used regularly in all intake studies. Expressions of feed intake are illustrated below (31).

Term	Coefficient of Variation
gm or Kg/animal/d	± 20 %
gm or Kg/100 lb(Kg) BW/d	± 14 %
gm or Kg/$BW_{Kg}^{0.75}$/d	± 13 %

$$\text{Relative Intake (RI)} = \frac{\text{gm daily forage DMI}}{80 \times (BW_{Kg}^{0.75})} \times 100$$

Intakes of good temperate grasses are normally higher than 80 g/d (81). However, most tropical grasses have lower maximum intake values with excellent forages having a value of 70 $g/Kg^{0.75}$. Yet maximum intake of chopped tropical forages by sheep was 83 g DM/Kg metabolic weight/day with most values generally below 80 g/d (81). Grieve and Osbourn (46) indicated that expressing voluntary intake of tropical forages based on metabolic size is valid since only 0.2% of the variation in feed intake was due to differences in metabolic size of the wethers.

2. Factors Controlling Voluntary Intake

A more complete discussion on factors influencing intake can be found in the reports by Balch and Campling (9), Conrad (29), Van Soest (117), Ingalls (51). Short statements about main factors that control forage intake follow.

2.1 Central nervous system (CNS) and the hypothalamus may control overall responses of feed intake and hunger drive.

2.2 Thermostatic regulation; warm temperature ($\geq$ 40 C) will decrease feed intake.

2.3 Chemostatic regulation; blood or rumen metabolites act on sensory mechanism so that high levels of ruminal VFA, quinine, NaCl or blood glucose will decrease voluntary intake.

2.4 Lipostatic regulation; increased body fatness decreases intake.

2.5 Oropharyngeal regulation; mouth is a metering system.

2.6 Caloric density; intake stops when enough energy is consumed.

2.7 Reticulo-ruminal size; gut fill limits intake.

2.8 Cell walls; 50-60% or more CW decreases intake.

2.9 Rate of digestion and passage; rapid digestion and passage will increase feed intake.

2.10 Activities and level of production; increased activities and milk production will increase intake.

2.11 Physical forms of feeds; grinding and pelleting increases intake; silage decreases DMI.

2.12 Protein and Mg; low Mg and CP ($\leq$ 7%) decrease intake. Highly fertilized forages (with high NPN) decrease intake.

2.13 Additives; urea decreases intake; molasses increases intake.

2.14 Water; there is a positive relation between water and dry matter intake.

2.15 Contamination; mold, feces, sand, hairy feeds tend to decrease intake.

2.16 Relative humidity; high temperature plus high humidity decrease intake.

2.17 Parasites decrease intake and digestibility.

2.18 Hormones; thyroxine and growth hormone increases feed intake.

c. Forage Digestibility

The common in vivo digestibility terms are DMD and OMD. Digestibility of forages is governed by many factors and some are discussed below.

1. Species and Cultivars

In general, temperate forages are more digestible than tropical forages due to lower CWC, ADF and lignin. Mean digestibility of tropical forages was found to be 12.8 units lower than that of temperate grasses (73,81). At a comparable age, alfalfa is more digestible than orchard grass (3). Leafy species are more digestible than stemmy varieties (73).

2. Age and Maturity

Digestibility decreases with advancing maturity (81). Immature forages and those with high CC are more

digestible because CC might supply readily available nutrients to the microbial population (39). Digestible dry matter of temperate grasses decreases steadily at the rate of 0.4-0.5 percentage unit per day from initial date of growth in the spring (3,51,93,94). The rate of change in DMD of tropical grasses ranges from a decrease of 0.7% to an increase of 1.3% units per day from initial growth (81). Minson and McLeod (80) reported that the digestibility of tropical grasses in Australia decreased at 0.2 percentage unit per day in summer regrowth compared with 0.1 unit/day in autumn regrowth.

However, Grieve and Osbourn (46) reported that tropical grasses in Trinidad showed an increase in DMD and GE up to 5 weeks of regrowth but the digestibility decreased rapidly after that period. The effect of stage of maturity on nutritive value of forages is presented in Table 1.

3. Chemical Composition

Chemical composition appears to be more related to digestibility than to intake (81). A decrease in CP, ash, soluble carbohydrates along with an increase in CWC, ADF, L, methoxyl and Si results in decreased digestibility.

4. Fertility Level

The effect of fertilization on digestibility is variable. The digestibility of fertilized "improved" tropical grasses is similar to that of the temperate

TABLE 1. The effects of stage of maturity on intake, DDM and NVI of two forages.

Forage and Maturity	DDM	DMI	NVI
Timothy Hay (Temperate)[a]	%		
Early Bloom	65	1593[c]	75
Half Bloom	57	1487	60
Full Bloom	51	1242	46
Post Bloom	48	1079	37
Bermuda (Tropical)[b]			
3-Week	59	70[d]	52
4-Week	65	88	73
6-Week	55	77	49

DDM = Digestible dry matter; DMI = Dry matter intake; NVI = Nutritive value index.

[a]Lloyd et al., 1961. J. Anim. Sci. 20:468.

[b]Grieve and Osbourn, 1965. J. Agr. Sci. 65:411.

[c]g/animal/d.

[d]$g/Kg^{0.75}/d$.

grasses (98). Fertilization with zero to 448 Kg N/ha increased protein digestibility but not DMD or CD of first and second cut timothy hay (97).

5. Feed Preparation

The digestibility of forages is greatly affected by particle size. The reduction of particle size by grinding or pelleting will enhance voluntary intake but decreases its digestibility (81,128).

6. Level of Feeding

The digestibility of forage is decreased when the level of feeding is increased. Forages probably do not remain in the rumen sufficiently long for maximum fermentation and degradation of CWC by rumen microorganisms (81).

7. Animal Species

The animals themselves may have different efficiencies for forage digestion. Sheep digest concentrate more efficiently than cattle whereas cattle digest dry roughage to a greater extent than do sheep (99). Butterworth (20) reported that the digestion of ruminants under tropical conditions may differ from that found in the temperate regions.

VII. RELATIONSHIPS AMONG CHEMICAL COMPONENTS, LABORATORY VALUES AND IN VIVO DATA

a. Relationships Among Chemical Components of Forages

The relationships among chemical constituents are influenced by stage of maturity, fertilization, heat, light, etc. as discussed previously. By definition, there is a negative relationship between CWC and CC and thus when CWC, ADF, C, L increase CP, sugars, lipids, vitamins, minerals and other soluble materials will decrease. The negative nature of the CP:CW relationship and the positive nature of relations among fractions of CW are given in Table 2 for both tropical and temperate forages.

TABLE 2. Some simple correlation coefficients among various chemical components.

Items	Temperate[1] Forages	Tropical[2] Forages
CWC vs. CP	- .78**	- .63**
CWC vs. C	0.71**	0.68**
CWC vs. L	0.17	0.45**
L vs. C	0.48**	0.38**
L vs. ADF	0.65**	0.65**

[1]Van Soest. 1965. J.A.S. 24:834.

[2]Kayongo-Male et al., 1972.

**p < .01

For abbreviations, see Appendix Table 1.

b. Relationships Between Cell Walls and Measures of Nutritive Value of Forages

Cell wall contents not only have a great influence on concentrations of other components, but also have significant effects on forage digestibilities, DMI and ADG as shown in Table 3.

An increase in CWC definitely decreases in vivo DDM, OMD, CWD, ED, IVDMD, IVTDMD of both tropical and temperate forages but with differing magnitudes of depression. High CWC will significantly lower the NVI of grasses and legumes. Dry matter intake and RI are significantly depressed by high CWC and finally ADG is reduced due to low DMI caused by high CW concentrations.

TABLE 3. Some correlations between CWC and measures of forage nutritive value.

Factors correlated	r	Forage Type	Reference
CWC vs. In Vivo DDM	-.48**	Grasses	88
" "	-.74**	Legumes	88
" "	-.86**	Alfalfa	88
" "	-.47**	Gra. + Leg.	88
" "	-.50**	Low ADF diet	8
" "	-.32**	Gra. + Leg.	58
" "	-.45**	Forages	123
" "	-.20	"	51
CWC vs. In Vitro DMD	-.84**	Trop. gra.	81
" "	-.14	Corn plant	12
" "	-.22*	Trop. gra.	64
CWC vs. In Vitro TDMD	-.69**	"	81
CWC vs. In Vivo DCW	0.85**	Low ADF diet	8
CWC vs. CWD	-.67**	Trop. gra.	81
CWC vs. DCW	0.73**	All forages	120
CWC vs. OMD	-.81**	Trop. gra.	81
CWC vs. ED	-.38**	Gra. + Leg.	58
CWC vs. NVI	-.63**	"	58
CWC vs. RI	-.56**	"	58
CWC vs. DMI	-.70**	All forages	51
"	-.66**	"	51
"	-.76**	"	123
"	-.77**	"	77
"	-.65**	"	117
CWC vs. ADG	-.80**	Trop. gra.	81

*P < .05 **P < .01

For abbreviations, see Appendix Table 1.

c. Relationships Between ADF and Nutritive Values of Forages

Acid-detergent fiber has a negative relationship to CP but positive relationships to CWC, C and L (64,117) and has significant negative effects on forage digestibilities, consumption and nutritive value as illustrated in Table 4.

There was a significant and negative relation between ADF and *in vivo* DDM, CD, DP, ED, IVDMD and IVTDMD for all forages studied (Table 4). This indicates that ADF alone can be used to predict forage digestibilities with moderate accuracy. The variation in correlation indicates that prediction equations should be developed for each species at each location. High concentrations of ADF decreased NVI of forages and depressed forage DMI except in one case.

d. Relationships Between MADF and In Vivo Data of Forages

Modified acid-detergent fiber (MADF) was highly correlated with *in vivo* DDM ($r = -.85$, $P < .001$) whereas the correlation coefficient between ADF and DDM was only $-.70$ ($P < .001$). The prediction of *in vivo* DDM from MADF had an error of 5.63% compared with 8.99% when using ADF. Besides, MADF was also highly correlated ($r = -.82$, $P < .001$) with DMI with about 7% standard error of estimate. Apparently MADF may be preferable to ADF to predict both intake and digestibility of forages and this method can be adapted to any routine forage evaluation system (25).

TABLE 4. Simple correlations between ADF and other nutritive values of forages.

Factors correlated	r	Forage Type	Reference
ADF vs. In Vivo DDM	-.39**	Grasses	88
" "	-.76**	Legumes	88
" "	-.84**	Alfalfa	88
" "	-.80**	Orchard	88
" "	-.53**	Gra. + Leg.	88
" "	-.85**	Forages	131
" "	-.78**	Gra. + Leg.	115
" "	-.75**	"	123
" "	-.74**	All forages	58
" "	-.70**	Gra. + Leg.	25
" "	-.66**	"	51
ADF vs. IVDMD	-.90**	Corn Plant	12
" "	-.38**	Trop. grasses	64
ADF vs. IVTDMD	-.82**	"	81
ADF vs. In Vivo CD	-.89**	Forages	131
ADF vs. Dig. ADF	+.50**	All forages	120
ADF vs. DP	-.85**	Forages	131
ADF vs. ED	-.76**	All forages	58
ADF vs. RI	-.31**	"	58
ADF vs. NVI	-.61**	"	58
ADF vs. DMI	-.64**	"	123
" "	-.53**	"	117
" "	+.37	"	51

*P < .05 **P < .01

See Appendix Table 1 for abbreviations.

e. Relationships Between Lignin and Measures of Forage Nutritive Value

Legumes contain up to 2-3 times more lignin than do the grasses (51,95,119,122). Lignin in grasses is more alkali-soluble than that of legumes (72,119). Lignin itself is not digestible but inhibits the digestibility of CWC, C and HC. However, L does not affect the digestibility of CC (122). Lignin probably decreases digestibility by forming incrustations and complexes with CHO, C, HC, etc. (115).

The use of L as a predictor of digestibility is excellent within the same forage species (123). Usually lignin is negatively related to other measures of *in vivo* nutritive value such as intake and digestibility as can be seen from Table 5.

As lignin content increased, there were significant decreases in CD, *in vivo* DDM, DP, ED, IVDMD and IVTDMD of all forages studied. The NVI of forages decreased slightly with an increase in L content. Also lignin had a low correlation with DMI but in one case L was positively correlated with DMI (51) and in another case the correlation between L and RI was positive (58). The positive correlation between lignin and intake when all forages are combined may be somewhat complicated by the greater intake of legumes than grasses and the greater lignin content of legumes as compared to grasses.

With high correlation coefficients between L and DDM in either grasses, forage legumes or within one species,

TABLE 5. Some correlations between lignin and forage nutritive values.

Factors correlated	r	Forage Type	Reference
L vs. In Vivo DDM	-.62**	Grasses	88
" "	-.81**	Legumes	88
" "	-.95**	Reed Canary	88
" "	-.46**	Gra. + Leg.	88
" "	-.72**	Low ADF diet	8
" "	-.50*	Forages	51
" "	-.88**	"	131
" "	-.80**	Alf.,B,Tim.	88
" "	-.82**	Grasses	115
" "	-.74**	Leg.	115
" "	-.40**	Gra. + Leg.	115
" "	-.95**	Mixed Forages	111
" "	-.64**	All Forages	58
L vs. IVDMD	-.76**	Trop. grasses	81
" "	-.69**	Corn plant	12
" "	-.92**	All forages	84
" "	-.96**	Dried grasses	84
" "	-.16	Trop. grasses	64
L vs. IVTDMD	-.80**	Trop. grasses	81
L vs. In vivo CD	-.88**	Forages	131
L vs. DP	-.84**	"	131
L vs. ED	-.60**	"	58
L vs. DMI	+.78**	"	51
" "	-.13	"	117
" "	-.10	"	123
L vs. RI	+.21	"	58
L vs. NVI	-.11	"	58

*P < .05 **P < .01

For abbreviations, see Appendix Table 1.

L can be used to predict forage digestibilities with higher accuracy than using CWC or ADF alone.

f. Relationships Between Cellulose and In Vivo Data of Forages

Cellulose content differs somewhat among grass species but on the average the content in both grasses and legumes is similar. Alfalfa C has greater resistance to hydrolysis by cellulase than C from grasses of similar digestibility (54). The relationships between C and other in vivo parameters are shown in Table 6.

TABLE 6. Some correlations between cellulose and measures of forage nutritive value.

Factors correlated	r	Forage Type	Reference
C vs. In vivo DDM	-.60**	Low ADF diet	8
" "	-.62**	Forages	131
" "	-.40**	Gra. + Leg.	60
" "	-.81**	" "	25
C vs. IVDMD	-.75**	Corn plant	12
" "	-.35**	Trop. grasses	64
C vs. IVTDMD	-.64**	" "	81
C vs. In vivo CD	-.60**	Forages	131
" "	0.25*	Gra. + Leg.	60
C vs. DP	-.64*	Forages	131
C vs. DC	0.95**	Low ADF diet	8
" "	0.67**	All forages	120
C vs. ED	-.46**	Gra. + Leg.	60
C vs. DMI	-.59**	All forages	117
C vs. RI	-.75**	Gra. + Leg.	60
C vs. NVI	-.78**	" "	60

*P < .05 **P < .01

For abbreviations, see Appendix Table 1.

All data indicate a negative relationship between C and *in vivo* DDM, CD, DP, ED, IVDMD and IVTDMD of both grasses and legumes. An increase in C content would significantly reduce the NVI of forages and result in a significant decrease in both RI and DMI. The predictability of *in vivo* DDM using C may not be satisfactory for some combined forages due to low correlation coefficients between these 2 factors but in many cases C content can predict DDM of each forage species with moderate to high accuracy.

g. Relationships Between HC and Other Nutritive Values of Forages

Grasses may contain up to 4 times the amount of HC found in legumes. Legume HC is less digestible than HC from grass species (120). Hemicellulose is neither chemically nor nutritionally uniform since it contains variable proportions of pentose, hexose and their derivatives as well as pectin (120,121). Its relationship to digestibility is low (Table 7). Therefore, HC may not be useful as a sole predictor for *in vivo* digestibility of forages.

h. Relationships Between Silica and Digestibility or Weight Gain

In temperate grasses, plant silica causes a decline in digestibility of about 3 units per 1 unit of Si (43). The correlation coefficient between Si and DDM in reed canary grass was highly significant ($r = -.86$, $P < .01$) whereas that between L and DDM was only -.58 (122).

TABLE 7. Some correlations between HC and other nutritive values of forages.

Factors correlated	r	Forage Type	Reference
HC vs. In vivo DMD	0.02	Low ADF diet	8
HC vs. IVDMD	-.13	Corn plant	12
" "	0.03	Trop. grasses	64
HC vs. Dig. HC	0.94**	All forages	120
HC vs. CP	-.26**	Trop. grasses	64
HC vs. IVTDMD	-.45**	"	62

**P < .01

For abbreviations, see Appendix Table 1.

Coward-Lord et al. (30) reported that plant Si caused a decline in in vivo dry matter digestibility of 3-5 units per 1 unit of silica in tropical grasses. However, Si did not significantly depress IVDMD and IVTDMD of tropical grasses (64,81). In addition, body weight gains of growing finishing lambs were significantly affected by adding soluble Si (sodium silicate) to their drinking water at a concentration of 800 mg/l (108). Feed efficiency of these lambs was also decreased. Silica may exist in forages in various forms thus quantitative relations between total Si and nutritive value may not be high.

i. Relationships Between CP and Other Measures of Forage Nutritive Value

Five to 10% of total N is bound with lignin in CWC and is indigestible (27,119). When CP level in forage is

less than 6%, digestibility of total carbohydrates is markedly decreased (73). There are highly positive correlations between CP and several measures of nutritive value but negative correlations are also noted for CWC and other fibrous constituents (Table 8).

An increase in CP level is normally followed by increases *in vivo* DDM, CD, DP, ED, NVI, RI, DMI, IVDMD, IVTDMD and IVCWD. However, the relationships between CP and forage digestibilities are variable depending on types of forages and other factors. The correlations between CP and intake or NVI are rather low and the use of CP as a sole predictor of *in vivo* intake or digestibility may not be satisfactory. The use of CP to predict fiber fractions or vice versa provides only moderate accuracy.

j. Relationships Between Two-stage In Vitro Fermentation Value and Other Measures of Forage Nutritive Value

The two-stage *in vitro* fermentation procedure (IVDMD or IVOMD) has been widely accepted as a useful technique to predict forage digestibility or its nutritive value. In tropical grasses, *in vivo* OMD can be predicted from IVOMD with a correlation of 0.93 ($P < .01$) and a standard error of 3.98%. The correlation coefficient between IVOMD and IVDMD in mixed forages was 0.98 ($P < .01$) (81).

The relationships between two-stage *in vivo* fermentation values and other measures of nutritive value are shown in Table 9. Digestible DM, true digestibility and

TABLE 8. Some correlations between CP and other measures of forage nutritive value.

Factors correlated	r	Forage Type	Reference
CP vs. In vivo DMD	0.21	Grasses	88
" "	0.76**	Legumes	88
" "	0.11	Low ADF diet	8
" "	0.85**	Forages	131
" "	0.58**	Gra. + Leg.	60
CP vs. IVDMD	-.24	Corn plant	12
" "	0.20**	Trop. grasses	64
CP vs. IVTDMD	0.46**	" "	64
" "	0.82**	" "	81
CP vs. In vivo CD	0.83**	Forages	131
" "	0.23*	Gra. + Leg.	60
CP vs. IVCWD	0.27**	Trop. grasses	64
CP vs. DP	0.97**	Low ADF diet	8
" "	0.99**	All forages	120
CP vs. ED	0.61**	Gra. + Leg.	60
CP vs. NVI	0.62**	" "	60
CP vs. RI	0.47**	" "	60
CP vs. DMI	0.54**	All forages	117
CP vs. EDDM	0.38**	Trop. grasses	64
CP vs. ETD	0.27**	" "	64
CP vs. CWC	-.63**	" "	64
CP vs. ADF	-.68**	" "	64
CP vs. C	-.69**	" "	64
CP vs. L	-.48**	" "	64
CP vs. Si	+.17	" "	64

*P < .05 **P < .01

For abbreviations, see Appendix Table 1.

ED of different feeds and forages can be satisfactorily predicted from IVDMD. With these high correlation coefficients, one prediction equation may be satisfactory to predict in vivo DDM of both grasses and legumes as suggested by Tilley and Terry (112).

The correlations between IVDMD and estimated digestibilities (summative equations) in tropical grasses are very low (r = 0.05 to 0.06) indicating that the summative equation developed from temperate forages may not be satisfactory with tropical forages.

The method (IVDMD) may be used to predict ADG and NVI of forages with moderate accuracy but it is not satisfactory to routinely predict either RI or DMI.

k. Relationships Between In Vitro True Dry Matter Digestibility (IVTDMD) and Forage Nutritive Value

Van Soest et al. (126) developed the method for IVTDMD and reported that in vivo apparent digestibility was positively and significantly related to IVTDMD (r = 0.96) and the prediction of apparent digestibility had a standard error of 2.8%. The correlation between IVTDMD and in vivo true digestibility was exceptionally high (r = 0.98) and the prediction of the latter had a standard error of only 1.7%.

The relationship between IVDMD (Tilley and Terry) and IVTDMD was also high (r = 0.95) with a standard error of prediction of 3%. Regarding chemical composition, Johnson and Pezo (62) reported that only CWC showed a high

TABLE 9. Some correlations between IVDMD and other measures of forage nutritive value.

Factors correlated	r	Forage Type	Reference
IVDMD vs. *In vivo* DDM	0.83**	Grasses	88
" "	0.97**	Legumes	88
" "	0.89**	Forages	131
" "	0.93**	"	11
" "	0.73**	Tall Fescue	18
" "	0.95**	Grasses	76
" "	0.97**	Alfalfa	76
" "	0.99**	Hi-roug. diet	76
" "	0.99**	Lo-roug. diet	76
" "	0.96**	Gra. + Leg.+woods	76
" "	0.97**	Forages	10
" "	0.93**	"	126
" "	0.90**	Gra. + Leg.	58
IVDMD vs. IVTDMD	0.95**	Forages	126
IVDMD vs. *In vivo* TDMD	0.92**	"	126
IVDMD vs. IVTDMD	0.65**	Trop. grasses	64
IVDMD vs. EDDM	0.05	" "	64
IVDMD vs. ETD	0.06	" "	64
IVDMD vs. ED	0.89**	Tall fescue	18
IVDMD vs. IVCWD	0.66**	Trop. grasses	64
IVDMD vs. VFA	0.81**	" "	2
IVDMD vs. ADG	0.78**	" "	81
IVDMD vs. DMI	0.51*	Forages	10
IVDMD vs. RI	0.08	All forages	58
IVDMD vs. NVI	0.46**	" "	58
" "	0.76**	" "	10

*P < .05 **P < .01

For abbreviations, see Appendix Table 1.

but negative correlation with IVTDMD for all grasses and legumes in temperate and tropical regions. Therefore, IVTDMD might be predicted from either CWC or the two-stage IVDMD.

Since the numerical values for IVTDMD are close to or equal to actual in vivo true digestibilities, this method was proposed as the most accurate one to predict in vivo digestibilities (126). The method requires less time than the two-stage IVDMD and it gives satisfactory results when used to evaluate both temperate and tropical forages (30,62,126).

1. Relationships Between Cell Wall Digestibility (CWD) and Other Measures of Nutritive Value of Forages

The digestibility of CWC or ADF in grasses is higher than that in legumes due to a lower lignin content in grass CWC. Cell wall digestibility decreases with advancing maturity along with the lowering of HC and C digestibilities (91). The rate of CWD was positively correlated ($r = 0.77$, $P < .05$) with cell contents even though CC did not contribute directly to a faster rate of CWD (110). On the other hand, rate of CWD or total CWD was negatively correlated with CWC, ADF, L, C, Si and L/ADF, L/C and L/HC ratios.

In tropical grasses, an increase in ADF was followed by a significant decrease in CWD whereas ratios of L/ADF, L/C did not have any significant effect on CWD. However, increasing L/HC seemed to decrease CWD in tropical forages.

In temperate forages, CWD was significantly decreased by an increase in L and L/C ratio whereas L/ADF ratio did not have significantly negative effect on CWD. Some data in Table 10 do not agree with that of Goering and Van Soest (43) who found a significantly negative correlation between L/ADF and CWD and used logarithm of L/ADF when calculating CWD.

In vitro CWD was highly correlated with two-stage IVDMD, in vitro true digestibility and OMD. This indicates that CWD is one of the main factors controlling in vivo forage digestibilities. In fact, the actual value of IVCWD is similar to that for in vivo CWD (36).

m. Relationships Between In Vitro Cellulose Digestibility (IVCD) and Other Nutritive Values of Forages

Cellulose digestibility in vitro is another laboratory technique used to estimate DMI, digestibility and NVI of forages. IVCD (12-hr) measurements are highly correlated with in vivo data for grasses (60). Cellulose digestibility also decreases with advancing maturity and the rate of CD is rapid within the first 12 hours then decreases (38).

The correlations between IVCD and other measurements in Table 11 indicate that in vivo DDM has a moderate relationship to IVCD. The correlation coefficients between IVCD and in vivo CWD or DE are sufficiently high to assure that these two parameters could be accurately predicted from IVCD. Dry matter intake of forages had a high correlation

TABLE 10. Some correlations between CWD and other measures of forage nutritive value.

Factors correlated	r	Forage Type	Reference
IVCWD vs. IVTDMD	0.92**	Trop. grasses	64
IVCWD vs. IVDMD	0.66**	"	64
IVCWD vs. EDDM	0.14	"	64
IVCWD vs. OMD	0.97**	"	81
IVCWD vs. CP	0.27**	"	64
IVCWD vs. CWC	-.21*	"	64
IVCWD vs. ADF	-.36**	"	64
IVCWD vs. L	-.26**	"	64
IVCWD vs. L/ADF	-.11	"	64
IVCWD vs. C	-.28**	"	64
IVCWD vs. L/C	-.16	"	64
IVCWD vs. HC	0.02	"	64
IVCWD vs. L/HC	-.23*	"	64
IVCWD vs. Si	-.13	"	64
IVCWD vs. L	-.88**	Temp. Gra. + Leg.	109
IVCWD vs. L/ADF	-.60	"	109
IVCWD vs. L/C	-.82**	"	109
IVCWD vs. CC	0.77*	"	110

*P < .05 **P < .01

For abbreviations, see Appendix Table 1.

with short-time, 12-hr IVCD. Longer time IVCD (36-48 hrs) showed high correlations with NVI and TDN and might be used to predict NVI and TDN of both tropical and temperate forages with reasonable accuracy.

TABLE 11. Some correlations between IVCD and measures of nutritive value.

Factors correlated	r	Forage Type	Reference
IVCD vs. In Vivo DMD	0.75**	Gra. + Leg.	88
" "	0.61**	All forages	58
" "	0.72**	Gra. + Leg.	60
" "	0.49**	Tall Fescue	18
IVCD(36-hr) vs. In Vivo DMD	0.95**	Trop. grasses	7
IVCD vs. In Vivo CD	0.88**	Forages	131
" "	0.89**	Tall Fescue	18
" "	0.48**	Gra. + Leg.	60
IVCD(24-hr) vs. In Vivo CD	0.93**	Non-legumes	86
IVCD(24-hr) vs. DE	0.90**	"	86
" "	0.73**	All forages	86
" "	0.87**	"	38
IVCD(36-hr) vs. DE	0.94**	Trop. grasses	7
IVCD vs. ED	0.76**	Gra. + Leg.	60
" "	0.64**	All forages	58
IVCD(12-hr) vs. DMI	0.83**	Forages	38
IVCD vs. RI	0.50**	"	60
IVCD vs. NVI	0.71**	"	60
IVCD(48-hr) vs. NVI	0.85**	Trop. grasses	7
IVCD(36-hr) vs. TDN	0.94**	"	7

**P < .01

For abbreviations, see Appendix Table 1.

n. Relationships Between Dry Matter Solubility (DMS) and Other Measures of Nutritive Value of Forages

The mere solubility of the dry matter under standardized conditions has been proposed as a simple but useful technique for evaluating several forages. It has high correlations with many in vivo parameters such as DDM, CD, ED

and intake (Table 12). As with many other laboratory techniques, the relationships are greater within one plant species than when species are combined. Most of the correlations between DMS and forage digestibilities are not as great as those for two-stage IVDMD. Therefore, the prediction of *in vivo* digestibilities using DMS may not be satisfactory. However, forage NVI might be estimated from DMS in both grasses and legumes since the correlation coefficients are 0.67 to 0.83.

TABLE 12. Some correlations between DMS and other measures of forage nutritive value.

Factors correlated	r	Forage Type	Reference
DMS vs. *In Vivo* DMD	0.60**	Grasses	88
" "	0.71**	"	60
" "	0.76**	Legumes	88
" "	0.87**	Alfalfa	60
" "	0.54**	Gra. + Leg.	88
DMS vs. ED	0.52**	Mixed forages	58
" "	0.71**	Grasses	60
" "	0.87**	Alfalfa	60
DMS vs. CD	0.68**	Grasses	60
" "	0.40	Alfalfa	60
DMS vs. RI	0.52**	Mixed forages	58
" "	0.79**	Grasses	60
" "	0.55**	Alfalfa	60
DMS vs. NVI	0.83**	"	60
" "	0.81**	Grasses	60
" "	0.67**	Mixed forages	58

*P < .05 **P < .01

For abbreviations, see Appendix Table 1.

o. Relationships Between Cellulose Solubility in CED and Other Measures of Nutritive Value of Forages

The solubility of cellulose or other plant material in cupriethylene diamine (1.0 M) has been used to measure forage nutritive value. There are significant correlations between cellulose solubility in CED and *in vivo* DDM, CD, ED, intake and NVI. The relationships between CED solubility and digestibilities were slightly higher than those between DMS and digestibilities. Again, the correlations within one plant species were greater than when species were combined. The relationships between CED and RI or CED and NVI were variable and low, so the use of CED as a predictor may not be satisfactory.

p. Relationships Between Turbidity Test and Other Components

Bennett and Archibald (13) and Archibald *et al.* (5) found highly significant correlations between turbidity in forage extracts and some chemical constituents. With various forage samples in two studies, the relationships between turbidity test and CP were positive ($r = 0.86$ and 0.52, $P < .01$) and negative with CF ($r = -.82$ and $-.45$, $P < .01$) and also positive with total ash ($r = 0.65$ and 0.53, $P < .01$). These relationships have never been used for prediction purposes.

q. Relationship Between Intake and Other Factors

Voluntary intake is not highly correlated with digestibility except in a limited number of forage species (10,81,117). However, DMI is highly correlated with

TABLE 13. Some correlations between cellulose solubility in CED and other measures of nutritive value.

Factors correlated	r	Forage Type	Reference
CED vs. In Vivo DMD	0.69**	Grasses	88
" "	0.92**	"	34
" "	0.57*	Alfalfa	60
" "	0.69**	Legumes	88
" "	0.67**	Gra. + Leg.	88
" "	0.52**	All forages	58
CED vs. In Vivo CD	0.92**	Grasses	34
" "	0.50*	Alfalfa	60
CED vs. In Vivo ED	0.90**	Grasses	34
" "	0.55**	Alfalfa	60
" "	0.46**	All forages	58
CED vs. RI	-.16	"	58
" "	0.71**	Alfalfa	60
" "	0.60*	Grasses	34
CED vs. NVI	0.76**	"	34
" "	0.76**	Alfalfa	60
" "	0.08	All forages	58

*P < .05 **P < .01

For abbreviations, see Appendix Table 1.

DDMI, and DEI (51). Among the chemical components, CWC seems to be one of the best predictors of intake (117). However, for tropical grasses ADF may be the better predictor of OM intake (81). Dry matter disappearance (DMD) after 6 hours of incubation with rumen fluids or rumen fluids plus pepsin gave very high correlations with DMI (0.83), DDMI (0.85), DEI (0.90), (10,51,77). Thus a 6-hr DMD value can be used to predict DMI with reasonable accuracy.

r. Relationship Between Digestibility and Other Factors

The relationships between in vivo digestibility and chemical composition have been previously discussed. Acid-detergent fiber, Si, L have significantly negative correlations with digestibility whereas IVDMD, IVOMD, IVCD, IVTDMD, TSAE, CP have significantly positive correlations with the digestibility (11,48,117,126). Digestibility and intake are positively correlated in some cases but are negatively correlated in others.

VIII. SOME CHARACTERISTIC DIFFERENCES BETWEEN TROPICAL AND TEMPERATE FORAGES

a. Histochemical Differences

In their review, Moore and Mott (81) tabulated the following differences between tropical and temperate grasses:

1. The carbon pathways for photosynthesis are different.
2. Photorespiration is lower in tropical than in temperate grasses.
3. The maximum level of photosynthesis is higher in tropical than in temperate grasses.
4. Transpiration may be less in tropical than in temperate grasses and tropical grasses use less water per gram of DM produced during growth.
5. Leaf anatomy differs especially with reference to the development and distribution of vascular bundles.

b. Differences in Chemical Composition

When compared with temperate grasses cut at similar stages of growth, tropical grasses, on the average, have lower levels of CP, TDN but higher CWC, ADF, CF, L, C and Si (30,73,81,98). Hemicellulose content of temperate grasses increases slightly with maturity (127) but it does not change with maturity in tropical grasses (30).

For tropical grasses, the contents of CWC, ADF, L and Si are higher than those of temperate grasses as illustrated below (2,30,64,81,122,123):

	Temperate grasses	Tropical grasses
CWC	34 - 73	45 - 83
ADF	18 - 46	21 - 57
L	1 - 11	2 - 12
Si	0.5 - 4	1 - 5

Some of these workers reported that CWC of most tropical grasses exceeds 65% while that for temperate grasses may be lower than this value. Grasses of temperate origin accumulate fructosans while common biennial and perennial legumes accumulate starch and sucrose (105). Grasses of subtropical and tropical origin accumulate starch and sugars such as sucrose, glucose, fructose and traces of glucofructosans (15,105). Besides, tannic acid is higher in many tropical forages such as Desmodium, Paspalum, and Digitaria (96). Large changes in chemical composition occur between 30 to 60 days for tropical forages. CWC, C, ADF, L increase with advancing maturity from 30 to 180 days of age (30).

c. Digestibility, Intake and Yield

Tropical grasses have lower digestibility and intake than temperate grasses cut at similar stages of growth (30,73,81,98). Dry matter and energy digestibility of tropical forages tends to increase up to 4 to 5 weeks of age then declines gradually thereafter and the rate of decline is less than that of temperate forages (30,46,81). Many tropical grasses have higher DM yields than temperate grasses (73).

IX. PREDICTION EQUATIONS

In order to have accurate prediction equations, *in vivo* data used in deriving such equations must also be accurate. Also any prediction equation is valid only for the type and species of forages used in its development. One desirable test before universal acceptance of any developed equation is to apply the equation to another or different set of samples having known animal values and thus verify the prediction equation (123). At present, there are hundreds of proposed prediction equations using different components and *in vitro* data and these are presented in Tables 14 to 22.

a. Prediction of In Vivo Digestible Dry Matter (DDM)

From Table 14, *in vivo* DDM can be predicted from many chemical components and *in vitro* data. Crude protein, CF and CF plus NFE show high correlations with DDM but the standard errors of prediction are too large (8 - 10%) to guarantee their application. Lignin and MADF may be better

TABLE 14. Equations used to predict *in vivo* digestible dry matter (DDM).

In Vivo DDM (Y) and/or IVDMD	Forage Type	r	SEE	Reference
$Y = 41.81 + 1.63\ CP$	TrG	0.94**	≤10	19
$Y = 120.03 - 1.778\ CF$	TrG	-.72**	--	19
$Y = 150.88 - 1.18\ (CF + NFE)$	TrG	0.98**	≤ 9	19
$Y = 100.37 - 6.08\ L$	TeM	-.94**	2.1	111
$Y = 107.30 - 1.32\ MADF$	TeM	-.85**	4.7	25
$Y = 59.20 - 2.75\ L - 0.24\ HC + 0.66\ CP + 0.50\ C$	TeM	0.80**	--	27
$Y = 0.98\ CC + CWC(147.3 - 78.9 \log \frac{L}{ADF}\ 100) - 12.9$	TeM	0.96**	2.7	118
$Y = 89.02 - 0.50\ ADF - 2.09(ADF\ P) - 0.36\ (TP - ADFP)$	TeM	0.75**	--	132
$Y = 15.50 + 0.67\ CED$	TeG	0.92**	--	34
$Y = 36.80 + 1.42\ \frac{(CED \times DMS)}{100}$	TeG	0.87**	--	60
$Y = 48.30 + 1.20\ \frac{(IVCD \times DMS)}{100}$	TeM	0.71**	--	60
$Y = 12.84 + 1.23(36\text{-hr IVCD})$	TrG	--	--	7
$Y = 0.99\ IVDMD - 1.01$	TeM	--	2.3	112
$Y = 0.85\ IVDMD + 8.37$	TeL	--	1.4	112
$Y = 16.70 + 0.74\ IVDMD$	TeM	0.88**	2.9	88
$Y = 0.96\ IVTDMD - 10.40$	TeM	0.96**	2.8	126
$Y = 1.04\ IVDMD - 4.30$	TeM	0.97**	1.3	10
$Y = 0.90\ IVDMD + 4.00$	TeM	0.93**	3.7	126

TABLE 14. Continued.

In Vivo DDM (Y) and/or IVDMD	Forage Type	r	SEE	Reference
Y = 7.10 + 0.83 IVDMD	TeM	--	2.1	58
Y = 0.75 IVDMD + 18.50	Sil	0.77**	4.3	89
Y = 24.51 + 0.63 IVDMD	TrG	0.84**	3.3	98
Y = 23.17 + 0.64 IVDMD	TrM	0.74**	4.4	98
Y = 85.00 - 0.48 (Days after regrowth)	TeG	--	1.6	93
IVDMD = 80.70 - 0.31 (Days after initial growth)	TrG	--	--	98
IVDMD = 75.92 - 0.22 (Days after initial growth)	TeG	--	--	98

TeG = Temperate grasses; TeL = Temperate legumes; TeM = Temperate mixed;
TrG = Tropical grasses; TrL = Tropical legumes; TrM = Tropical mixed;
Sil = Silages; r = correlation coefficient;
SEE = Standard error of estimate;
*p < .05 **p < .01

For abbreviations, see Appendix Table 1.

than CP, CF, CF + NFE because they give high correlations with DDM and the standard errors of prediction are much more smaller (2 - 5%).

The use of multiple variables as combinations of CC, CWC, L/ADF (118), L, HC, CP, C (27), ADF, ADF-P, TP-ADFP (132) to predict DDM did not significantly increase the magnitude of the correlation coefficient nor decrease the standard error of estimate. For instance Van Soest's equation using CC, CW, L/ADF gave standard error of estimate similar to that for the equation using L alone (compare Equations for Ref. 111 with that for 118, Table 14). Cellulose solubility in CED or CED x DMS may be satisfactory to predict DDM for grasses but the prediction of DDM from IVCD x DMS gave lower accuracy for mixed forages than did previously mentioned factors.

The two-stage IVDMD has proven to be an excellent method to predict in vivo DDM since it gives high predictability for DDM in temperate forages with standard errors ranging from 1.3 to 3.7%. However, the method gives low but acceptable predictability for DDM in tropical forages and in silages with standard errors ranging from 3.3 to 4.4%.

b. Prediction of In Vivo True Digestibility

In vivo true digestibility (Table 15) can be precisely predicted from in vitro TDMD with small standard errors (1.7 to 2.0%). The two-stage IVDMD procedure can also be used to predict in vivo true digestibility

TABLE 15. Equations proposed for use in predicting *in vivo* true digestible dry matter (TDDM).

In vivo TDDM (Y) and/or IVTDMD	Forage Type	r	SEE	Reference
Y = 0.88 (*In Vivo* App. Dig) + 21.20	TeM	0.98**	1.8	126
Y = 0.81 IVDMD + 23.70	TeM	0.92**	3.4	126
Y = 0.89 IVTDMD + 8.60	TeM	0.98**	1.7	126
Y = 6.20 + 0.92 IVTDMD	TeM	0.98**	2.0	75
Y = 42.70 + 0.61 TSAE (cellulase)	TeM	0.87**	10.4	75
IVTDMD = 0.92 IVDMD + 16.20	TeM	0.95**	3.0	126
IVTDMD = 116.90 - 0.596 CW - 1.677 L	TrM	0.79**	CV 12%	62
IVTDMD = 117.20 - 0.761 C - 0.586 HC - 1.926 Per. Lignin	TrM	0.80**	CV 12%	62
IVTDMD = 110.40 - 0.68 CWC	TrM	0.71**	CV 14%	62
IVTDMD = 110.00 - 0.81 CWC	TrL	0.87**	CV 7%	62

but with a larger standard error (3.4%) than the first method. Other laboratory methods such as TSAE gave low predictability for true digestibility with a very large standard error (10.4%).

True digestibility *in vitro* has been predicted from a two-stage IVDMD procedure with reasonable accuracy. The use of cell wall constituents to predict IVTDMD in tropical legumes gave moderate accuracy whereas combinations of CWC, L or C, HC, L still gave low predictability for IVTDMD.

c. Prediction of In Vivo Organic Matter Digestibility (OMD)

Like apparent DDM, *in vivo* OMD (Table 16) can not be satisfactorily predicted from CP or CF + NFE because of large standard errors, but OMD *in vivo* can be predicted more precisely from *in vitro* OMD with a low standard error of about 2%. Lignin in OM can be satisfactorily used to predict *in vivo* OMD in either grasses or legumes but L gives larger standard error for mixed forages. A summative equation for *in vivo* OMD using CC, CWC, L/ADF ratio gives satisfactory predictability with a 3% standard error. Total residue after cellulase (TRAC) and TSAE can be used to predict *in vivo* OMD or DOM of forages with reasonable accuracy. For silages, *in vivo* OMD or DOM can be predicted from *in vitro* OMD with about a 4% standard error.

TABLE 16. Equations proposed for predicting *in vivo* organic matter digestibility (OMD) and digestible organic matter (DOM).

In Vivo Digestibility	Forage Type	r	SEE	Reference
OMD = 46.05 + 1.415 CP	TrG	0.89**	≤10	19
OMD = 142.50 - 1.045 (CF + NFE)	TrG	0.93**	10	19
OMD = 11.88 + 0.82 (IVOMD)	TeG	0.97**	2.3	129
OMD = 84.00 - 3.097 L(in OM)	TeM	-.83**	4.8	17
OMD = 91.87 - 5.594 L(in OM)	TeG	-.93**	3.0	17
OMD = 90.83 - 3.422 L(in OM)	TeL	-.96**	1.9	17
OMD = 0.912 IVOMD + 6.10	TeM	0.96**	2.3	17
OMD = 0.98CC + CWC (1.57 - 0.926 log L/ADF x 100) - 5.81	TeM	0.94**	3.0	17
OMD = 126.96 - 1.035 (TRAC)	TeM	-.92**	3.2	54
OMD = 19.50 + 0.78 (IVOMD)	Sil	0.81**	4.2	89
DOM = 30.52 + 0.836 (TSAE)	TeM	0.92**	2.8	54
DOM = 15.50 + 0.81 (IVDOM)	Sil	0.83**	3.8	89

**p < .01

For abbreviations, see Appendix Table 1.

d. Prediction of In Vivo Cellulose Digestibility

Cellulose digestibility in vivo for grasses can be precisely predicted from either IVCD or cellulose solubility in CED with a small standard error of about 2%.

TABLE 17. Equations used to predict cellulose digestibility.

In Vivo CD (Y)	Forage Type	r	SEE	Reference
Y = 8.84 + 0.807 (CED)	TeG	0.92**	---	34
Y = 6.64 + 1.02 (IVCD)	TeG	0.97**	2.0	129

e. Prediction of Digestible Protein

Digestible protein in both tropical and temperate forages can be predicted from CP or CP plus CF with very high correlations. These prediction equations may be accurate for forages having no heat damage such as green and new forages. The equation proposed by Holter and Reid (50) has been verified for both fertilized and non-fertilized forages. Many scientists criticize these equations because CP may vary with N fertilization and high CP level does not always give high DP value probably due to over heating and drying of forages. Goering et al. (44) reported high correlations between DN and ADF-N, acid-detergent soluble N, pepsin-soluble N, AD insoluble N, pepsin-insoluble N and many equations have been proposed to predict DN in heat-damaged forages. Along with this new idea, some scientists suggested the use of ADF-protein, total protein and their

TABLE 18. Equations proposed for predicting digestible protein

TABLE 18. Equations proposed for predicting digestible protein.

Items	Forage Type	r or R	SEE	Reference
DP = 39.83 log CP - 31.56	TrG	0.80**	1.4	23
DP = 0.929 CP - 3.48	TeM	0.99**	0.5	50
DP = 0.9596 CP - 3.55	TrG	0.99**	---	19
DCCP = 81.5 log CP - 22.1	TrM	---	---	20
DCCP = 21.0 + 80.1 log CP - 1.3CF	TrM	---	---	20
DCCP = 100.89 log CP - 44.45	TrG	0.97**	---	19
DCCP = 5.14 CP	TrG	0.93**	---	19
DCCP = 9.588 + 4.284 CP	TrG	0.95**	---	19
DCN = 72.96 - 1.02 $\frac{(\text{ADF N}}{\text{TN}}$ x 100)	Tem	0.93**	8.5	44
DN = 0.78 (AD Sol.N) - 0.25	TeM	0.94**	0.3	44
DN = 0.87 (pepsin Sol.N) - 0.07	TeM	0.96**	0.2	44
DN = 0.23 + 0.68 (AD Sol.N) - 0.60 (AD Insol.N)	TeM	0.96**	0.2	44
DN = 0.26 + 0.84 (pepsin Sol.N) - 0.31 (pepsin Insol.N)	TeM	0.97**	0.2	44

TABLE 18. Continued.

Items	Forage Type	r or R	SEE	Reference
$DCN = 82.45 - 1.8\ (ADFP/TP) + 0.01\ (ADFP/TP)^2$	TeM	0.94**	---	132
$DCN = 60.13 + 1.05\ (TP) - 5.51\ (ADFP) - 0.41\ \frac{(ADFP)}{TP}$	TeM	0.94**	---	132

DP = Digestible Protein;
DCN = Dig Coef. of Total N;
ADFN = Acid-detergent nitrogen;
ADFP = Acid-detergent protein;
DCCP = Dig. Coef. of CP;
DN = Digestible N;
TN = Total nitrogen;
TP = Total protein.

For other abbreviations, see Appendix Table 1.

ratio to estimate the digestion coefficient of N in normal and heated forages (132).

f. Prediction of TDN

From Table 19, all prediction equations for TDN using proximate analysis were based on CP + CF and CF + NFE. Even though the correlation between TDN and these components is high, prediction of TDN using these components may not be satisfactory due to the large standard error. However, TDN can be successfully predicted from 36-hr *in vitro* cellulose digestibility for tropical grasses.

TABLE 19. Equations proposed for predicting TDN.

TDN (Y)	Forage Type	r	SEE	Ref.
Y = 7.76 + 0.8192 (*In vivo* DDM)	TrG	0.99**	4.0	19
Y = 129.39 - 0.9419 (CF + NFE)	TrG	0.93**	9.0	19
Y = 74.43 + 0.35 CP - 0.73 CF	TeL	---	---	1
Y = 50.41 + 1.04 CP - 0.07 CF	TeG	---	---	1
Y = 65.14 + 0.45 CP - 0.38 CF	TeM	---	---	1
Y = 77.07 - 0.75 CP - 0.07 CF	Sil	---	---	1
Y = 17.79 + 0.906 (36-hr IVCD)	TrG	---	---	7

g. Prediction of Energy Digestibility

Digestible energy (Table 20) can be precisely predicted from either acid-insoluble lignin or two-stage IVDMD values with a standard error of prediction of 2.3%.

TABLE 20. Equations proposed to predict energy digestibility.

In vivo Digestibility

TABLE 20. Equations proposed to predict energy digestibility.

In vivo Digestibility	Forage Type	r	SEE	Reference
DE = 98.0 - 6.21 (Acid-Insol. Lig.)	TeM	-.94**	2.3	111
DE (Cal.) = 1494.1 + 19.8 (24-hr IVCD)	TrM	---	9.9	86
DE (Cal.) = 1310.9 + 282.6 (VFA)	TrM	---	9.3	41
DE (Cal.) = 1245.3 + 286.8 (IV Org. Acid)	TrM	---	9.4	41
DE = 13.50 + 0.664 (CED)	TeG	0.90**	---	34
DE (KCal/g) = 0.219 + 0.0418 (DMD)	TrG	0.86**	---	21
DE (KCal/g) = 0.152 + 0.0407 (OMD)	TrG	0.82**	---	21
DE = 23.5 + 0.75 (30-hr IVCD)	TeG	0.86**	2.7	24
DE = 1.80 (36-hr IVCD) - 11.76	TrG	---	---	7
DE = 0.79 (IVDMD) + 8.40	TeM	---	2.3	58

TABLE 20. Continued.

TABLE 20. Continued.

In vivo Digestibility	Forage Type	r	SEE	Reference
DE = 0.89 (IVDOM) + 12.6	Sil	0.79**	4.8	89
DE = 72.32 - 40.56 (ADFN)	TeM	0.90**	4.4	44
ME (KCal/g) = 3.92 - 0.175 L(in OM)	TeG	r***	4.7	6
ME (KCal/g) = 1.89 + 0.067 CP (in OM)	TeG	r***	6.0	6
N_m (KCal/g) = 1.30 + 0.056 CP (in OM)	TeG	r***	8.9	6
N_f (KCal/g) = 0.35 + 0.068 CP (in OM)	TeG	r***	12.0	6
N_m (KCal/g) = 3.04 - 0.16 L (in OM)	TeG	r***	5.5	6
N_f (KCal/g) = 2.32 - 0.16 L (in OM)	TeG	r***	13.3	6

**P <.01 ***P < .001

For abbreviations, see Appendix Table 1.

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Cellulose solubility in CED or 30-hr IVCD can be used to predict DE of grasses with reasonable accuracy. Digestible energy in silage could be predicted from IVDOM but volatile fatty acid and organic acid production in vitro are not useful predictors for DE.

Metabolizable energy in temperate grasses can be predicted from L (in OM) with moderate accuracy. In addition, CP or L in forage organic matter can be used to predict ME or NE of temperate grasses but with relatively large standard errors.

h. Prediction of Voluntary Intake

From Table 21, 6-hr in vitro fermentation (IVDMD) was one of the more accurate items used to predict DMI, DEI and DDMI. Besides, DMI can be satisfactorily predicted from CWC, MADF, 18-hr IVCD or a combination of buffer + pepsin and IVDMD. Relative intake can be predicted from C, DMS, CED, CED x DMS or IVCD x DMS with moderate accuracy.

i. Prediction of Nutritive Value Index (NVI)

Data in Table 22 indicate that NVI was predicted from CED, CED x DMS or 18-hr IVCD with moderate accuracy. The precision for predicting NVI was improved when DMS, pepsin DMD, 12-hr IVCD, IVCD x DMS, 18-hr IVCD x 30-hr IVCD, or a combination of 48-hr buffer + rumen fluid and IVDMD values were used as predictors. Dry matter NVI was satisfactorily predicted from DDMI, DEI, or 6-hr IVDMD.

TABLE 21. Equations used for predicting volumetric [illegible]

TABLE 21. Equations used for predicting voluntary intake.

Prediction of Intake	Forage Type	r	SEE	Reference
DMI = 43.1 + 1.38 (18-hr IVCD)	TeM	0.81**	4.68	24
DMI = 118.6 - 1.80 (MADF)	TeM	-.82**	7.00	25
DMI = 63.5 + 1.72 (Buf + Pep) -0.95 (IVDMD)	TeM	0.91**	---	10
DMI = 110.4 - 1716/CC	TeM	-.65**	---	43
$DMI/_{cwt}$ = 1.020 + 0.062 (6-hr IVDMD)	TeM	0.77**	0.30	51
$DEI/_{cwt}$ = 0.220 + 0.051 (6-hr IVDMD)	TeM	0.90**	0.14	51
$DDMI/_{cwt}$ = 0.462 + 0.044 (6-hr IVDMD)	TeM	0.85**	0.15	51
RI = 10.01 + 1.00 (CED)	TeG	0.60*	---	34
RI = 36.56 + 1.08 (CED)	TeL	0.68**	7.09	35
RI = 8.59 + 3.42 DMS	TeM	0.78**	---	35
$RI = 56.1 + 2.16 \frac{(CED \times DMS)}{100}$	TeM	0.47**	---	60
$RI = 59.7 + 3.29 \frac{(IVCD \times DMS)}{100}$	TeM	0.70**	---	60
RI = 165.7 - 2.51 C	TeM	0.68**	8.41	60

*P < .05 **P < .01

For abbreviations, see Appendix Table 1.

TABLE 22: Equations used to predict

TABLE 22. Equations used to predict nutritive value index.

NVI (Y)	Forage Type	r	SEE	Reference
$Y = 1.126\ (CED) - 30.27$	TeG	0.76**	---	34
$Y = 2.615\ DMS - 9.15$	TeM	0.83**	---	35
$Y = 12.4 + 2.52\ \frac{(CED \times DMS)}{100}$	TeM	0.74**	---	60
$Y = 24.0 + 3.05\ \frac{(IVCD \times DMS)}{100}$	TeM	0.88**	---	60
$Y = 28.4 + 0.24\ (18\text{-hr IVCD}) \times (30\text{-hr IVCD})$	TeG	0.82**	5.0	24
$Y = 25.5 + 1.20\ (18\text{-hr IVCD})$	TeG	0.70**	5.1	24
$Y = 1.314\ (12\text{-hr IVCD}) - 7.8$	TeM	0.91**	---	38
$Y = 1.60\ (\text{pepsin DMD}) - 0.75$	TeM	0.95**	---	40
$Y = -62.6 - 2.46\ (48\text{-hr Buf + RF}) + 3.89\ (IVDMD)$	TeM	0.94**	---	10
$DMNVI = 2.39 + 29.31\ DDMI$	TeM	0.97**	2.1	51
$DMNVI = 9.57 + 26.01\ DEI$	TeM	0.95**	2.7	51
$DMNVI = 13.2 + 1.395\ (6\text{-hr IVDMD})$	TeM	0.89**	3.7	51

**P < .01

For abbreviations, see Appendix Table 1.

EXPERIMENTAL PROCEDURES

I. MATERIALS

a. Forages

Forage samples were obtained from both tropical and temperate regions. There were 24 alfalfa (*Medicago sativa*) 10 bromegrass (*Bromus inermis*), 9 tall fescue (*Festuca arundinacea*) samples with known *in vivo* data and some chemical estimates from Purdue University and 6 alfalfa, 6 bromegrass, and 6 reed canary (*Phalaris arundinacea*) grass samples with known *in vivo* data from the Department of Dairy Science, Michigan State University. Also, 40 samples of 5 grasses (8 for each of bromegrass, orchard (*Dactylis glomerata*), reed canary grass, Kentucky bluegrass (*Poa pratensis*), tall fescue) grown at Department of Crop Science fields, Michigan State University were cut at 30, 45, 60, 75, 90, 105, 120, 135 days of age after regrowth in the spring with the first cutting on May 20, 1972.

There were five para grass (*Brachiaria mutica*), 5 Napier grass (*Pennisetum purpureum*), 5 speargrass (*Imperata cylindrica*), 5 centrosema (*Centrosema pubescens*) and 3 mung bean (*Phaseolus aureus*) samples cut at 30, 45, 60, 75, 90 days of age after initial growth from Thailand. Forty grass samples from various species in genera

Pennisetum, Panicum, Paspalum, Brachiaria, Cenchrus, Digitaria, Andropogon, Tripsacum, Cynodon, Sorghastrum and Eriochloa were from Mayaguez, Puerto Rico and were cut after 30 days of regrowth. In addition, some statistical analyses of forage samples from previous experiments at the Department of Dairy Science were used in this study.

Using data obtained from 1961 to 1970 from digestion trials and laboratory analyses on forages at Michigan State University, multiple correlations and regression equations were calculated to more accurately predict in vivo dry matter digestibility, dry matter intake, digestible dry matter intake, total digestible nutrients, digestible energy, weight gain. Forage samples were composed of alfalfa, birdsfoot trefoil, Siberian reed canary grass, bromegrass, ryegrass, timothy and legume silage.

These samples had been analyzed for CP, CF, EE, ash, NFE by standard methods outlined in AOAC (4) and by Woodman (130). Cell walls, ADF, L were determined according to Goering and Van Soest (43). Dry matter solubility followed the method outlined by Dehority and Johnson (35). In vitro dry matter disappearance (6 and 36-hr) for samples and standard was the same as that reported by Ingalls (51) and Allinson et al. (3). Two-stage in vitro fermentation was according to Tilley and Terry (112) with some modifications developed by various personnel at the Department of Dairy Science, Michigan State University.

Various sequential combinations of variables selected from these laboratory estimates (CP, CF, EE, ash,

NFE, CW, ADF, L, CC, DMS, 6-hr DMD, 36-hr DMD, TT DMD, L/ADF ratio and DMD of standard) were used to develop multiple regression equations by means of a LSD program on a CDC 3600 computer.

b. Enzymes

Samples of different cellulases (Cellulase 36, Marschall, Novo, Onozuka) were obtained from commercial companies. Takadiastase (amylase) was obtained from Miles Laboratories sold under the name of "Clarase 900." Pepsin (1:10,000) was purchased from Nutritional Biochemicals Corporation, Cleveland, Ohio.

c. Methods for Studies on Enzyme Activities

Several commercial cellulases, Clarase 900 (amylase) and pepsin were used to determine appropriate pH levels, ratio of enzyme to substrate, length of incubation and the kind of cellulase that would give the greatest solubilizing activity on forages. Two hundred mg of Whatman cellulose powder, 300 mg of high (H) or low (L) strain or common alfalfa hays and unbeaten paper ground to pass a 40-mesh screen in a Wiley mill were incubated with these enzymes.

Different concentrations of cellulases and amylase were dissolved in sodium acetate: acetic acid buffer having different pH levels from 3.0 to 6.0. Various concentrations of pepsin were suspended in HCl with different pH levels (1.5 to 3.0). A mixture of cellulase was filtered through Whatman filter paper No. 1 to remove the residue

whereas clear solutions of amylase and pepsin were used directly.

The samples plus enzymes were kept in 50-ml Erlenmyer flasks with rubber stoppers and the flasks incubated at 38-39 C for different intervals of time with occasional shaking by hand. At the end of incubation time, the mixture was filtered using a Millipore apparatus and tared prefilter paper. The residue was dried and weighed to determine total residue after enzyme and dry matter loss after enzymatic incubation.

II. METHODS OF ANALYSIS

a. Chemical Analysis

Forages were analyzed for moisture content, CP, total ash by standard methods outlined in AOAC (4). Neutral-detergent fiber or CW, ADF, ADL, permanganate lignin, insoluble ash, silica values were determined according to the procedures of Goering and Van Soest (43). Hemicellulose was calculated as the difference between neutral-detergent fiber and ADF and cellulose as the weight loss upon ashing the permanganate treated ADF residue or after treating ADF residue with 72% H_2SO_4.

b. Two-stage In Vitro Fermentation (IVDMD)

This procedure was adapted from that of the Tilley-Terry method (112) with some modifications made by various personnel in this laboratory. Half a gram of sample as well as alfalfa standard was placed into 50-ml centrifuge

tubes. Several samples were done simultaneously along with separate determinations for dry matter content. Phosphate buffer was used and composed of 4.08 g KH_2PO_4, 8.72 g Na_2HPO_4, 1.5 g $MgSO_4.7\ H_2O$, 0.5 g KCl, 0.1 g $CaCl_2$, 25 mg $Na_2S\ .9\ H_2O$, 10 ml urea (8% solution), 20 ml Na_2CO_3 (15.73% solution) per liter. The buffer was warmed up in a water bath (38-39 C) and bubbled with CO_2 until the solution became clear and the pH was about 6.8.

Rumen fluid was squeezed out through 5 layers of cheesecloth from a fistulated cow that had been fed good quality alfalfa hay 2 hours previously, and 1 hour previously the remaining hay removed and access to water prevented. The rumen fluid was allowed to settle at 37-38 C for a short time and the bottom layer which was free from feed particles was drawn into a flask kept warm at the same temperature. Carbon dioxide was then bubbled into rumen fluid constantly. Ten ml of buffer were added to the sample in the tubes 15-40 minutes before the rumen fluid and the tubes kept in water bath at 38-39 C. Twelve ml of rumen fluid were added to the tubes followed by a flush of CO_2 after which tubes were capped immediately with rubber stoppers fitted with a bunsen valve. The sample tubes were incubated in a water bath (38-39 C). This bath was covered with a plastic sheet and CO_2 bubbled into the enclosure. Tubes were incubated for 48 hours and shaken twice per day.

After 48 hours, 0.9 ml of 6 N HCl was added to each tube to stop microbial fermentation and bring a pH to 1.7 to 2.0 and then 0.5 ml of 20% pepsin solution was added,

and tubes were incubated in the water bath for another 48 hours with occasional shaking by hand. After 96 hours, the fermentation mixture was filtered through a tared sintered glass crucible with dry matter remaining determined gravimetrically and the loss of dry matter calculated as percent of initial dry matter and called IVDMD.

c. In Vitro Cell Wall and True Digestibilities (IVCWD and IVTDMD)

The method of in vitro fermentation was the same as the first-stage IVDMD. After 48 hours of incubation, the fermentation mixture was transferred into a beaker and boiled for 1 hour with neutral-detergent solution according to the procedures outlined by Van Soest et al. (126) or Goering and Van Soest (43). In vitro CWD and IVTDMD were calculated as percentages of original CW or DM lost during the fermentation or the boiling procedure.

d. Cellulase Digestion

The methods for cellulase digestion were similar to those described by Jarrige et al. (54), Guggolz et al. (48) and Moore et al. (82) with modifications.

A buffer used for cellulase digestion was a mixture of sodium acetate and acetic acid prepared and adjusted to have a pH of 3.85 to 3.90 according to the techniques outlined by Dawson et al. (33). Three hundred mg of air-dry samples ground to pass a 40-mesh screen in a Wiley mill, were placed in 50-ml Erlenmyer flasks followed by an addition of 10 ml distilled water to moisten the samples. Ten

ml of the buffer containing 300 mg cellulase were added to the flasks which were stoppered and placed in an incubator at 38-39 C for 60 hours with occasional stirring. At the end of the incubation time, the mixture was filtered using Millipore apparatus and tared prefilter paper.[1] The residue was dried and weighed to determine total residue after cellulase (TRAC) and dry matter loss from cellulase digestion.

e. Cellulase and Amylase Digestion

The digestion of forages by cellulase was the same as that discussed above. At the end of 30-hr incubation, pH of the mixture was adjusted to 5.5 using saturated sodium acetate. Immediately, 10 ml of 2% Takadiastase solution in water were added to the flasks which were stoppered and incubated at 38-39 C for another 30 hours. Since cellulase and Takadiastase contained some carbohydrates, an enzyme blank treated as sample was necessary for proper calculations.

At the end of the incubation time (60 hours in total), the mixture was filtered using Millipore apparatus and prefilter paper as described above. The filtrate was received in a suction flask for carbohydrate determination (TACAE). The residue was dried and weighed to determine total solubles after enzymes (TSAE) and total residues after

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[1]Obtained from Millipore Corporation, Bedford, Massachusettes, 01730, Cat. No. AP 2504700.

enzymes (TRAE). The filtrate was transferred to 500-ml volumetric flask and treated with 2-3 ml of 10% lead acetate to precipitate excess protein or enzyme. Subsequent procedures were according to Smith (105). For the determination of sugars, 0.5 ml of aliquot was used for Nelson's test according to procedures outlined by Clark (26) using 10 to 100 µg glucose as standard. Total sugars minus sugars in the blank were expressed as total available carbohydrates after enzymes (TACAE).

f. Cellulase and Pepsin Digestion

The digestion of forages by cellulase was the same as that described earlier. At the end of 30-hr incubation, pH of the mixture was adjusted to 1.75 to 1.85 using 6 N HCl. Immediately, 1 ml of 20% pepsin solution in water was added to the flasks which were stoppered and incubated at 38-39 C for another 30 hours with occasional agitation. At the end of incubation time (60 hrs in total), the mixture was filtered using Millipore apparatus and prefilter paper as discussed earlier. The residue was dried and weighed to determine total DMD due to these enzymes.

g. Pepsin Digestion

The method of pepsin digestion by Donefer et al. (39) was used with some modifications. Three hundred mg of sample ground to pass a 40-mesh screen were placed in 50-ml Erlenmyer flasks followed by an addition of 35 ml of 0.075 N HCl containing 200 mg pepsin. The flasks were stoppered and incubated at 38-39 C for 60 hours with

occasional agitation. At the end of incubation the mixture was filtered using the Millipore apparatus and prefilter paper. The residue was dried and weighed to determine total DMD due to pepsin digestion.

h. Amylase Digestion and TNC

A buffer solution for amylase digestion was a mixture of sodium acetate-acetic acid prepared and adjusted to have a pH of 5.5 according to Dawson et al. (33). Three hundred mg of samples ground to pass a 40-mesh screen were placed in 50-ml Erlenmyer flasks to which were added 10 ml of distilled water. The rest of procedures were the same as outlined by Smith (105) except that 1% Takadiastase solution in water was used and the residue was filtered using the Millipore apparatus and prefilter paper. The residue was dried and weighed to determine total DMD due to this enzyme. The filtrate (0.5 ml) was used for the determination of total nonstructural carbohydrates (TNC) according to procedures outlined by Clark (26) using 10 to 100 µg glucose as standard.

i. Water-soluble Carbohydrates and Turbidity Test

Half a gm of sample ground to pass a 1-mm screen was placed into screwcap test tubes followed by an addition of 25 ml distilled water and 2 drops of concentrated acetic acid. The tubes were placed on a shaker for 30 minutes at about 200 strokes/minute. The mixture was filtered through glasswool and the extract was used for turbidity test according to the procedures outlined by Bennett and Archibald

(13). Another portion of the mixture was filtered through Whatman filter paper No. 30 and the filtrate was analyzed for water-soluble carbohydrates according to the procedures described by Johnson et al. (61).

III. STATISTICAL ANALYSIS

Simple and multiple correlations as well as regressions among in vivo data, in vitro fermentations, enzyme digests, and chemical components were calculated. Some prediction equations for in vivo parameters from selected laboratory estimates were also calculated using a CDC 3600 computer.

RESULTS AND DISCUSSION

I. STUDIES ON ENZYME ACTIVITIES

a. Comparisons of Cellulases

Existing techniques using cellulases, pepsin and amylase for forage evaluation have considerable variation in procedural details especially in terms of buffer composition, pH, incubation time, kinds and concentration of enzymes and incubation techniques (39,48,54,63,75,82,105). The cellulolytic activity of four enzyme preparations using pure cellulose and alfalfa hays as substrate is shown in Table 23.

Onozuka enzyme digested the greatest amount of pure cellulose (8%) compared to only approximately 2% for cellulase 36, Marschall and Novo enzymes. For the alfalfa sample, the Marschall enzyme solubilized 50% of the DM compared with 46 to 48% for the other three enzymes. The DMD for Marschall enzyme was statistically greater ($P < .05$) than values for the other 3 enzymes. Also, DMD of alfalfa hay due to cellulase alone was greatest for Marschall enzyme. Due to its slightly greater DMD value and enzyme availability, Marschall enzyme was used in subsequent studies.

TABLE 23. Composition and activities of four different cellulases[a] on two substrates.

Enzymes	DM %	CP % of DM	Pure Cellulose 200 mg	Alfalfa (L) 300 mg	
				Total solubility	Due to Enzyme[b]
				% DMD[c]	
Cellulase 36 (300mg)	91.86	15.43	1.64	46.11	8.20
Marschall (300mg)	87.43	32.93	2.47	50.07	12.16
Novo (300mg)	94.48	54.87	1.80	47.94	10.03
Onozuka (300mg)	94.00	6.25	8.16	46.37	8.46

[a]Cellulase 36: obtained from Rohm and Haas, Philadelphia, Pennsylvania.
Marschall: obtained from Miles Labs., Elkhart, Indiana.
Novo: obtained from Novo Enzyme Corporation, Omaha, Nebraska.
Onozuka: obtained from All Japan Biochemicals, Co., Ltd., 8-21 Shingikancho, Nishinomiya, Japan.
Each enzyme was dissolved in the buffer (pH 4.8) and incubated at 38-39 C for 64 hrs.

[b]DMD of enzyme treated alfalfa minus buffer treated alfalfa.

[c]Each value was average of duplicate samples.

DM = Dry Matter; CP = Crude protein;
DMD = Dry matter disappearance.

b. pH Levels for Maximum Enzymic Activity

The amount of enzymic activity at various pH levels for cellulases, Clarase 900 (amylase) and pepsin is presented in Table 24. Both Marschall and Novo enzymes showed the greatest activity at a pH between 3.5 to 4.0 with both alfalfa and cellulose as substrates. On the other hand, Clarase 900 had maximum solubilizing activity at a pH

TABLE 24. Effects of pH levels on activities of two cellulases, an amylase and pepsin.

pH	Enzymes and Substrates (amounts used in mg)					
	Marschall 300 Alfal.(H) 300	Novo 300 Alfal.(L) 300	Novo 300 Unbeaten Paper 300	Clarase 900 (300) Alfal.(H) 300	Pepsin (200) Alfal. No. 158 (300)	
	% DMD				pH	% DMD
3.0	51.42	49.98	22.45	ND	1.50	39.99
3.5	53.31	51.68	29.78	ND	1.85	41.36
4.0	53.30	48.44	30.35	33.27	2.50	34.54
4.5	50.78	46.02	29.12	35.66	3.00	35.50
5.0	47.37	43.31	21.11	36.68		
5.5	ND	41.10	16.88	36.78		
6.0	ND	ND	ND	37.00		

Clarase 900 is Takadiastase (amylase) from Miles Labs., Indiana and contained 91.82% DM, 16.94% CP (DM basis).
Incubated at 38-39 C, 64 hrs for cellulases, amylase and 52 hrs for pepsin.
ND = Not determined.

above 5.5. Pepsin could maximally solubilize alfalfa dry matter at a pH of 1.85. Therefore, a pH of 3.85 to 4.0 was adopted for subsequent cellulase incubations and that of 5.5 for Takadiastase and 1.85 for pepsin studies.

c. Incubation Time

The effects of length of incubation times on enzymatic activities are shown in Table 25. All three enzymes namely cellulase, Clarase 900 and pepsin appeared to have maximum activity near 60 hours of incubation. Therefore, an incubation time of 60 hours was used for single enzyme incubations and for a sequential hydrolysis by 2 enzymes (30 hrs for first enzyme followed by 30 hrs for second enzyme).

d. Concentrations of Enzymes Used

The ratios of enzymes to substrates for maximum enzymatic activity are presented in Table 26. Both Novo and Marschall enzymes solubilized relatively large amounts of alfalfa hay and cellulose powder at concentrations between 200 to 600 mg cellulase per 200 to 300 mg cellulose powder or hay. Clarase 900 and pepsin solubilized large amounts of alfalfa hay at a concentration of 200 mg enzyme per 300 mg hay. Therefore, 300 mg of Marschall, 200 mg Clarase 900 and 200 mg pepsin per 300 mg substrates were adopted for enzymic evaluation of forages.

Cellulase action on cellulose showed a curvilinear response but cellulase on alfalfa did not have a decrease in substrate solubilization at a high enzyme concentration.

TABLE 25. Effects of length of incubation on various enzyme activities.

Incubation Time	Marschall Cellulase (300 mg) Alfalfa No. 18 (300mg)	Clarase 900 (100 mg) Alfalfa No. 6 (300mg)	Pepsin 1:10,000 (200 mg) Alfalfa No. 158 (300mg)
(hrs)		% DMD	
16	43.57	40.96	33.75
24	44.31	41.98	34.48
30	46.14	42.92	34.88
36	46.33	42.17	34.96
42	46.58	43.07	36.06
48	47.64	43.73	36.83
54	48.27	43.68	37.42
60	48.02	43.28	37.50
66	48.74	44.18	37.60
72	48.52	43.71	38.10

Cellulase dissolved in sodium acetate-acetic acid buffer at pH 3.85.
Clarase 900 dissolved in sodium acetate-acetic acid buffer at pH 5.5.
Pepsin dissolved in HCl buffer at pH 1.85.
All incubated at 38-39 C.
Values were averages of duplicate samples.

TABLE 26. Effects of enzyme concentrations on dry matter disappearance (DMD) of various substrates.

Enzyme Conc.	Enzymes and Substrates				
	Novo Alfal.(H) 300 mg	Novo Cellulose 200 mg	Marschall Alfal. No. 70 300 mg	Clarase 900 Alfal. No. 70 300 mg	Pepsin Alfal. No. 70 300 mg
	% DMD				
10 mg	39.26	ND	ND	ND	ND
50 mg	47.62	ND	ND	ND	ND
100 mg	49.32	ND	44.24	41.79	36.62
200 mg	53.66	2.19	47.96	43.66	39.98
300 mg	53.05	2.66	50.27	42.93	39.12
400 mg	53.63	3.12	51.46	43.01	39.01
500 mg	54.87	2.97	51.93	42.22	38.93
600 mg	53.87	2.99	ND	ND	ND
700 mg	54.22	2.53	ND	ND	ND
800 mg	54.94	2.35	ND	ND	ND

Novo and Marschall cellulases dissolved in sodium acetate-acetic acid buffer at pH 3.85.
Clarase 900 dissolved in sodium acetate-acetic acid buffer at pH 5.50.
Pepsin dissolved in HCl buffer at pH 1.85.
All incubated at 38-39 C for 60 hrs.
ND = Not determined.

Continuous agitation for 60-64 hours was not necessary because there was no significant difference between DMD values (52.34% versus 53.33%) for shaken and non-shaken samples, respectively. A mixture of 300 mg cellulase (Marschall) and 200 mg Takadiastase incubated with low-strain alfalfa samples at pH 3.85 for 60 hours gave 47.8% DMD and this value was not significantly different from 46.7% DMD when using cellulase alone. Probably the pH (3.85) used for this incubation was not appropriate for Takadiastase activity. However, when the pH was raised to 5.5 using saturated sodium acetate solution at end of a 30-hr cellulase incubation and then Takadiastase added and incubated for another 30 hours, an increase of about 4-7% DMD over that when using cellulase alone (60 hrs) was obtained.

A mixture of 300 mg Marschall cellulase (in sodium acetate-acetic acid buffer, pH 3.85) and 70 mg pepsin (in HCl, pH 1.85) was incubated with alfalfa hay for 60 hours and gave higher DMD (52.5%) compared with 35.5 or 50.3% DMD when using pepsin or cellulase alone for the same incubation time. Since all 3 enzymes have a different pH for maximum activity, a sequential incubation with pH adjustments was made when any two enzymes were used in subsequent experiments.

In this study, maximum cellulolytic activity was not observed when incubated at pH 4.5 to 4.8 as proposed by many workers (48,75,82). The use of pH 4.45 for Takadiastase as proposed by Smith (105) was also not observed

to be maximal. An incubation time of 72 hours for cellulase as proposed by others (48,75) was unnecessary.

II. RESULTS OF A COMPARATIVE STUDY ON TEMPERATE AND TROPICAL FORAGES

a. Chemical Composition and Digestibility in Temperate and Tropical Forages

Literature values indicate differences between temperate and tropical forages in many chemical components as well as in digestibility (2,81,98). Tabulations showing differences for forages of the present study are given in Tables 27 and 28. All forages from Purdue University, Departments of Dairy Science, Crop and Soil Sciences, Michigan State University, Thailand and Puerto Rico were used in comparing digestibility and chemical composition and only Thai and MSU grasses were used for comparing the effects of maturity on forage nutritive value.

On the average, temperate forages had 1.05 times greater *in vitro* DMD than tropical forages (54.54% vs. 51.87%). However, this difference may not be significant due to large standard deviations for both groups of forages. Both tropical grasses and legumes had lower IVDMD values than those for their temperature counterparts. Crude protein content in tropical forages was also lower than that for temperate forages (12.63 vs. 15.67%). This difference (a factor of 0.80) was due to lower concentration of CP in both tropical grasses and legumes. However, tropical forages were greater than temperate forages by a factor of 1.11, 1.05, 1.21 for CWC, ADF and ash,

TABLE 27. Chemical composition and in vitro digestibility of

TABLE 27. Chemical composition and *in vitro* digestibility of temperate and tropical forages.[2]

Items	Combined		Grasses		Legumes	
	Temperate	Tropical	Temperate	Tropical	Temperate	Tropical
	% of dry matter					
IVDMD	54.54±11.77[1] (n=73)	51.87±10.05 (n=63)	53.16±12.34 (n=60)	50.76±10.02 (n=55)	60.92±5.30 (n=13)	59.50±6.59 (n=8)
CP	15.67± 6.02 (n=101)	12.63± 4.32 (n=63)	13.85± 5.84 (n=71)	11.60± 3.37 (n=55)	19.97±3.91 (n=30)	19.72±3.40 (n=8)
CW	57.68±11.17 (n=95)	64.25± 9.74 (n=63)	63.69± 6.18 (n=65)	67.26± 5.72 (n=55)	44.65±7.96 (n=30)	43.56±5.28 (n=8)
ADF	36.97± 5.92 (n=101)	38.82± 4.56 (n=63)	37.61± 5.83 (n=71)	39.82± 3.57 (n=55)	35.45±5.95 (n=30)	31.96±4.98 (n=8)
Lig	6.24± 2.27 (n=101)	5.43± 1.37 (n=63)	5.59± 2.17 (n=71)	5.17± 1.17 (n=55)	7.78±1.70 (n=30)	7.16±1.46 (n=8)
Ash	8.06± 1.46 (n=101)	9.74± 2.55 (n=63)	8.11± 1.54 (n=71)	9.93± 2.59 (n=55)	7.93±1.27 (n=30)	8.50±1.94 (n=8)

IVDMD = *In vitro* dry matter disappearance; CP = Crude protein; CW = Cell Walls; ADF = Acid-detergent fiber; Lig = Lignin.

[1]Mean and standard deviation.

[2]Temperate = Purdue and all MSU forages; Tropical = Thai and Puerto Rican forages.

respectively. In addition, lignin in tropical grasses and legumes was lower than that for temperate forages.

Results of this study in terms of IVDMD, CP, CWC, and ADF concentrations are in accord with studies by many others (2,30,62,81,98,122,123). On the contrary, lignin value did not agree with that reported by these workers who found slightly lower L in temperate forages whereas L in temperate forages for this study was about 1.15 times that of tropical forages.

b. Chemical Composition and Digestibility at Different Stages of Maturity in Temperate and Tropical Grasses

Crude protein contents and *in vitro* digestibility values of both tropical and temperate grasses declined gradually with advancing maturity (Table 28). The rates of decline in CP or *in vitro* digestibilities for temperate grasses were greater than those for tropical grasses probably due to a faster rate of increase in CW for temperate grasses. The rate of decline in IVDMD calculated by averaging that from weeks 4 through 12 was 0.42 percentage unit/day for temperate grasses compared with 0.09 unit for tropical grasses. These rates of decline in digestibility for both types of forages are in agreement with studies by other workers (78,80,81,93,94). At 11 to 12 weeks of age, both temperate and tropical grasses had similar *in vitro* digestibilities even though CP level was significantly higher for temperate grasses.

The quantity of cell walls of tropical grasses was essentially unchanged from 4 to 12 weeks of age. Coward-Lord

TABLE 28. Comparisons of chemical composition and digestibilities between tropical and temperate grasses with advancing maturity (30 to 90 days).

Items		Days--30 / Weeks-- 4	45 / 6	60 / 8	75 / 10	90 / 12	Ave.	Average change/d	Mean Diff.	Sig. Level
		% of dry matter								
CP	Temp. grass	17.1	17.3	11.9	10.1	9.4	13.2	-.13	5.6	P<.05
	Trop. grass	10.8	7.7	6.7	7.5	5.4	7.6	-.09		
CW	Temp. grass	56.3	63.9	63.7	64.9	67.7	63.3	+.19	6.6	P<.01
	Trop. grass	69.9	69.9	69.5	69.9	70.3	69.9	+.01		
IVDMD	Temp. grass	72.2	62.5	57.5	53.9	47.1	58.6	-.42	9.2	P<.10
	Trop. grass	48.4	51.6	50.6	48.9	47.6	49.4	-.09		
IVTDMD	Temp. grass	70.5	64.1	59.4	57.6	52.8	60.9	-.30	4.2	P<.30
	Trop. grass	55.4	59.4	57.5	56.9	54.3	56.7	-.10		

Temp. grasses: All figures were mean values of bromegrass, orchard, reed canary, tall fescue, Kentucky bluegrass from Department of Crop Science, Michigan State University, n = 5 for each mean.

Trop. grasses: All figures were mean values of Para grass, Napier, speargrass from Thailand, n = 3 for each mean.

CP = Crude protein; CW = Cell walls; IVDMD = In vitro dry matter disappearance; IVTDMD = In vitro true dry matter disappearance.

et al. (30) and Grieve and Osbourn (46) also reported that CW fractions of tropical forages in Puerto Rico increased rapidly between 4-5 weeks of age with small changes thereafter. Tropical grasses are low in nutritive value and this is due to an integrated action of low CP, high CW and ADF as well as probably the presence of some inhibitors.

III. RESULTS OF STUDIES ON FORAGES FROM PURDUE UNIVERSITY AND DEPARTMENT OF DAIRY SCIENCE, MICHIGAN STATE UNIVERSITY

a. Correlations Among Various Chemical Components

Correlation coefficients between several chemical components and enzyme values for forages from Purdue and Michigan State University are presented in Table 29. Forages from Purdue University contained 56% legume (alfalfa) whereas those from MSU contained only 33% legume (alfalfa) and the rest were grasses. There were negative relationships between CP and all cell wall fractions for the Purdue forages but MSU forages showed positive and non-significant correlations between CP and CW or HC. An increase in ADF, C and L would take the space of other nutrients and therefore produced a decrease in CP content. Ash and CP were positively correlated with both groups of forages. Cell walls had negative correlations with L and ash but positive correlations with C and HC. High correlation between CW and HC ($r = 0.96$, $P < .01$) in the MSU forages indicates that HC accounted for most of the fiber in CW probably because 67% of the samples were grasses and 33% of them were legumes. These grasses contained higher levels of HC (25.62%) than

TABLE 29. Correlation coefficients among various chemical components and enzymatic incubation values.

Factors correlated	Purdue Univ.		Dairy, MSU	
	n	r	n	r
CP vs. CWC	43	-.91**	12	0.10
CP vs. ADF	43	-.83**	18	-.83**
CP vs. L	43	-.12	18	-.53*
CP vs. C	43	-.92**	18	-.78**
CP vs. HC	43	-.67**	12	0.34
CP vs. Ash	43	0.64**	18	0.30
CP vs. TNC	14	-.02	---	---
CP vs. TACAE	39	0.17	---	---
CWC vs. ADF	43	0.74**	12	-.25
CWC vs. L	43	-.09	12	-.66**
CWC vs. C	43	0.90**	12	0.44
CWC vs. HC	43	0.87**	12	0.96**
CWC vs. Ash	43	-.44**	12	-.02
CWC vs. TNC	14	0.17	---	---
CWC vs. TACAE	39	0.08	---	---
ADF vs. L	43	0.55**	18	0.74**
ADF vs. C	43	0.93**	18	0.85**
ADF vs. HC	43	0.31*	12	-.50
ADF vs. Ash	43	-.70**	18	-.24
ADF vs. TNC	14	0.06	---	---
ADF vs. TACAE	39	-.42**	---	---
L vs. C	43	0.20	18	0.28
L vs. HC	43	-.53**	12	-.81**
L vs. Ash	43	-.60**	18	-.35

TABLE 29. Continued.

Factors correlated	Purdue Univ. n	Purdue Univ. r	Dairy, MSU n	Dairy, MSU r
L vs. TNC	14	0.27	---	---
L vs. TACAE	39	-.64**	---	---
C vs. HC	43	0.59**	12	0.22
C vs. Ash	43	-.56**	18	-.07
C vs. TNC	14	-.01	---	---
C vs. TACAE	39	-.22	---	---
HC vs. Ash	43	-.10	12	0.12
HC vs. TNC	14	0.45	---	---
HC vs. TACAE	39	0.43**	---	---
Ash vs. TNC	14	-.48	---	---
Ash vs. TACAE	39	0.35*	---	---
TNC vs. TACAE	14	0.14	---	---

CP = Crude protein; CW = Cell walls;
ADF = Acid-detergent fiber; L = Lignin;
C = Cellulose; HC = Hemicellulose;
TNC = Total nonstructural carbohydrates;
TACAE = Total available carbohydrates after enzymes.

*P < .05 **P <.01

did legumes (9.20% HC) and the percentages of HC in CW for these grasses and legumes (alfalfa) were 41.4 and 20.6, respectively. High concentrations of HC were related to low concentrations of ADF (r = -.50) and consequently the correlation between ADF and CW was negative (r = -.25) in the MSU forages. A negative relationship between CW and L for both groups of forages indicates that L tends to decrease when CW increases.

Total nonstructural carbohydrates (TNC) had low correlations (r = +0.45 to -0.48) with other chemical components. Correlations between TACAE and CP, CW, HC, Ash, TNC were low and positive (0.08 to 0.43**) but those between TACAE and ADF, L, C were slightly higher and negative (-.22 to -.64**).

The negative relationships between CP or ash to CW and other fibrous fractions are in accord with those reported by Kayongo-Male et al.(64) and Van Soest (117). The positive changes between CP and ash are in agreement with the report by Smith (104) and Van Riper and Smith (114). Low correlations between TNC, TACAE and CP or CW in these forages indicate that changes in CP or CW were not related to changes in these nonstructural or available carbohydrates. Similarly, Van Riper and Smith (114) reported that TAC (or TNC) was variable and did not show definite trends with changes in other chemical constituents or with advancing maturity. High levels of CP are generally accepted as an indication of good quality in forages. Therefore, an increase in CW and other fibrous fractions with correlated decrease in CP would definitely result in a decrease of forage nutritive value.

b. In Vivo Dry Matter Digestibility (DDM) vs. Chemical Components

The relationships between chemical components and five in vivo parameters are presented in Table 30. In vivo DDM was positively and significantly correlated with CP (r = 0.66 to 0.76) or ash (r = 0.46 to 0.64) for both MSU

TABLE 30. Correlation coefficients of *in vivo* parameters with chemical components and enzymatic incubation values.

	In Vivo DDM				In Vivo DMI				In Vivo DDMI				TDN		DE	
	Purdue Univ.		Dairy MSU		Purdue Univ.		Dairy MSU		Purdue Univ.		Dairy MSU		Dairy MSU		Dairy MSU	
Items	n	r	n	r	n	r	n	r	n	r	n	r	n	r	n	r
CP	43	0.76**	18	0.66**	43	0.68**	18	-.11	43	0.75**	18	0.16	12	0.65*	14	0.61*
CW	43	-.66**	12	-.17	43	-.70**	12	0.31	43	0.73**	12	0.19	6	-.50	8	-.30
ADF	43	-.82**	18	-.65**	43	-.47**	18	0.21	43	-.65**	18	-.07	12	-.70*	14	-.62*
L	43	-.49**	18	-.54*	43	0.08	18	0.02	43	-.15	18	-.20	12	-.55	14	-.54*
C	43	-.74**	18	-.50*	43	-.58**	18	0.29	43	-.69**	18	0.06	12	-.66*	14	-.48
HC	43	-.33*	12	0.01	43	-.64**	12	0.40	43	-.56**	12	0.32	6	-.35	8	0.09
Ash	43	0.64**	18	0.46*	43	0.34*	18	0.15	43	0.48**	18	0.30	12	0.43	14	0.59*
L/ADF	43	-.12	18	-.47*	43	0.33*	18	-.03	43	0.18	18	-.21	12	-.45	14	-.45
L/C	43	-.11	18	-.47*	43	0.35*	18	-.02	43	0.19	18	-.20	12	-.45	14	-.45
L/HC	42	0.12	12	-.15	43	0.56**	12	-.43	43	0.42**	12	-.39	6	0.30	8	-.27
HC/C	43	-.08	12	0.06	43	-.50**	12	0.44	43	-.37*	12	0.37	6	-.32	8	0.20
TNC	14	0.18			14	-.08			14	0.03						
TACAE	39	0.30			39	0.01			39	0.12						

DDM = Digestible dry matter; DMI = Dry matter intake; DDMI = Digestible dry matter intake; TDN = Total digestible nutrients; DE = Digestible energy.

See footnote Table 29 for other abbreviations.

*P < .05 **P < .01

and Purdue forages. These were consistent and significantly negative correlations between DDM and ADF, L, C, and CW. Lignin-fiber ratios had relatively low correlations with DDM for both groups of forages. Total nonstructural carbohydrates and TACAE had positive but low correlations with *in vivo* DDM.

The significant and negative relation (r = -.65 to -.82) between ADF content and *in vivo* DDM is in agreement with other studies (25,88,115,117,131). Lignin did not have as marked depressing effect (r =-.49 to -.54) on DDM as that reported in the other studies mentioned above. Based on the present study the use of ADF as a single predictor of *in vivo* DDM may be the most preferable since it had a higher correlation than any other chemical constituents with DDM.

Total nonstructural carbohydrates and TACAE had such low correlations (r = 0.18 to 0.30) that they can not be used as useful predictors of DDM. This finding is not in agreement with that of Wilkins and Minson (129) who reported good relationship between TAC (TNC) and *in vivo* OMD and actually suggested TAC as a useful single predictor of forage digestibility.

c. Dry Matter Intake (DMI) vs. Chemical Components

Forage dry matter intake (DMI) for both groups showed opposite signs of relationships (+ vs. -) to all chemical components (CP, CW, ADF, C, HC, L-fiber ratios) except L and ash (Table 30). For Purdue forages, there

were significant, positive correlations between DMI and CP ($r = 0.68$) or L/HC ratio ($r = 0.56$) but negative correlations ($P < .01$) with CW ($r = -.70$), ADF, C, HC and HC/C ratio. All chemical components had low and non-significant relationships to DMI for MSU forages. The most important components depressing DMI for Purdue and MSU forages were CW and L/HC, respectively. For Purdue forages, CW would be the most appropriate single predictor for estimating forage intake. This finding is in agreement with that reported by Van Soest (117) who found that CW had significant correlation with DMI and developed a prediction equation for DMI using a reciprocal of 100-CW. Neither TNC nor TACAE could be used to predict DMI due to their extremely low correlations with forage intake.

The differences in trends and magnitude of correlation coefficients for these two groups of forages emphasize the fact that differences do exist between any two populations and in this case the differences might be due to unequal proportions of grasses and legumes in each forage group and that MSU forages consisted of only 18 samples and not all had complete chemical analysis.

d. Digestible Dry Matter Intake (DDMI) vs. Chemical Components

The correlations between digestible dry matter intake (DMI x DDM) and chemical components were not consistent for both groups of forages (Table 30). Crude protein, ash and L/HC ratio had positive and significant correlations with DDMI whereas CW, ADF, C, HC and HC/C

ratio had significantly negative correlations with DDMI for Purdue forages. Total nonstructural carbohydrates and TACAE showed very low correlations with DDMI. All chemical components had low and non-significant correlations with DDMI for MSU forages. For Purdue forages, an increase in CW followed by a decrease in CP would result in a significant decrease in DDMI. Either crude protein or CW seemed to be the appropriate single predictor for estimating DDMI of Purdue forages but no single chemical components would be satisfactory to predict DDMI for MSU forages.

e. Total Digestible Nutrients (TDN) vs. Chemical Components

Total digestible nutrients were significantly correlated ($r = 0.65$, $P < .05$) with CP but had low and positive correlations with ash and L/HC ratio for MSU forages. All other CW fractions and fiber ratios had negative correlations ($r = -.32$ to $-.70$) with TDN. Acid-detergent fiber, C and CP seemed to be the three important factors controlling the concentration of TDN in forages. Acid-detergent fiber showed a reasonably high correlation with TDN ($r = -.70$, $P < .05$) and this value might be sufficient to use ADF as a single predictor for mixed forages. In this case, ADF might predict TDN more accurately than CP or C alone. Adams et al. (1) proposed the use of CP and CF to predict TDN in various forages (Table 19). Probably a combination of factors (ADF, CP, C, etc.) in a multiple regression equation may be able to predict TDN more

precisely than using single predictors. At present, there are no reports using these three factors.

f. Digestible Energy (DE) vs. Chemical Components

The correlations of digestible energy with chemical components for MSU forages followed a pattern similar to that for TDN. There were significant correlations between DE and CP or ash but negative correlations with ADF, L, CW, C, and L-fiber ratios. Among chemical components, ADF tended to be the most important single constituent ($r = -.62$, $P < .05$) controlling energy digestibility. This finding is in agreement with that reported by Johnson and Dehority (58) who found a high correlation between ADF and energy digestibility ($r = -.76$, $P < .01$). In addition, lignin also showed a marked depressing effect on DE. Sullivan (111) used acid-insoluble L to predict DE with reasonable accuracy ($r = -.94$, $P < .01$, SEE = 2.3). However, there are no useable prediction equations using ADF or a combination of ADF, CP, L, ash, etc. to predict digestible energy.

g. In Vivo Dry Matter Digestibility (DDM) vs. In Vitro Fermentations and Enzymatic Incubations

In vivo DDM was significantly and positively correlated ($r = 0.76$ to 0.88) with in vitro DMD by the Tilley-Terry method for both groups of forages (Table 31). In vitro organic matter and dry matter disappearance had similar correlation coefficients with DDM. All enzymatic incubations using cellulase, amylase, buffer, a sequential

TABLE 31. Correlation coefficients of *in vivo* parameters with *in vitro* fermentations and enzymatic incubations for forages from two sources.

Items	*In Vivo* DDM				*In Vivo* DMI				*In Vivo* DDMI				TDN		DE	
	Purdue Univ.		MSU		Purdue Univ.		MSU		Purdue Univ.		MSU		MSU		MSU	
	n	r	n	r	n	r	n	r	n	r	n	r	n	r	n	r
IVDMD	15	0.88**	18	0.76**	15	0.73**	18	0.40	15	0.81**	18	0.66**	12	0.79**	14	0.87**
IVOMD	15	0.89**			15	0.74**			15	0.81**						
Cell	14	0.87**	18	0.33	14	0.90**	18	0.37	14	0.92**	18	0.46*	12	0.32	14	0.65*
Pep			18	0.49*			18	0.07			18	0.25	12	0.49	14	0.62*
Amy	14	0.78**			14	0.88**			14	0.87**						
Cell+Amy	39	0.76**			39	0.69**			39	0.73**						
Cell+Pep			18	0.43			18	0.26			18	0.40	12	0.41	14	0.70**
Buf	39	0.67**	18	0.21	39	0.61**	18	0.19	39	0.65**	18	0.26	12	0.21	14	0.43
TEE	39	0.75**			39	0.66**			39	0.70**						

IVDMD = *In vitro* dry matter disappearance (Tilley-Terry method); IVOMD = *In vitro* organic matter digestibility; Cell = Cellulase incubation; Pep = Pepsin incubation; Amy = Amylase incubation; Cell+Amy = Cellulase incubation followed by amylase incubation; Cell+Pep = Cellulase incubation followed by pepsin incubation; Buf = Buffer extract; TEE = Total enzyme extract (total solubles after enzymes minus buffer extract).

See Footnote Table 30 for other abbreviations.
*p < .05 **p < .01

hydrolysis by cellulase plus amylase and total enzyme extract had significant and positive correlations (r = 0.67 to 0.87) with DDM for Purdue forages. Only pepsin incubation had a significant but low correlation (r = 0.49) with DDM for the MSU forages with the correlation for cellulase plus pepsin approaching significance.

This study confirms many other studies (10,11,58, 76,88,98,112,126,131) that the two-stage in vitro fermentation (IVDMD or IVOMD) has high and significant correlations with DDM. In addition, other laboratory estimates such as cellulase, amylase or cellulase plus amylase could be used to predict in vivo dry matter digestibility. All in vitro measurements and values for enzymatic incubations were mutually and positively correlated ($P < .01$). Incubation with cellulase has some advantages over IVDMD or IVOMD since it requires less incubation time (60 hrs vs. 98 hrs), less laboratory manipulations and requires no rumen fluid. However, data in Table 31 indicate that cellulase alone may be just as or more accurate in predicting DDM as a sequential incubation using cellulase plus amylase.

h. Dry Matter Intake (DMI) vs. In Vitro Fermentations and Enzymatic Incubations

For Purdue forages, DMI was significantly and positively correlated with all in vitro fermentations and enzymatic incubation values with r's ranging from 0.61 to 0.90. There were no significant correlations between these measurements and DMI for MSU forages, but all were positive. In the Purdue forages, cellulase incubation value

had a very high correlation with DMI ($r = 0.90$, $P < .01$) and this absolute figure was slightly greater than the correlation ($r = -.70$, $P < .01$) between DMI and CW (Table 30). The use of cellulase as a predictor of DMI may be more accurate than CW but determination of CW requires less time and equipment. Data in Table 31 indicate that both cellulase and amylase incubations could be used to predict intake as or more accurately than other in vitro methods. Jarrige et al. (54) also reported that in vitro incubation with cellulase (24 hrs) could predict intake with moderate accuracy. The low correlation would indicate that the two-stage IVDMD was not a reliable predictor of intake. This finding is in agreement with those by Barnes (10) and Ingalls (51). For the MSU forages, pepsin incubation had very low correlation with intake whereas others noted a high correlation between intake and pepsin-soluble dry matter (79).

i. Digestible Dry Matter Intake (DDMI) vs. In Vitro Fermentations and Enzymatic Incubations

Digestible dry matter intake was significantly and positively correlated with all in vitro fermentations and enzymatic incubations for the Purdue forages but only IVDMD and cellulase had significant correlations with DDMI for the MSU forages. The correlation coefficients between amylase or cellulase values and DDMI ($r = 0.87$ to 0.92) were slightly higher than the correlation between IVDMD and DDMI ($r = 0.81$). This finding indicates that both enzymes preferably cellulase incubation could be used to predict

DDMI more accurately than *in vitro* fermentations. For the MSU forages, IVDMD was still a better predictor of DDMI than the enzymatic incubations used in that part of the study. Ingalls (51) reported that a 6-hr IVDMD could predict *in vivo* DDMI with great accuracy ($r = 0.85$, $P < .01$, SEE = 0.15 lb/cwt). Although the correlations between enzymic incubations and DDMI were low, cellulase incubation seemed to be superior to the other enzymic incubations.

j. Total Digestible Nutrients (TDN) vs. In Vitro Fermentations and Enzymatic Incubations

In the MSU forages, TDN was significantly and positively correlated with IVDMD ($r = 0.79$, $P < .01$). None of the three enzymatic incubations had significant correlations with TDN but pepsin was superior to cellulase or the combination. When Guggolz *et al.* (48) used cellulase and pronase (a proteolytic enzyme) in a sequential hydrolysis of forages and crop residues, they found that total solubles after enzymes (TSAE) were positively and significantly correlated with TDN when TDN ranged from 16 to 85% ($r = 0.902$, $P < .01$, $n = 24$). In this study, 12 forages had a narrow range of TDN (52 to 64%) and this technique did not have a significant correlation with TDN. In the present study, the two-stage IVDMD was the best predictor of TDN for this sample of forages. In addition, TDN could be satisfactorily predicted from 36-hr *in vitro* cellulose digestibility (7).

k. Digestible Energy (DE) vs. In Vitro Fermentations and Enzymatic Incubations

Digestible energy was positively and significantly correlated with IVDMD, cellulase, pepsin and cellulase followed by pepsin. The greatest correlation ($r = 0.87$, $P < .01$) was found for IVDMD indicating that DE could be accurately predicted from this two-stage IVDMD. This finding is in accord with that by Johnson and Dehority (58) who developed a prediction equation for DE using IVDMD with only a 2.3% standard error of estimate. Among enzymatic incubations, the sequential hydrolysis by cellulase and pepsin gave the highest correlation ($r = 0.70$, $P < .01$) with DE. Pepsin alone or cellulase alone had significant but lower correlations with DE ($r = 0.62$ to 0.65, $P < .05$) than the cellulase plus pepsin sequence.

IV. RESULTS OF STUDIES ON GRASSES FROM MICHIGAN STATE UNIVERSITY

a. Nutritive Value vs. Stage of Maturity

A simple correlation analysis between age of grasses and laboratory measures of nutritive value is presented in Table 32. There were significant and negative correlations between age or maturity and CC, CP, HC, soluble ash and *in vitro* digestibilities but positive correlations with CW, fibrous fractions, L, Si, L-fiber ratios and turbidity test (O.D.) with advancing maturity. Crude protein, HC, soluble ash, CC, HC/C ratio, IVDMD, IVOMD, IVCWD, IVTDMD and predicted intake decreased significantly ($P < .01$) whereas CW, ADF, L, C, Si, insoluble ash,

TABLE 32. Correlation coefficients between age and chemical composition and digestibilities for MSU grasses during 30 to 135 days of growth.[1]

Items correlated	n	r	Items correlated	n	r
Age vs. CP	40	-.82**	Age vs. L/ADF	40	0.71**
Age vs. CW	40	0.54**	Age vs. L/C	40	0.76**
Age vs. ADF	40	0.76**	Age vs. L/HC	40	0.85**
Age vs. L	40	0.79**	Age vs. HC/C	40	-.77**
Age vs. C	40	0.51**	Age vs. Turbid.	40	0.73**
Age vs. HC	40	-.54**	Age vs. WS-CHO	40	0.15
Age vs. Total ash	40	-.21	Age vs. IVDMD	40	-.87**
Age vs. Insol. ash	40	0.74**	Age vs. IVOMD	40	-.86**
Age vs. Sol. ash	40	-.60**	Age vs. IVCWD	40	-.86**
Age vs. Si	40	0.77**	Age vs. IVTDMD	40	-.83**
Age vs. OM	40	0.21	Age vs. Prd. Intake	40	-.54**
Age vs. CC	40	-.54**			

[1]MSU grasses = Brome, Orchard, Reed Canary, Tall Fescue and Kentucky Bluegrass grown during April 15 through September 4, 1972.

Si = Silica; OM = Organic matter; CC = Cell contents; Turbid = Turbidity; WS-CHO = Water-soluble carbohydrates; IVCWD = In Vitro cell wall digestibility; Prd. Intake = Predicted intake (Goering and Van Soest, 1970). See other abbreviations in Tables 28, 29, 31.

*P < .05 **P < .01

L-fiber ratios and turbidity increased significantly. The results of this study are in accord with those of other workers (30,80,98,104,114). Levels of water-soluble carbohydrates (WS-CHO) changed slightly (r = 0.15) with

advancing maturity probably because they served as precursors for polysaccharide synthesis. Deriaz (37) also found the same trend for WS-CHO in ryegrass.

b. Correlations Among Chemical Components

The relationships among chemical constituents (Table 33) demonstrate that CP had positive correlations with total ash and HC but significant, negative correlations with all other fibrous fractions, L, Si, turbidity and WS-CHO. Cell walls were significantly and positively correlated with all fibrous fractions, L, Si, and turbidity test but negatively correlated with ash and WS-CHO. Acid-detergent fiber, C, L, Si, and turbidity were mutually and positively correlated. Hemicellulose was negatively correlated with ADF, L, Si, ash, turbidity and WS-CHO. The correlations between WS-CHO and ADF, L, C, ash or turbidity were all negative.

A decrease in CP was followed by a significant increase in CW, ADF, L, C, Si. These relationships among chemical components are in agreement with data on forages from Purdue and studies by other workers (30,46). The high correlation ($r = 0.92$) for CW with lignocellulose (ADF) and C might be expected since CW is mainly composed of ADF and C. Similarly, ADF is composed of L and C and these correlations are 0.85 and 0.92, respectively.

The negative and significant relationship between CP and turbidity (O.D.) and the positive correlation between fibrous fractions and turbidity do not agree with

TABLE 33. Correlation coefficients among chemical components for MSU grasses during 30 to 135 days of growth.

Factors Correlated	n	r	Factors Correlated	n	r
CP vs. CW	40	-.44**	ADF vs. WS-CHO	40	-.35*
CP vs. ADF	40	-.65**	L vs. C	40	0.65**
CP vs. L	40	-.50**	L vs. HC	40	-.30
CP vs. C	40	-.52**	L vs. Si	40	0.53**
CP vs. HC	40	0.51**	L vs. Tot. Ash	40	-.16
CP vs. Si	40	-.59**	L vs. Turbid.	40	0.78**
CP vs. Tot. Ash	40	0.23	L vs. WS-CHO	40	-.33*
CP vs. Turbid.	40	-.47**	C vs. HC	40	0.02
CP vs. WS-CHO	40	-.37*	C vs. Si	40	0.28
CW vs. ADF	40	0.92**	C vs. Tot. Ash	40	-.08
CW vs. L	40	0.73**	C vs. Turbid.	40	0.41**
CW vs. C	40	0.93**	C vs. WS-CHO	40	-.42**
CW vs. HC	40	0.21	HC vs. Si	40	-.69**
CW vs. Si	40	0.25	HC vs. Tot. Ash	40	-.25
CW vs. Tot. Ash	40	-.22	HC vs. Turbid.	40	-.30
CW vs. Turbid.	40	0.55**	HC vs. WS-CHO	40	-.43**
CW vs. WS-CHO	40	-.52**	Si vs. Tot. Ash	40	0.29
ADF vs. L	40	0.85**	Si vs. Turbid.	40	0.62**
ADF vs. C	40	0.92**	Si vs. WS-CHO	40	0.09
ADF vs. HC	40	-.19	Tot. Ash vs. Turbid.	40	0.02
ADF vs. Si	40	0.54**	Tot. Ash vs. WS-CHO	40	-.32*
ADF vs. Tot. Ash	40	-.12	Turbid. vs. WS-CHO	40	-.28
ADF vs. Turbid.	40	0.67**			

See footnote of Tables 31, 32 for abbreviations.

*P < .05 **P < .01

relationships noted by Bennett and Archibald (13) and Archibald et al. (5). These grass samples were cut at different stages of growth and old grasses contained seeds or starchy material that might increase the extract turbidity of forages. The decrease in WS-CHO concentrations when levels of CP, CW, ADF, C, L, HC increased may be explainable by the fact that WS-CHO are readily available energy sources for the synthesis of these nutrients.

c. In Vitro Digestibilities vs. Chemical Composition

The simple correlation analysis between chemical composition and in vitro digestibilities presented in Table 34 indicates that there were significant and positive correlations between CP, soluble ash or HC/C ratio and IVDMD, IVOMD, IVCWD or IVTDMD. However, CW, ADF, L, C, Si, insoluble ash, L/fiber ratios and OD turbidity were significantly and negatively correlated with all measures of in vitro digestibilities.

In this study, L seemed to be the most important factor depressing in vitro DMD, IVOMD, IVCWD and IVTDMD and this is in agreement with data on forages from MSU Department of Dairy Science but differed from that of the Purdue forages in which C was the most important factor depressing fermentation values. In vitro CWD, IVDMD and IVOMD were inhibited more by L/C or L/HC ratios than by L/ADF ratio as reported by Van Soest (120). This may be related to the greater relationship obtained between L and digestibility estimate than that obtained by Van Soest.

TABLE 34. Correlation coefficients between chemical components and digestibilities for MSU grasses during 30 to 135 days of growth.

Items	n	IVDMD	IVOMD	IVCWD	IVTDMD
CP	40	0.60**	0.60**	0.56**	0.56**
CW	40	-.76**	-.78**	-.56**	-.78**
ADF	40	-.86**	-.87**	-.69**	-.85**
L	40	-.90**	-.90**	-.80**	-.87**
C	40	-.63**	-.65**	-.40**	-.63**
HC	40	0.24	0.22	0.31*	0.17
Tot. Ash	40	0.22	0.20	0.19	0.20
Insol. Ash	40	-.62**	-.62**	-.67**	-.61**
Sol. Ash	40	0.54**	0.53**	0.54**	0.52**
Si	40	-.64**	-.63**	-.67**	-.61**
WS-CHO	40	0.27	0.28	0.09	0.27
OD Turbid	40	-.83**	-.84**	-.78**	-.79**
L/ADF	40	-.80**	-.79**	-.75**	-.76**
L/C	40	-.84**	-.83**	-.81**	-.80**
L/HC	40	-.88**	-.87**	-.81**	-.84**
HC/C	40	0.67**	0.67**	0.53**	0.61**

For abbreviations, see footnotes Tables 28, 31 and 32.

*P < .05 **P < .01

Turbidity test values had high correlations (P < .01) with *in vitro* digestibilities indicating the possibilities of using this simple technique to predict *in vitro* or possibly *in vivo* digestibilities of grasses cut at different

stages of growth. However, total ash and water-soluble carbohydrates could not be used as predictors of any digestibility estimates because of low correlations with these parameters.

Relationships among the *in vitro* digestibility estimators such as IVDMD, IVOMD, IVCWD and IVTDMD were all mutually and significantly correlated ($r = 0.93$ to 0.99, $P < .01$) and the correlation coefficient between IVDMD and IVTDMD was 0.98. Thus IVTDMD could be precisely predicted from the two-stage *in vitro* fermentation. Van Soest *et al.* (126) reported that the values for *in vitro* true digestibility were similar to actual *in vivo* true digestibility.

V. RESULTS OF STUDIES ON FORAGES FROM THAILAND AND PUERTO RICO

a. Nutritive Value vs. Stage of Maturity

A simple correlation analysis between age of forages and measures of nutritive value for Thai forages is presented in Table 35. There were no significant correlations between age or maturity and all measures of nutritive value such as chemical composition or *in vitro* digestion values. However, CP, CC, total ash, L/fiber ratios and digestibilities tended to decrease with advancing maturity whereas fibrous fractions and TNC increased slightly with maturity. These trends are similar to those of other forages (Table 32). The rates of decline in CP and digestibilities for tropical forages were slower than those for temperate forages as can be seen from the magnitudes of correlations

TABLE 35. Correlation coefficients between maturity and measures of nutritive value for Thai forages during 30 to 90 days of growth.[4]

Factors Correlated	n	r	Factors Correlated	n	r
Age vs. IVDMD	23	-.15	Age vs. CC	23	-.13
AGE vs. IVCWD	23	-.12	Age vs. L/ADF	23	-.13
Afe vs. IVTDMD	23	-.18	Age vs. L/C	23	-.16
Age vs. CP	23	-.22	Age vs. L/HC	23	-.14
Age vs. CW	23	0.13	Age vs. HC/C	23	-.01
Age vs. ADF	23	0.16	Age vs. Cell	15	-.09
Age vs. L	23	-.08	Age vs. Amy	15	-.04
Age vs. C	23	0.20	Age vs. Buf	15	-.12
Age vs. HC	23	0.10	Age vs. TNC	15	0.44
Age vs. Si	23	-.01	Age vs. Prd. DDM[1]	23	-.15
Age vs. Tot. Ash	23	-.30	Age vs. Prd. DDM[2]	23	-.22
Age vs. Ins. Ash	23	-.09	Age vs. Prd. DMI[3]	23	-.10
Age vs. Sol. Ash	23	-.34			
Age vs. OM	23	0.30			

[1]Data from Reid et al. (98).

[2]Data from Bredon et al. (19).

[3]Data from Goering and Van Soest (43).

[4]Thai forages = Para Grass, Napier grass, speargrass, mung bean and centrosema grown during October 10, 1971 through January 15, 1972.

For abbreviations, see footnote of Tables 31, 32 and 33.

for CP, IVDMD or IVTDMD in Thai and MSU grasses. Minson and McLeod (78,80), Reid et al. (98) also reported that in vitro digestibility decreased 0.1 to 0.2 digestibility unit per day in tropical forages whereas the decrease for temperate grasses was 0.3 to 0.4 unit/d (Table 28). Total nonstructural carbohydrates (TNC) in tropical forages increased slightly with advancing maturity probably because these grasses accumulate sugars and starch (105). Lignin and Si did not increase with advancing maturity even though these forages were grown on sandy loam soils in southern Thailand.

b. Relationships Among Chemical Components

Correlation coefficients among chemical components for Thai and Puerto Rican forages are presented in Table 36. Thai forages contained 35% legumes, 65% grasses and all were cut at various ages whereas Puerto Rican forages were all grasses cut at one stage of maturity. Crude protein for both groups of forages was significantly and negatively correlated with CW, ADF, and C whereas HC, Si had negative correlations with CP but with differential significances. Lignin had a positive correlation with CP for the Thai forages because both components tended to decrease with advancing maturity but a negative correlation for the Puerto Rican grasses. There were positive correlations between CP and ash for both forages. Cell walls had positive correlations with the fibrous fractions (ADF, C, and HC) but had a negative correlation with L for Thai forages

TABLE 36. Correlation coefficients among chemical components for Thai and Puerto Rican forages.

Factors correlated	Thailand		Puerto Rico	
	n	r	n	r
CP vs. CW	23	-.83**	40	-.68**
CP vs. ADF	23	-.73**	40	-.73**
CP vs. L	23	0.25	40	-.47**
CP vs. C	23	-.78**	40	-.80**
CP vs. HC	23	-.84**	40	-.24
CP vs. Si	23	-.41*	40	-.06
CP vs. Ash	23	0.11	40	0.42**
CP vs. TNC	15	-.38	16	0.47
CW vs. ADF	23	0.93**	40	0.69**
CW vs. L	23	-.22	40	0.38*
CW vs. C	23	0.97**	40	0.71**
CW vs. HC	23	0.97**	40	0.72**
CW vs. Si	23	0.35	40	0.10
CW vs. Ash	23	-.28	40	-.63**
CW vs. TNC	15	0.03	16	-.38
ADF vs. L	23	-.09	40	0.71**
ADF vs. C	23	0.98**	40	0.87**
ADF vs. HC	23	0.81**	40	-.00
ADF vs. Si	23	0.45*	40	0.28
ADF vs. Ash	23	-.11	40	-.37*
ADF vs. TNC	15	0.08	16	-.39
L vs. C	23	-.21	40	0.39*
L vs. HC	23	-.28	40	-.16
L vs. Si	23	-.54**	40	0.21

TABLE 36. Continued.

Factors correlated	Thailand		Puerto Rico	
	n	r	n	r
L vs. Ash	23	-.27	40	-.39
L vs. TNC	15	-.49	16	-.54*
C vs. HC	23	0.88**	40	0.14
C vs. Si	23	0.38	40	0.06
C vs. Ash	23	-.21	40	-.32*
C vs. TNC	15	0.18	16	-.35
HC vs. Si	23	0.26	40	-.13
HC vs. Ash	23	0.37	40	-.52**
Si vs. Ash	23	0.69**	40	0.20
Si vs. TNC	15	0.25	16	0.13
Ash vs. TNC	15	-.04	16	-.22

For abbreviations, see footnotes of Tables 30, 31, 32 and 33.

*P < .05 **P < .01

and a positive correlation for Puerto Rican grasses. In general, the correlations between total nonstructural carbohydrates (TNC) and other constituents were variable and not statistically significant. Lignin had a significant and negative correlation with Si in Thai forages but a positive correlation in the Puerto Rican forages. Ash and Si had positive correlations for both groups and that for Thai forages was significant.

In tropical forages, an increase in CW fractions was followed by a decrease in CP and total ash. Cell walls

of tropical grasses contained 47.9% C, 40.8% HC, 2.9% Si and 7.7% L whereas cell walls of temperate grasses contained 47.0% C, 39.0% HC, 3.4% Si and 10.4% L. In Thai grasses alone, total ash contained 32.4% Si whereas Michigan grasses had only 27.2% Si in the total ash. Therefore, Si seemed to be a major component of total ash in some tropical forages grown on sandy loam soils.

c. Relationships Among Measures of Nutritive Value of Tropical Forages

The relationships between in vitro digestibilities and chemical components (Table 37) indicate that in vitro DMD and IVTDMD were positively correlated with CP, total ash and soluble ash but negatively correlated with CW, ADF, C, L and TRAC. Total nonstructural carbohydrates (TNC) had low and non-significant correlations with all three in vitro estimates of digestibility. Lignin/ADF ratio had significant and positive correlations with in vitro digestibilities in the Thai forages but not in Puerto Rican grasses.

Many patterns of relationships between IVCWD and chemical components were different for Thai and Puerto Rican forages. Crude protein and IVCWD were significantly and negatively correlated ($r = -.58$) for Thai forages but positively correlated ($r = 0.29$) in Puerto Rican grasses. Cell walls and ADF were positively correlated with IVCWD in Thai forages but significantly and negatively correlated with IVCWD in Puerto Rican grasses. All three in vitro digestibility techniques and enzymatic incubations as well

TABLE 37. Correlation coefficients among measures of nutritive value for Thai and Puerto Rican forages.

	IVDMD				IVTDMD				IVCWD				Correlations Among Measures of Nutritive Value (All tropical Grasses)	
	Thailand		Puerto Rico		Thailand		Puerto Rico		Thailand		Puerto Rico			
Items	n	r	n	r	n	r	n	r	n	r	n	r		
CP	23	0.47*	40	0.15	23	0.49*	40	0.46**	23	-.58**	40	0.29		
CW	23	-.81**	40	-.30	23	-.83**	40	-.67**	23	0.23	40	-.42**	IVDMD vs. IVCWD	0.50**
ADF	23	-.79**	40	-.45**	23	-.77**	40	-.67**	23	0.18	40	-.56**	IVDMD vs. IVTDMD	0.78**
L	23	-.09	40	-.25	23	-.06	40	-.39*	23	-.47*	40	-.36*	IVCWD vs. IVTDMD	0.70**
C	23	-.81**	40	-.36*	23	-.81**	40	-.62**	23	0.19	40	-.49**	IVDMD vs. Cell	0.59**
HC	23	-.77**	40	0.02	23	-.80**	40	-.29	23	0.24	40	-.05	IVDMD vs. Amy	0.59**
Ash	23	0.59**	40	0.13	23	0.63**	40	0.41**	23	0.58**	40	0.25	IVDMD vs. Buf	0.60**
S Ash	23	0.72**	40	0.20	23	0.74**	40	0.48**	23	0.49*	40	0.30	IVTDMD vs. Cell	0.83**
Si	23	0.05	40	-.18	23	0.09	40	-.21	23	0.69**	40	-.21	IVTDMD vs. Amy	0.74**
TNC	15	-.02	16	-.51	15	0.04	16	-.28	15	0.12	16	-.46	IVTDMD vs. Buf	0.84**
TRAC	15	-.96**	16	-.35	15	-.94**	16	-.52*	15	-.85**	16	-.41		
L/ADF	23	0.43*	40	-.04	23	0.44*	40	-.12	23	-.43*	40	-.14		
L/C	23	0.47*	40	-.11	23	0.50*	40	-.15	23	-.41*	40	-.18		
L/HC	23	0.42*	40	-.19	23	0.50*	40	-.14	23	-.40	40	-.24		
HC/C	23	-.55**	40	0.22	23	-.62**	40	0.10	23	0.30	40	0.25		

TRAC = Total residue after cellulase; S Ash = Soluble Ash. For other abbreviations see footnotes Tables 30, 31 and 32.

*P < .05 **P < .01

as buffer extract were positively and significantly ($P < .01$) correlated among themselves ($r = 0.50$ to 0.84). The correlation coefficient between buffer extract and IVTDMD ($r = 0.84$) was slightly higher than those between IVTDMD and enzymatic incubation values ($r = 0.74$ to 0.83).

With these tropical forages, increases in fibrous fractions (CS, ADF, C, HC) would also be accompanied by a decrease in CP and ash and both trends would result in a decrease in IVDMD and IVTDMD. Cell walls, ADF, and C were the most important factors depressing digestibilities in Thai and Puerto Rican grasses. Silica and lignin had much less relationships to *in vitro* digestibilities in these tropical forages than they had in temperate grasses (Table 34). High correlations between IVTDMD and IVDMD or IVCWD, enzymatic and buffer incubations indicate that IVTDMD could be predicted from these laboratory measurements. Buffer extract was a more accurate predictor of IVTDMD than was IVDMD or enzymatic incubations for tropical forages.

VI. RESULTS OF STUDIES ON GRASSES AND LEGUMES

a. Relationships Among Chemical Components

Results of studies in previous sections indicate that each group of forages has different relationships among chemical components, *in vitro* measurements, *in vivo* parameters and other measures of nutritive value and this is especially so when each forage group has different proportions of grasses to legumes. Data in Table 27

illustrate that grasses have higher CW, ADF, HC than legumes whereas legumes have higher amounts of CP, digestibility values than do grasses. Furthermore, temperate forages had higher levels of CP and digestibility than tropical grasses. These data and those of others (2,81,98) indicate that legumes should be examined separately from grasses. The correlations among chemical components for these two classes of forages are presented in Table 38.

A statistical analysis of overall forages indicates that CP was significantly and negatively correlated with CW, ADF, L, and Si but positively correlated with ash. Cell walls had significant and positive correlations with ADF and Si but tended to have a negative relationship to ash. Acid-detergent fiber was positively and significantly correlated with L (L is part of ADF) and Si but negatively correlated with ash. Lignin had a positive correlation with Si but a negative correlation with ash. However, ash and Si were positively correlated. The patterns for relationships among these components for the grasses and legumes alone followed similar trends to those relationships for combined forages but the magnitude of the correlation coefficients was very different between grasses and legumes.

In temperate forages, the relationships among chemical components for both grasses and legumes followed the same trends and had similar levels of significance even though the correlation coefficients were of different magnitude. Tropical grasses and legumes showed many differences. Crude protein in tropical grasses had significant

TABLE 38. Correlation coefficients among chemical components for grasses and legumes from temperate and tropical regions.

	Overall Forages						Temperate Forages					Tropical Forages		
	Combined		Grasses		Legumes		Grasses		Leg.[1]	Combined		Gra.[2]	Leg.[3]	Comb.[4]
Items	n	r	n	r	n	r	n	r	r	n	r	r	r	r
CP vs. CW	158	-.80**	120	-.64**	38	-.75**	65	-.67**	-.91**	95	-.81**	-.57**	0.37	-.72**
CP vs. ADF	164	-.77**	126	-.80**	38	-.65**	71	-.82**	-.85**	101	-.79**	-.68**	0.32	-.70**
CP vs. L	164	-.20**	126	-.62**	38	-.53**	71	-.68**	-.60**	101	-.32**	-.62**	-.23	-.07
CP vs. Si	103	-.45**	95	-.40**	8	-.06	40	-.59**	---	40	-.59**	-.29*	-.06	-.36**
CP vs. Ash	164	0.16*	126	0.30**	38	0.36*	71	0.45**	0.62**	101	0.40**	0.55**	-.35	0.24
CW vs. ADF	158	0.75**	120	0.84**	38	0.90**	65	0.92**	0.94**	95	0.72**	0.73**	0.83**	0.82**
CW vs. L	158	-.07	120	-.54**	38	0.75**	65	0.73**	0.78**	95	0.06	0.39**	0.56	-.19
CW vs. Si	103	0.21*	95	0.02	8	0.70*	40	0.25	---	40	0.25	-.06	0.70*	0.20
CW vs. Ash	158	-.09	120	-.42**	38	-.47**	65	-.50**	-.64**	95	-.27**	-.73**	-.05	-.23
ADF vs. L	164	0.51**	126	0.75**	38	0.83**	71	0.85**	0.84**	101	0.67**	0.62**	0.73*	0.17
ADF vs. SI	103	0.42**	95	0.34**	8	0.65	40	0.54**	---	40	0.54**	0.24	0.65	0.36**
ADF vs. Ash	164	-.21**	126	-.26**	38	-.40*	71	-.42**	-.61**	101	-.45**	-.47**	0.32	-.19
L vs. Si	103	0.23*	95	0.29**	8	0.48	40	0.53**	---	40	0.53**	0.05	0.48	0.04
L vs. Ash	164	-.44**	126	-.41**	38	-.43**	71	-.42**	-.64**	101	-.44**	-.53**	0.24	-.47**
Si vs. Ash	103	0.24*	95	0.21*	8	0.56	40	0.29	---	40	0.29	0.30*	0.56	0.35**

[1] n = 30; [2] n = 55; [3] n = 8; [4] n = 63.

See footnote of Table 29 for abbreviations.

*P < .05 **P < .01

and negative correlations with CW and ADF but the correlations were positive and non-significant in tropical legumes. Lignin and Si were significantly and negatively correlated with CP in grasses but non-significant in legumes. The correlation between CP and ash was positive for tropical grasses but negative for the legumes. The correlation between CW and Si was slightly negative in tropical grasses but significantly positive in legumes. Cell walls had a significant, negative correlation with ash in grasses but practically no relationship for tropical legumes. In tropical grasses, ash had significant and negative correlations with ADF and L but non-significant and positive correlations in tropical legumes. Evidently there were many opposite relationships among chemical components for grasses and legumes. Besides, the rates of change in chemical composition and digestibilities were slower for tropical forages (Table 28). Tropical legumes had only two species of forages and a small number of samples which might not be representative of a larger population of tropical legumes. All these reasons might also contribute to overall differences between temperate and tropical forages.

b. In Vivo Parameters vs. Laboratory Estimates

The relationships between in vivo digestibility, DMI and laboratory estimates are presented in Table 39. In temperate forages, in vivo DDM had significant but low correlations with DMI, CP, ash, and all enzymatic incubations

TABLE 39. Correlation coefficients between digestibility or intake and measures of nutritive value in temperate forages.

Factors correlated	Combined		Grasses		Legumes (Alfalfa)	
	n	r	n	r	n	r
In vivo DDM vs. DMI	61	0.44**	31	0.41*	30	0.46**
In vivo DDM vs. IVDMD	33	0.85**	20	0.92**	13	0.86**
In vivo DDM vs. IVOMD	15	0.89**	8	0.98**	7	0.95**
In vivo DDM vs. Cell	32	0.63**	26	0.85**	6	0.59
In vivo DDM vs. Amy	20	0.53*	14	0.78**	6	0.52
In vivo DDM vs. Buf	57	0.62**	29	0.61**	28	0.74**
In vivo DDM vs. CP	61	0.69**	31	0.65**	30	0.79**
In vivo DDM vs. CW	55	-.62**	25	-.71**	30	-.86**
In vivo DDM vs. ADF	61	-.74**	31	-.70**	30	-.79**
In vivo DDM vs. L	61	-.40**	31	-.75**	30	-.70**
In vivo DDM vs. Ash	61	0.55**	31	0.65**	30	0.48**
In vivo DMI vs. IVDMD	33	0.64**	20	0.61**	13	0.62*
In vivo DMI vs. IVOMD	15	0.74**	8	0.51	7	0.74
In vivo DMI vs. Cell	32	0.53**	26	0.52**	6	0.22
In vivo DMI vs. Amy	20	0.69**	14	0.88**	6	0.08
In vivo DMI vs. Buf	57	0.42**	29	0.45*	28	0.10
In vivo DMI vs. CP	61	0.52**	31	0.63**	30	0.26
In vivo DMI vs. CW	55	-.45**	25	-.45*	30	-.22
In vivo DMI vs. ADF	61	-.37**	31	-.60**	30	-.22
In vivo DMI vs. L	61	-.04	31	-.64**	30	-.28
In vivo DMI vs. Ash	61	0.33**	31	0.53**	30	0.20

See footnotes of Tables 29, 30, and 31 for abbreviations.

*P < .05 **P < .01

(r = 0.44 to 0.69). Digestibility was significantly and negatively correlated with CW, ADF and lignin. Two-stage *in vitro* fermentations (IVDMD or IVOMD) had highly significant correlations with *in vivo* DDM (r = 0.85 to 0.89). In this study, ADF seemed to be an important factor depressing forage digestibility. Due to low correlations, all enzymatic values would not be satisfactory to estimate *in vivo* DDM for combined forages. Even buffer extract was as good as the two enzymatic incubations. The two-stage *in vitro* fermentation was an excellent technique and IVOMD was better than IVDMD in predicting *in vivo* DDM.

The relationships between *in vivo* DDM and DMI or other measures of nutritive value for temperate grasses were similar to those for temperate legumes in terms of plus and minus signs. However, the correlations between DDM and cellulase or amylase incubations were highly significant (r = 0.78 to 0.85) for grasses but non-significant for legumes. Many laboratory methods had high relationships to DDM within either grasses or legumes indicating that these measurements can be used to predict *in vivo* DDM of that particular forage type.

Forage DMI had significant but low correlations with IVDMD, enzymatic incubations, buffer extract, CP and ash (r = 0.33 to 0.69) whereas it had negative relationships to CW, ADF and L when forages were combined. *In vitro* OMD seemed to be a better predictor of DMI for combined forages than other laboratory estimates. Amylase incubation was slightly better than cellulase in predicting DMI. Among

chemical components, CP was better than CW and this finding is not in agreement with that by Van Soest (117) who found CW to be a better predictor of DMI than CP. Relationships between DMI and laboratory estimates for temperate grasses and legumes were similar but the magnitudes of correlation coefficients for both forages were very different. Amylase incubation seemed to be a good predictor of DMI for grasses but not for legumes. In vitro DMD might be used to predict DMI for either grasses, legumes or combined forages but with low accuracy. Cell walls were not satisfactory ($r = -.45$) to predict DMI of forages even though grasses contain higher amounts of CW than do legumes. On the other hand, L was a better predictor of DMI than CP, CW, ADF in grasses.

VII. PREDICTIONS OF NUTRITIVE VALUE FROM LABORATORY ESTIMATES AND STAGE OF MATURITY

In the present study, there were different relationships among in vivo parameters and laboratory values for the various groups of forages studied. Therefore, some laboratory methods may be useful in predicting nutritive value of certain forages but not for other forages. To illustrate this point, several selected laboratory values that could be used to predict in vivo nutritive value of specified forage types are presented in Tables 40 to 42. Prediction equations for nutritive value from stage of maturity are shown in Table 43. Various aspects of these prediction equations will now be discussed.

a. Predictions of In Vivo Dry Matter Digestibility (DDM)

The in vivo dry matter digestibility of alfalfa and some temperate grasses can be precisely predicted from several values obtained by laboratory procedures (Table 40). Cell walls could be used to predict DDM of alfalfa more accurately ($r = -.86$, $P < .01$, SEE = 3.9) than for temperate grasses ($r = -.71$, $P < .01$, SEE = 5.6). Oh et al. (88) also reported a high correlation ($r = -.86$, $P < .01$) between CW and DDM for alfalfa. In vivo DDM of alfalfa and temperate grasses could also be predicted by using ADF, L and CP with moderate to high accuracy (Items 3 to 13, Table 40) with standard error of estimate of 2.9 to 5.6. Acid-detergent fiber or L could predict in vivo DDM of reed canary grass more accurately than they could predict DDM for alfalfa, bromegrass, tall fescue and mixed grasses. Many scientists have also reported that ADF and L had high correlations with DDM (58,88,115,131) and that DDM of temperate grasses was precisely predicted ($r = -.94$, SEE = 2.1) from their lignin content (111).

Crude protein could predict DDM of alfalfa with only moderate accuracy (SEE = 4.7) in this study. Bredon et al. (19) reported that CP was not a reliable predictor of DDM for mixed tropical grasses (SEE $\leq$ 10.0). Generally speaking, all these chemical components (CW, ADF, L, CP) could be used to predict DDM of a specified forage type but with large standard errors of estimate. An acceptable standard error for predicting DDM should be kept below 3

TABLE 40. Regression equations for estimating *in vivo* digestibility and *in vitro* true dry matter disappearance from laboratory analytical values.

DDM or IVTDMD vs. Laboratory Estimates	Forage Type	n	r	SEE
Predictions of DDM (%)				
1. DDM = 98.840-0.790CW	Alfalfa	30	-.86**	3.9
2. DDM = 108.900-0.760CW	Temp. gra.	25	-.71**	5.6
3. DDM = 97.800-0.966ADF	Alfalfa	30	-.79**	4.5
4. DDM = 96.025-0.959ADF	Brome	16	-.70**	5.6
5. DDM = 112.426-1.635ADF	Reed Can.	6	-.84*	3.4
6. DDM = 103.000-1.115ADF	Tall Fes.	9	-.86**	4.4
7. DDM = 93.819-0.912ADF	Temp. gra.	31	-.70**	5.2
8. DDM = 86.665-2.970L	Alfalfa	30	-.70**	5.3
9. DDM = 79.317 - 3.584L	Brome	16	-.79*	4.8
10. DDM = 87.499-8.029L	Reed Can.	6	-.89*	2.9
11. DDM = 83.038-5.115L	Tall Fes.	9	-.93**	3.3
12. DDM = 76.746-3.444L	Temp. gra.	31	-.75**	4.9
13. DDM = 34.300+1.460CP	Alfalfa	30	0.79**	4.7
14. DDM = 1.369IVDMD-23.923	Alfalfa	12	0.88**	4.4
15. DDM = 0.960 IVDMD+4.579	Brome	11	0.93**	2.8
16. DDM = 1.138IVDMD-7.786	Reed Can.	6	0.88*	3.1
17. DDM = 0.934IVDMD+5.716	Temp. gra.	20	0.92**	2.6
18. DDM = 1.525IVOMD-30.411	Alfalfa	6	0.96**	2.8
19. DDM = 1.077IVOMD+1.621	Temp. gra.	8	0.98**	1.8
20. DDM = 29.460+0.650Cell	Alfalfa	6	0.58	5.4
21. DDM = 38.570+0.660Cell	Brome	13	0.83**	3.8
22. DDM = 33.810+0.810Cell	Reed Can.	6	0.76	4.1
23. DDM = 31.340+0.880Cell	Tall Fes.	7	0.89**	4.6
24. DDM = 35.660+0.740Cell	Temp. gra.	26	0.85**	3.9
25. DDM = 28.257+0.865Amy	Alfalfa	6	0.52	5.6
26. DDM = 43.062+0.723Amy	Brome	7	0.78*	5.4

TABLE 40. Continued.

DDM or IVTDMD vs. Laboratory Estimates	Forage Type	n	r	SEE
Predictions of DDM (%)				
27. DDM = 35.283+0.961Amy	Tall Fes.	7	0.79*	6.1
28. DDM = 39.229+0.836Amy	Temp. gra.	14	0.78**	5.4
29. DDM = 10.264+1.506 Pep	Alfalfa	5	0.90*	3.0
30. DDM = 39.934+0.734 Pep	Temp. gra.	10	0.74*	3.3
31. DDM = 20.090+0.904(Cell+Amy)	Alfalfa	22	0.77**	4.4
32. DDM = 35.784+0.678(Cell+Amy)	Brome	9	0.85**	5.2
33. DDM = 32.230+0.806(Cell+Amy)	Tall Fes.	8	0.89**	4.3
34. DDM = 34.684+0.722(Cell+Amy)	Temp. gra.	17	0.86**	4.6
35. DDM = 23.526+0.729(Cell+Pep)	Alfalfa	6	0.63	5.0
36. DDM = 39.106+0.584(Cell+Pep)	Brome	6	0.87*	2.4
37. DDM = 23.589+0.960(Cell+Pep)	Reed Can.	6	0.93**	2.3
38. DDM = 32.449+0.743(Cell+Pep)	Temp. gra.	12	0.88**	2.4
39. DDM = 30.498+1.267Buf	Alfalfa	22	0.75**	4.5
40. DDM = 38.788+1.101Buf	Tall Fes.	8	0.74*	6.4
41. DDM = 40.584+1.035Buf	Temp. gra.	17	0.64**	6.9
Predictions of IVTDMD (%)				
42. IVTDMD = 19.760+0.700IVDMD	Temp. gra.	40	0.97**	2.4
43. IVTDMD = 31.230+0.930Cell	Trop. gra.	31	0.83**	5.5
44. IVTDMD = 26.086+1.548Amy	Trop. gra.	31	0.74**	6.5
45. IVTDMD = 26.400+1.721Buf	Trop. gra.	31	0.84**	5.3

DDM = *In vivo* dry matter digestibility; IVTDMD = *In vitro* true dry matter disappearance; CW = Cell walls; ADF = Acid-detergent fiber; L = Lignin; CP = Crude protein; IVDMD = *In vitro* dry matter disappearance (Tilley-Terry method); IVOMD = *In vitro* organic matter digestibility; Cell = Cellulase incubation; Amy = Amylase incubation; Pep = Pepsin incubation; Buf = Buffer extract; r = Correlation coefficients; SEE = Standard error of estimate.

*P < .05 **P < .01

digestibility units (112,117). A prediction equation for a given forage type was usually unsuitable for another forage type. For example, prediction equations in items 8 and 9 using lignin to predict DDM of alfalfa and bromegrass might be different because they had different intercepts or constants (86.7 vs. 79.3) and different slopes (3.0 vs. 3.6) for alfalfa and bromegrass, respectively.

Two-stage in vitro fermentations (IVDMD and IVOMD, Items 14 to 19, Table 40) could predict in vivo DDM of alfalfa and temperate grasses more accurately than could chemical components discussed above. The respective standard errors of estimate ranged from 1.8 to 4.4 compared to 2.9 to 5.6. In vitro OMD was better than IVDMD in predicting DDM of both alfalfa and grasses. Several scientists also reported that the two-stage fermentations were excellent techniques to predict DDM of grasses or legumes (10,58,88,112,126).

Cellulase, amylase, pepsin and a sequential hydrolysis by these enzymes (Items 20 to 38, Table 40) could be used to predict in vivo DDM of alfalfa and temperate grasses with standard errors of estimate ranging from 2.3 to 6.1. Cellulase was efficient in predicting DDM of bromegrass and/or combined temperate grasses but it was not a reliable predictor of DDM for alfalfa. Amylase could predict DDM of temperate grasses slightly better than it could for alfalfa, but pepsin could predict DDM of alfalfa better than for grasses. A sequential hydrolysis by

cellulase plus amylase could predict DDM of alfalfa and grasses with similar accuracy whereas the hydrolysis by cellulase plus pepsin could predict DDM of grasses much more accurately than it could for alfalfa.

The amount of material solubilized by only buffer could predict DDM of alfalfa more accurately than it could for temperate grasses. However, the standard errors of estimate for buffer incubations were too large (4.53 to 6.86) for acceptable prediction purposes.

In temperate grasses, IVDMD could be used to predict in vitro true dry matter disappearance (IVTDMD) accurately (SEE = 2.4) whereas cellulase, amylase and buffer incubations could predict IVTDMD of tropical grasses but with larger standard errors (5.3 to 6.5).

In this study, the two-stage in vitro fermentations (IVDMD or IVOMD) were the most accurate methods to predict in vivo DDM of legumes as well as grasses. Chemical components (CW, ADF, L, CP) were as accurate predictors as enzymatic incubation values in predicting DDM. Among these three enzymes, cellulase and pepsin were comparable and were slightly better than amylase in predicting DDM. Cellulase tended to be the preferred enzyme for predicting DDM of grasses while pepsin was the preferred enzyme for legumes. This may be related to the fact that legumes (alfalfa) contain higher level of CP (20.0% compared with 13.8%) which may serve as substrate for pepsin while grasses contain more cellulose than alfalfa (32.0 compared with

27.7, Table 27, C = ADF - L). A sequential hydrolysis by cellulase plus amylase did not measurably improve the accuracy of DDM prediction over the use of one enzyme alone. Yet, in some cases, the sequence of two enzymes increased precision of estimation over that of only one enzyme. For example, a sequence of cellulase plus pepsin greatly improved the prediction of DDM for grasses by reducing standard errors from 3.9 (for cellulase alone) and 3.3 (for pepsin alone) to 2.4 digestibility units. Similarly cellulase plus amylase reduced standard errors of predicting DDM for alfalfa from 5.4 (cellulase alone) and 5.6 (amylase alone) to 4.4.

The regression analysis in Table 40 indicates that CW and ADF had a smaller effect on digestibility of grasses than for alfalfa but lignin had a larger effect in grasses than in legumes (compare the regression coefficients (b-values) for Equations 1 vs. 2, 3 vs. 7 and 8 vs. 12, Table 40). The intercept values (constants) for these equations indicate that the digestibility of alfalfa having no ADF or L (97.8, 86.7) exceeds that of grasses having no ADF or L (93.8, 76.7). The intercept values for Equations 1 and 2 approximate 100 indicating that CW may be responsible for DDM varying from 100.

b. Predictions of Dry Matter Intake (DMI)

Dry matter intake ($gm/BW_{Kg}^{0.75}$) of temperate grasses predicted from CW or L content had standard errors of estimate of 13.4 and 10.2 and were too large to be acceptable

(Table 41). Other workers also reported that CW had low but significant correlations (r = -.65 to -.77) with DMI (51,77,117).

Two-stage IVDMD was not a reliable predictor of DMI with standard errors of prediction of 14.3 and 12.0 for alfalfa and temperate grasses, respectively. This finding is in agreement with studies by others (10,51). Cellulase incubation values were an excellent predictor of DMI for tall fescue and bromegrass (SEE = 3.2 to 4.1) but not for mixed grasses. Amylase incubation was also a reliable predictor of DMI for bromegrass, tall fescue and mixed grasses with standard errors of 4.3 to 5.2. A sequential hydrolysis by cellulase then amylase could predict DMI of bromegrass, tall fescue and mixed grasses more precisely than using amylase alone. Buffer extract could predict DMI of bromegrass, tall fescue and mixed grasses more accurately than could CW, L or IVDMD as buffer incubation values had greater correlations and lower standard errors of estimate.

In this study, enzymatic incubations were more accurate than chemical components (CW, L), IVDMD or buffer extract in predicting forage DMI. Even incubation with buffer only was slightly better than IVDMD or chemical components. All three types of enzymatic incubations had similar degrees of accuracy for predicting forage intake with cellulase preferable for the individual grasses. In practice, a single enzymatic incubation would be simpler

TABLE 41. Regression equations for estimating dry matter intake from laboratory analytical values.

In Vivo DMI vs. Laboratory Estimates	Forage Type	n	r	SEE
Predictions of DMI ($gm/BW_{Kg}^{0.75}$)				
1. DMI = 123.070-0.940CW	Temp. gra.	24	-.46*	13.4
2. DMI = 87.790-5.240L	Temp. gra.	31	-.64**	10.2
3. DMI = 2.063IVDMD-47.948	Alfalfa	13	0.62*	14.3
4. DMI = 1.409IVDMD-14.746	Temp. gra.	20	0.61**	12.0
5. DMI = 1.070Cell+16.770	Brome	7	0.94**	4.1
6. DMI = 0.800Cell+30.650	Tall Fes.	7	0.93**	3.2
7. DMI = 0.910Cell+32.900	Temp. gra.	26	0.51**	12.0
8. DMI = 1.203Amy+22.701	Brome	7	0.91**	5.2
9. DMI = 0.922Amy+32.899	Tall Fes.	7	0.87*	4.3
10. DMI = 1.061Amy+27.951	Temp. gra.	14	0.88**	4.7
11. DMI = 0.910(Cell+Amy)+21.365	Brome	9	0.96**	3.4
12. DMI = 0.778(Cell+Amy)+30.166	Tall Fes.	8	0.93**	3.4
13. DMI = 0.836(Cell+Amy)+26.006	Temp. gra.	17	0.92**	3.8
14. DMI = 1.587Buf+24.104	Brome	9	0.77*	7.4
15. DMI = 1.053Buf+36.702	Tall Fes.	8	0.76*	5.7
16. DMI = 1.307Buf+30.579	Temp. gra.	17	0.75**	6.4

DMI = Dry matter intake.

For other abbreviations, see Table 40.

*P < .05 **P < .01

than a sequence of two enzyme incubations. Cellulase or amylase incubation values would be a satisfactory predictor of DMI for several temperate grasses. For rapid and inexpensive screening of grasses, incubations with buffer only could be used to estimate dry matter intake.

c. Predictions of Total Digestible Nutrients (TDN), Digestible Dry Matter (DDM) and Digestible Energy (DE)

Since only a few values for TDN of legumes were available, the following discussion will serve as an exploration to the use of some laboratory estimates to predict TDN. Crude protein, two-stage IVDMD, cellulase, pepsin and a sequential hydrolysis by cellulase then pepsin had positive and non-significant correlations with TDN for legumes. Data in Table 42 indicate that these laboratory values might not be reliable predictors of TDN because some of them gave large standard errors of estimate (4.4 to 6.5). Acid-detergent fiber might be an accurate predictor of TDN for legumes since it had high correlation with TDN and gave small standard error of estimate (SEE = 1.8).

The same samples of temperate grasses were used to compare the predictability of DDM, TDN and DE from similar laboratory values (Items 7 through 24, Table 42). A sequential hydrolysis of cellulase plus pepsin tended to be a more accurate predictor of DDM than chemical components, IVDMD, cellulase or pepsin alone (SEE = 2.9 for cellulase + pepsin compared with 3.3 to 4.2 for the others).

In this study, the two-stage IVDMD and enzymatic incubations did not excel chemical components (CP, ADF) in predicting TDN of temperate grasses. Acid-detergent fiber tended to be a reliable predictor of TDN with a standard error of 2.6.

TABLE 42. Regression equations for estimating total digestible nutrients, digestible dry matter and digestible energy from laboratory analytical values.

In Vivo Parameters vs. Laboratory Estimates	Forage Type	n	r	SEE
a. Predictions of TDN (%)				
1. TDN = 13.722+2.724CP	Alfalfa	4	0.87	4.4
2. TDN = 269.077-5.633ADF	"	4	-.98*	1.8
3. TDN = 1.334IVDMD-22.329	"	4	0.84	4.9
4. TDN = 0.898Cell+15.920	"	4	0.69	6.5
5. TDN = 1.250Pep+13.795	"	4	0.69	6.5
6. TDN = 0.946(Cell+Pep)+10.401	"	4	0.72	6.3
b. Predictions of DDM (%)				
7. DDM = 34.937+1.236CP	Temp. gra.[1]	7	0.86*	3.3
8. DDM = 115.658-1.724ADF	"	8	-.84**	3.6
9. DDM = 1.042IVDMD-0.271	"	8	0.84**	3.5
10. DDM = 0.858Cell+32.797	"	8	0.83**	3.7
11. DDM = 0.955Pep+32.742	"	8	0.77*	4.2
12. DDM = 0.786(Cell+Pep)+31.306	"	8	0.90**	2.9
c. Predictions of TDN (%)				
13. TDN = 37.974+0.971CP	Temp. gra.[1]	7	0.77*	3.6
14. TDN = 106.305-1.506ADF	"	8	-.87**	2.6
15. TDN = 0.829IVDMD+9.841	"	8	0.80*	3.3
16. TDN = 0.711Cell+35.194	"	8	0.82*	3.2
17. TDN = 0.715 Pep+37.430	"	8	0.69	4.0
18. TDN = 0.615(Cell+Pep)+35.350	"	8	0.84**	3.0
d. Predictions of DE (%)				
19. DE = 39.164+0.914CP	Temp. gra.[1]	7	0.70	4.2
20. DE = 100.125-1.309ADF	"	8	-.74*	3.8
21. DE = 0.904IVDMD+5.402	"	8	0.85**	2.9
22. DE = 0.773Cell+33.135	"	8	0.87**	2.8

TABLE 42. Continued.

In Vivo Parameters vs. Laboratory Estimates	Forage Type	n	r	SEE
23. DE = 0.723Pep+37.217	Temp. gra.	8	0.68	4.1
24. DE = 0.650(Cell+Pep)+34.035	"	8	0.87**	2.8

TDN = Total Digestible nutrients; DDM = Digestible dry matter; DE = Digestible energy. See Table 40 for other abbreviations.

[1]Same samples for all three parameters (DDM, TDN, DE).

*P < .05 **P < .01

Digestible energy of grasses could be satisfactorily predicted from either cellulase, cellulase plus pepsin or the two-stage IVDMD with standard errors ranging from 2.8 to 2.9. Pepsin was not as efficient as cellulase in predicting DE of grasses. In this study, IVDMD, cellulase and cellulase plus pepsin excelled chemical components (CP, ADF) in predicting digestible energy. This finding is in accord with those by Butterworth (21), Johnson and Dehority (58). Even though a sequence of cellulase plus pepsin was also excellent, cellulase alone might be sufficient for predicting DE because a cellulase incubation has less manipulations than the two-enzyme sequence.

d. Predictions of Crude Protein and In Vitro Digestibilities from Stages of Maturity

Correlation coefficients and prediction equations for CP levels and in vitro digestibilities based on stages of maturity of grasses are presented in Table 43. Crude

TABLE 43. Regression equations for estimating forage nutritive value from stage of maturity.

Nutritive Value vs. Maturity	Forage Type	n	r	SEE
Crude Protein (CP, %)				
1. CP = 17.309-0.093X	Brome	8	-.96**	1.1
2. CP = 18.414-0.096X	Orchard	8	-.89**	1.9
3. CP = 20.810-0.120X	Reed Canary	8	-.93**	1.9
4. CP = 19.695-0.103X	Tall Fescue	8	-.91**	1.9
5. CP = 20.034-0.071X	Ky. Bluegrass	8	-.78*	2.3
6. CP = 19.252-0.096X	Temp. grasses	40	-.83**	2.3
7. CP = 11.162-0.066X	Para grass	5	-.74	1.6
8. CP = 14.032-0.092X	Napier	5	-.83	1.7
9. CP = 10.880-0.062X	Speargrass	5	-.99**	0.2
10. CP = 12.024-0.073X	Trop. grasses	15	-.77**	1.4
11. CP = 19.470+0.035X	Centrosema	5	-.30	3.1
In Vitro Dry Matter Disappearance (IVDMD, %)				
12. IVDMD = 72.854-0.228X	Brome	8	-.93**	3.7
13. IVDMD = 86.999-0.422X	Orchard	8	-.99**	2.0
14. IVDMD = 74.680-0.214X	Reed Canary	8	-.82*	5.8
15. IVDMD = 82.889-0.423X	Tall Fescue	8	-.98**	3.1
16. IVDMD = 81.304-0.457X	Ky. Bluegrass	8	-.98**	3.5
17. IVDMD = 79.745-0.349X	Temp. grasses	40	-.87**	7.0
18. IVDMD = 55.358-0.013X	Para grass	5	-.14	2.6
19. IVDMD = 60.764-0.052X	Napier	5	-.82	1.0
20. IVDMD = 39.278-0.018X	Speargrass	5	-.21	2.4
21. IVDMD = 60.102-0.063X	Centrosema	5	-.36	4.5
22. IVDMD = 51.133-0.028X	Trop. grasses	15	-.07	9.0
In Vitro True Dry Matter Disappearance (IVTDMD, %)				
23. IVTDMD = 73.028-0.175X	Brome	8	-.90**	3.4
24. IVTDMD = 81.764-0.312X	Orchard	8	-.98**	2.8
25. IVTDMD = 75.190-0.163X	Reed Canary	8	-.85**	4.1
26. IVTDMD = 77.164-0.295X	Tall Fescue	8	-.97**	2.8
27. IVTDMD = 70.954-0.262X	Ky. Bluegrass	8	-.98**	2.1

TABLE 43. Continued.

Nutritive Value vs. Maturity	Forage Type	n	r	SEE
28. IVTDMD = 75.620-0.241X	Temp. grasses	40	-.83**	5.7
29. IVTDMD = 58.538-0.030X	Trop. grasses	15	-.07	9.6

X = No. of days elapsing from first regrowth (April 15 through September 4, 1972 for temperate grasses; October 10, 1971 through January 15, 1972 for tropical forages).

r = Correlation coefficients; SEE = Standard error of estimate.

*P < .05 **P < .01

protein levels in bromegrass, orchard grass, reed canary, tall fescue and Kentucky bluegrass decreased significantly (P < .05 to < .01) with advancing maturity. From the day of first cutting in the spring (May 20) through September 4, CP levels of reed canary and tall fescue decreased in excess of 0.10% per day whereas that for Kentucky bluegrass decreased only 0.07% per day. On the average, CP in temperate grasses decreased at the rate of 0.10% per day and this was statistically significant (P < .01). There was greater precision for estimating percentage of CP in bromegrass than in Kentucky bluegrass or all grasses combined from maturity data. Regression equations to predict CP from maturity for orchard grass, reed canary and tall fescue had similar standard errors (SEE = 1.9).

For the tropical grass samples, all had a decrease in protein with advancing maturity but only speargrass and

combined tropical grasses had statistically significant, negative correlations (Table 43). The rate of decline in CP for Napier grass was greatest (0.09% per day) compared with 0.06 and 0.07% per day for speargrass and Para grass, respectively. On the other hand, CP level in Centrosema, a tropical legume, tended to increase probably due to only 5 cuttings where CP increased from 17% (week 4, first cutting) to 24, 23, 23, 20% for second, third, fourth, and fifth cutting, respectively. The rate of change was obviously non-linear. On the average, CP in tropical grasses declined at a slower rate (0.07 vs. 0.10% per day) and this was not statistically different from that for temperate grasses. Crude protein in speargrass could be precisely predicted from Equation 9 with only 0.2% standard error and, Equation 10 could predict CP in all tropical grasses with a standard error of only 1.4%.

In vitro DMD of both temperate and tropical forages decreased with advancing maturity. The rates of decline in IVDMD for five temperate grasses ranged from 0.21 to 0.46% unit per day with an average of 0.35 ($P < .01$). The IVDMD of orchard grass could be predicted with the least standard error of any grass whereas the IVDMD levels in other four grasses could be predicted with standard errors of 3.1 to 5.8.

In vitro DMD of all tropical grasses and the legume decreased with advancing maturity but the correlations and regression coefficients were not statistically significant. The rates of decline in IVDMD for tropical forages ranged

from 0.01 to 0.06 percentage unit per day with an average rate of 0.03% unit per day and this was significantly different ($P < .01$) from a value of 0.35 for temperate grasses.

In vitro true dry matter disappearance (IVTDMD) of temperate grasses was significantly decreased ($P < .01$) with advancing maturity while that for tropical grasses was not affected. The rate of decline in IVTDMD for orchard grass was greatest (0.31) and that for reed canary grass was the least (0.16% unit per day). On the whole, the rate of digestibility decline for temperate grasses was greater ($P < .05$) than that for tropical grasses (0.24 vs. 0.03% unit per day). Regression equation for Kentucky bluegrass could accurately predict its IVTDMD at any stage of growth with 2.1% standard error. Equations for other temperate grasses gave larger standard errors (2.8 to 4.1).

VIII. PREDICTIONS OF FORAGE NUTRITIVE VALUE USING MULTIPLE REGRESSION TECHNIQUE

In many cases, simple correlation and regression was not satisfactory for predicting in vivo forage nutritive value due to the low relationships and predictability obtained between the laboratory estimators and the in vivo parameters. In many cases, inclusion of two or more variables in a multiple regression equation can greatly increase the accuracy of the prediction equation.

a. Predictions of In Vivo Digestibility and Intake for Purdue and Michigan State University Forages

Multiple regression equations for predicting in vivo DDM and DMI of Purdue and MSU forages from laboratory analytical values are presented in Table 44. Combinations of chemical components used to predict DDM and DMI of temperate grasses (Equations 1,2) gave large standard errors (5.0 to 5.3 for DDM and 9.9 to 7.5 for DMI prediction). The multiple correlation coefficients for DDM in Equations 1,2 were only slightly greater than their individual simple correlation coefficients (see Table 44) and low partial correlation coefficients indicate that these predictor combinations were little or no more reliable predictors of DDM than were CW, ADF or L alone. The latter three each used alone had r value of 0.70 or more and could predict DDM of grasses with standard errors of estimate similar to that for the multiple regression (Table 40). However, prediction of DMI from combined chemical predictors (Equations 1 or 2, Table 44) was slightly more accurate than using CW or L alone (Table 41).

For multiple correlation and regression analysis in this case, the multiple correlation coefficient (R) measures the closeness of relationship between in vivo DDM or DMI and combined effects of two or more variables. The numerical value of an R lies between zero (no relationship) and +1.0 (greatest relationship) and generally the value is always at least as large as that of any simple or partial correlation coefficient. Partial correlation is a

TABLE 44. Multiple regression equations for estimating in vivo digestibility and intake of temperate grasses and legumes from laboratory analytical values (Purdue University and MSU forages).

Items	In Vivo Dry Matter Digestibility (%)	Dry Matter Intake ($gm/BW_{Kg}^{0.75}$)
	Temperate Grasses	
Equation 1. (n = 31)	R = 0.77** SEE = 5.0	R = 0.70** SEE = 9.9
bo Constant	76.36	40.42
X1 CP	0.14 (0.65**,[1] 0.07[2])	1.38 (0.63**,[1] 0.33[2])
X2 ADF	-0.20 (-.70**, -.08)	0.97 (-.60**, 0.18)
X3 Lig	-2.41 (-.75**, -.33)	-5.36 (-.64**, -.36)
X4 Ash	0.05 (0.65**, 0.01)	-1.19 (0.52**, -.12)
Equation 2. (n = 25)	R = 0.79** SEE = 5.3	R = 0.88** SEE = 7.5
bo Constant	87.18	18.84
X1 CP	0.41 (0.76**, 0.14)	1.84 (0.77**, 0.40)
X2 CW	-0.33 (-.71**, -.17)	1.81 (-.45*, 0.56**)
X3 ADF	0.16 (-.75**, 0.04)	-1.41 (-.67**, -.24)
X4 Lig	-2.48 (-.75**, -.24)	-5.44 (-.73**, -.36)
X5 Ash	-0.81 (0.63**, -.14)	-2.40 (0.54**, 0.29)
Equation 3. (n = 23)	R = 0.81** SEE = 5.3	R = 0.92** SEE = 7.0
bo Constant	162.29	126.48
X1 Buffer	-1.17 (0.61**, -.34)	-0.83 (0.49*, -.19)
X2 CP	0.59 (0.75**, 0.16)	-0.17 (0.78**, -.03)
X3 CW	-1.01 (-.71**, -.36)	2.77 (-.46*, 0.62**)
X4 ADF	-0.01 (-.74**, -.00)	-4.58 (-.68**, -.51*)

TABLE 44. Continued.

Items	In Vivo Dry Matter Digestibility (%)	Dry Matter Intake ($gm/BW_{Kg}^{0.75}$)
X5 Lig	-2.14 (-.73**, -.21)	-7.27 (-.76**, -.48*)
X6 Ash	-1.58 (0.61**, -.26)	-1.72 (0.55**, -.22)
Equation 4. (n = 20)	R = 0.90** SEE = 3.7	R = 0.95** SEE = 5.8
bo Constant	-52.64	90.87
X1 Cellulase	1.31 (0.86**, 0.77**)	-0.73 (0.54*, -.40)
X2 CP	0.37 (0.68**, 0.15)	1.29 (0.80**, 0.32)
X3 CW	-0.09 (-.69**, -.05)	2.63 (-.46*, 0.69**)
X4 ADF	1.47 (-.70**, 0.38)	-3.67 (-.68**, -.55*)
X5 Lig	0.48 (-.65**, 0.07)	-7.18 (-.77**, -.56*)
X6 Ash	1.41 (0.49*, 0.31)	-2.08 (0.54*, -.29)
Equation 5. (n = 14)	R = 0.98** SEE = 1.7	R = 0.98** SEE = 6.1
bo Constant	0.36	124.17
X1 IVDMD	1.37 (0.94**, 0.95**)	-1.49 (0.71**, -.71**)
X2 CP	-1.07 (0.68**, -.73**)	4.56 (0.93**, 0.79**)
X3 CW	0.15 (-.38, 0.25)	1.08 (-.67**, 0.46)
X4 ADF	-0.31 (-.62*, -.23)	-2.26 (-.88**, -.44)
X5 Lig	-0.52 (-.56*, -.19)	-0.49 (-.84**, -.06)
X6 Ash	-0.02 (0.36, -.01)	-2.91 (0.64*, -.45)
Equation 6. (N = 20)	R = 0.93** SEE = 3.4	R = 0.96** SEE = 5.7
bo Constant	40.41	188.76
X1 Cellulase	1.23 (0.86**, 0.79**)	-0.81 (0.54*, -.45)

TABLE 44. Continued.

Items	In Vivo Dry Matter Digestibility (%)	Dry Matter Intake ($gm/BW_{Kg}^{0.75}$)
X2 Buffer	-1.08 (0.58**, -.49)	-1.13 (0.48*, -.33)
X3 CP	0.03 (0.68**, 0.01)	0.92 (0.80**, 0.24)
X4 CW	-0.48 (-.69**, -.27)	2.22 (-.45*, 0.62**)
X5 ADF	0.64 (-.70**, 0.18)	-4.55 (-.68**, -.62**)
X6 Lig	-0.04 (-.65**, -.01)	-7.73 (-.77**, -.60*)
X7 Ash	0.79 (0.49*, 0.20)	-2.72 (0.54*, -.38)
Equation 7. (n = 12)	R = 0.99** SEE = 1.7	R = 0.98** SEE = 6.2
bo Constant	-36.74	287.92
X1 IVDMD	1.29 (0.94**, 0.96**)	-1.40 (0.71**, -.72)
X2 Buffer	0.32 (0.26, 0.29)	-2.09 (0.72**, -.48)
X3 CP	-0.28 (0.67*, -.22)	2.30 (0.93**, 0.45)
X4 CW	0.03 (-.36, 0.03)	1.39 (-.69*, 0.49)
X5 ADF	0.32 (-.56, 0.22)	-4.53 (-.92**, -.66)
X6 Lig	1.11 (-.48, 0.36)	-5.86 (-.90**, -.49)
X7 Ash	0.07 (0.23, 0.06)	-3.11 (0.70*, -.56)
Equation 8. (n = 11)	R = 0.99** SEE = 1.0	R = 0.99** SEE = 4.0
bo Constant	-42.19	94.21
X1 IVDMD	0.93 (0.96**, 0.96**)	-2.44 (0.67*, -.91*)
X2 Cellulase	0.64 (0.83**, 0.87*)	2.52 (0.78**, 0.86*)
X3 CP	-0.11 (0.56, -.16)	2.92 (0.94**, 0.73)
X4 CW	-0.18 (-.30, -.48)	1.51 (-.67*, 0.74)
X5 ADF	0.58 (-.48, 0.53)	-3.45 (-.91**, -.67)

TABLE 44. Continued.

Items	In Vivo Dry Matter Digestibility (%)	Dry Matter Intake ($gm/BW_{Kg}^{0.75}$)
X6 Lig	2.18 (-.31, 0.82)	2.60 (-.90**, 0.38)
X7 Ash	0.93 (-.04, 0.68)	0.65 (0.66*, 0.16)
Equation 9. (n = 17)	R = 0.93** SEE = 2.4	R = 0.63 SEE = 13.2
bo Constant	14.30	-7.71
X1 IVDMD	0.83 (0.90**, 0.75**)	0.31 (0.56,* 0.08)
X2 Cellulase	0.33 (0.80,** 0.35)	1.18 (0.61,** 0.24)
X3 Buffer	-0.65 (0.34, -.55)	0.85 (0.49,* 0.16)
	Temperate Legumes	
Equation 10. (n = 30)	R = 0.88** SEE = 3.8	R = 0.40 SEE = 13.3
bo Constant	89.03	-7.21
X1 CP	0.49 (0.79**, 0.18)	2.80 (0.26, 0.29)
X2 CW	-0.77 (-.86**, -.43)	0.88 (-.22, 0.15)
X3 ADF	0.47 (-.79**, 0.23)	1.10 (-.22, 0.15)
X4 Lig	-1.32 (-.70**, -.26)	-5.38 (-.28, -.30)
X5 Ash	-0.91 (0.48**, -.22)	-1.35 (0.20, -.10)
Equation 11. (n = 28)	R = 0.87** SEE = 4.0	R = 0.54 SEE = 12.8
bo Constant	83.30	109.35
X1 Buffer	-0.19 (0.74**, -.09)	-2.43 (0.10, -.33)
X2 CP	0.82 (0.81**, 0.25)	3.00 (0.28, 0.29)
X3 CW	-0.74 (-.84**, -.37)	0.32 (-.24, 0.05)
X4 ADF	0.58 (-.76**, 0.26)	0.63 (-.26, 0.09)
X5 Lig	-1.41 (-.65**, -.28)	-6.54 (-.36, -.39)
X6 Ash	-0.95 (0.49**, -.22)	-2.02 (0.26, -.15)
Equation 12. (n = 13)	R = 0.91* SEE = 5.0	R = 0.83 SEE = 13.8
bo Constant	55.59	-217.29
X1 IVDMD	0.27 (0.85**, 0.12)	2.69 (0.62*, 0.40)

TABLE 44. Continued.

Items	In Vivo Dry Matter Digestibility (%)	Dry Matter Intake ($gm/BW_{Kg}^{0.75}$)
X2 CP	1.56 (0.72**, 0.39)	4.42 (0.54, 0.40)
X3 CW	-0.17 (-.85**, -.11)	3.65 (-.40, 0.67)
X4 ADF	-0.07 (-.80**, -.03)	-1.86 (-.51, -.26)
X5 Lig	-2.14 (-.56*, -.38)	-4.34 (-.32, -.28)
X6 Ash	-1.47 (0.47, -.27)	-2.57 (0.26, -.17)
Equation 13. (n = 11)	R = 0.90 SEE = 7.0	R = 0.90 SEE = 14.2
bo Constant	55.12	-22.77
X1 IVDMD	0.50 (0.85**, 0.18)	4.77 (0.62*, 0.65)
X2 Buffer	0.04 (0.71*, 0.01)	-2.43 (0.35, -.37)
X3 CP	1.04 (0.79**, 0.19)	-1.74 (0.56, -.16)
X4 CW	-0.12 (-.83**, -.07)	3.18 (-.39, 0.67)
X5 ADF	-0.37 (-.81**, -.11)	-6.17 (-.64*, -.65)
X6 Lig	-1.80 (-.50, -.31)	-1.13 (-.42, -.10)
X7 Ash	-1.36 (0.50, -.25)	-1.96 (0.35, -.18)
Equation 14. (n = 11)	R = 0.86** SEE = 5.0	R = 0.64 SEE = 15.9
bo Constant	-26.06	-62.35
X1 IVDMD	1.19 (0.85**, 0.69*)	2.81 (0.62*, 0.58)
X2 Buffer	0.55 (0.71*, 0.26)	-1.29 (0.35, -.19)

R = Multiple correlation coefficients; SEE = Standard error of estimate; CP = Crude protein; CW = Cell walls; ADF = Acid-detergent fiber; Lig = Lignin; IVDMD = Two-stage in vitro fermentation (Tilley-Terry); Cell = Cellulase incubation; Buf = Buffer extract.

[1]Simple correlation coefficient with in vivo parameter.

[2]Partial correlation coefficient with in vivo parameter.

*P < .05 ** P < .01

measure of association of *in vivo* DDM and one variable with a fixed value of the second or third variable. Partial regression coefficient also indicates the magnitude of contribution of each variable to the *in vivo* digestibility.

A study of an R, simple correlation (r), partial correlation coefficient, and partial regression coefficient will give the degree of contribution of each variable in multiple regression analysis. Generally, multiple correlation and regression give more accurate prediction and more precise relationships among the variables than does a series of simple regression analysis.

The inclusion of the value for buffer solubility to various chemical components gave a multiple regression with an R value of only 0.81 which is not considered satisfactory for good precision of predicting DDM but buffer value added slightly improved the precision for DMI (Equation 3, Table 44). The inclusion of cellulase alone or cellulase plus buffer to these chemical components did reduce the standard error of estimate for both DDM and DMI predictions ($R \geq 0.90$ for Equations 4 and 6). The addition of the two-stage IVDMD value alone or IVDMD and buffer solubility values to various chemical components greatly improved the prediction of DDM and DMI (Equations 5 and 7, $R \geq 0.97$ and SEE = 1.7 and 6.1, respectively). One of the most reliable combinations to predict DDM and DMI of grasses was to include both IVDMD and cellulase

values to values for chemical components (Equation 8). This gave an R value of 0.99 and a standard error of only 1.0 for DDM and 4.0 for DMI prediction which is excellent for prediction purposes. A combination of values for IVDMD, cellulase, buffer solubility (Equation 9) could accurately predict DDM (R of 0.93 and SEE = 2.4) but was not sufficiently useful for predicting DMI of grasses with an R of only 0.63.

For temperate legumes, a combination of chemical components (Equation 10) tended to predict DDM more accurately than did chemical components plus values for buffer solubility or IVDMD (Equations 11 to 13). The combination of IVDMD and buffer solubility was unsatisfactory (Equation 14). However, all these combinations when used to predict DDM had R values of above 0.86 with rather large standard errors (SEE = 3.8 to 7.0) similar to those for CW, ADF, lignin, CP, IVDMD or enzymatic incubation values alone (Table 40). No combinations of items were satisfactory to predict DMI of legumes due to large standard errors of estimate (SEE = 12.8 to 15.9).

In this study, no significant improvement in predicting DDM was noted by using multiple correlation coefficients when compared to that of the best individual predictors. Oh et al. (88) also found similar results that multiple regression or correlations did not improve the relationship above that of simple correlation for

combined forages but greatly improved the correlations for some particular species.

b. Predictions of In Vivo Dry Matter Digestibility (DDM) for Michigan Forages

Several multiple regression equations were developed using a large number of samples and different predictor combinations for predicting in vivo parameters of temperate legumes, grasses and silages are arranged in the order of increasing standard errors of estimate (SEE) in Tables 45 through 49. The samples comprise a series of data collected during the past 10 years (1961 to 1970). However, not all laboratory determinations had been conducted on every sample. Thus the number of samples (n) for equations in the following tables might not be the same.

Dry matter digestibility in vivo of temperate legumes could be satisfactorily predicted from a combination of CP, ADF and 36-hr IVDMD with or without ash giving a standard error of estimate of 2.2 percentage units (Equations 1 and 2, Table 45). The multiple correlation coefficient ($R = 0.80$, $P < .01$) was much greater than that for simple correlation coefficients using individual variables. Each of these variables had moderately high partial correlation coefficients with DDM and these variables accounted for 64% ($R^2 = 0.64$) of the variation in DDM. However, ash contributed little to this combination. A combination of ADF and TTDMD (Equation 3) was also an

TABLE 45. Multiple regression equations for estimating in vivo digestibility from laboratory analytical values (Michigan forages).

Prediction of In Vivo DDM (Y) %	n	R	SEE	Corr. coefs. of DDM with variables: Simple correlations	Corr. coefs. of DDM with variables: Partial correlations
LEGUMES					
1. Y = 55.43+0.49CP-0.25Ash-0.36ADF +0.23(36-hr IVDMD)	42	.81[b]	2.2	0.53,[b] -.08, -.68,[b] 0.50[b]	0.41,[b] -.24, -.50,[b] 0.45[b]
2. Y = 51.37+0.43CP-0.34ADF +0.27(36-hr IVDMD)	42	.80[b]	2.2	0.53,[b] -.68,[b] 0.50[b]	0.37,[a] -.47,[b] 0.54[b]
3. Y = 59.83-0.46ADF+0.28(TTDMD)	38	.76[b]	2.4	-.67,[b] 0.53[b]	-.64,[b] 0.49[b]
4. Y = 55.52+0.14CP-0.42ADF+0.29(TTDMD)	43	.75[b]	2.5	0.49,[b] -.65,[b] 0.54[b]	0.11, -.52,[b] 0.47[b]
5. Y = 60.72+0.38CP-0.51Ash-0.38ADF +10.28(TTDMD/StndX100)	43	.74[b]	2.6	0.49,[b] -.11, -.65,[b] 0.54[b]	0.28, -.42,[b] -.42,[b] 0.21
6. Y = 73.94+0.41CP-0.48Ash-0.48ADF	44	.73[b]	2.6	0.49,[b] -.09, -.65[b]	0.29, -.38,[a] -.55[b]
7. Y = 85.47-0.64ADF+0.21DMS -8.42(6-hr DMD/StndX100)	31	.70[b]	2.8	-.63,[b] 0.30 0.30	-.64,[b] 0.35,[a] -.30
8. Y = 59.99+0.22CP-0.38ADF +9.50(TTDMD/StndX100)	43	.68[b]	2.8	0.49,[b] -.65,[b] 0.54[b]	0.16, -.39,[b] 0.18
9. Y = 72.24+0.25CP-0.47ADF	44	.67[b]	2.8	0.48,[b] -.65[b]	0.18, -.52[b]

TABLE 45. Continued.

Prediction of *In Vivo* DDM (Y) %	n	R	SEE	Corr. coefs. of DDM with variables: Simple correlations	Corr. coefs. of DDM with variables: Partial correlations
10. Y = 79.22+0.27CP-0.58CF-0.76Ash	34	.68[b]	3.0	0.50,[b] -.52,[b] -.03	0.17, -.50,[b] -.48[b]
GRASSES					
11. Y = 81.99(TTDMD/StndX100)-0.17VS-8.11	12	.97[b]	1.0	0.96,[b] 0.52	0.96,[b] -.43
12. Y = 40.96-0.79ADF+0.28(36-hr IVDMD) +32.16(TTDMD/StndX100)	24	.91[b]	1.8	-.60,[b] 0.58,[b] 0.76[b]	-.74,[b] 0.61,[b] 0.67[b]
13. Y = 21.88+0.20CP-0.75Ash-0.36ADF +54.35(TTDMD/StndX100)	24	.88[b]	2.2	0.33, -.04, -.60,[b] 0.76[b]	0.24, -.40,[a] -.26, 0.80[b]
14. Y = 65.09+0.59CP+2.38CF+1.19Ash -2.77ADF	12	.89[b]	2.2	0.11, 0.29, -.33, -.49	0.52, 0.85,[b] 0.47, -.86[b]
15. Y = 37.16+0.01CP-0.67ADF +46.43(TTDMD/StndX100)	24	.86[b]	2.3	0.33, -.60,[b] 0.76[b]	0.02, -.48,[a] 0.76[b]
16. Y = 73.15+0.56CP+1.91CF-2.28ADF	12	.86[b]	2.4	0.11, 0.29, -.45	0.46, 0.81,[b] -.83[b]
17. Y = 2.38+0.29CP+53.80(TTDMD/StndX100)	24	.81[b]	2.6	0.33, 0.76[b]	0.45,[a] 0.79[b]
18. Y = 157.52-1.59ADF-5.36L-0.64CC	12	.81[a]	2.8	-.49, -.66,[b] 0.26	-.62,[a] -.73,[b] -.47

TABLE 45. Continued.

Prediction of In Vivo DDM (Y) %	n	R	SEE	Corr. coefs. of DDM with variables: Simple correlations	Corr. coefs. of DDM with variables: Partial correlations
19. Y = 7.30+54.94(TTDMD/StndX100)	24	.76[b]	2.8	0.76[b]	
20. Y = 68.60-0.16CP-1.06ADF+0.50(TTDMD)	24	.77[b]	2.9	0.33, -.60,[b] 0.55[b]	-.18, -.59,[b] 0.60[b]
SILAGES					
21. Y = 69.96+0.88CP-0.39CF-3.49Ash +0.31ADF	12	.85[a]	2.2	0.32, -.48, -.36, 0.04	0.58,[a] -.60,[a] -.77,[b] 0.45
22. Y = 44.21+0.63CP+0.81NFE-139.34L/ADF	12	.82[a]	2.2	0.32, 0.34 -.38	0.59,[a] 0.75,[b] -.75[b]

DDM = In vivo dry matter digestibility;
ADF = Acid-detergent fiber;
L = Lignin;
NFE = Nitrogen-free extract;
IVDMD = In vitro dry matter disappearance;
TTDMD = Two-stage in vitro dry matter disappearance by Tilley-Terry method;
VS = 0.98(100 - CW) + CW(147.3 - 78.9 Log L/ADF X 100), where CW = Cell walls;
R = Multiple correlation coefficient;
Superscript a = P < .05; b = P < .01

CP = Crude protein;
CF = Crude fiber;
CC = Cell contents;
DMS = Dry matter solubility;
Stnd = DMD of standard sample;
SEE = Standard error of estimate;

accurate predictor of DDM (R = 0.76, P < .01, SEE = 2.4) and both these two variables contributed significantly to the variation in DDM as evidenced by their partial correlations. The addition of CP to ADF plus TTDMD (Equation 4) did not improve accuracy and the partial correlations indicate that CP contributed little to this equation.

Combinations of chemical components (CP, ash, ADF; CP, ADF; CP, CF, ash) in Equations 6 to 10 had large standard errors (2.6 to 3.0) and small multiple correlation coefficients (0.73 to 0.67). Prediction equation including CP in combination with ADF or CF tended to have low multiple correlations with DDM and low predictability. In these equations protein had a relatively low partial correlation coefficient yet protein itself had higher simple correlations with DDM than many other constituents.

For legume forages, a combination of chemical components plus *in vitro* fermentation values was superior to a combination of chemical components alone in predicting *in vivo* DDM. Using a combination of predictors from the proximate analysis scheme (CP, CF, ash) was less accurate in predicting DDM than using predictors from Van Soest's system of analysis when either was used with an *in vitro* rumen value. Any acceptable multiple regression should have great accuracy and includes predictors that can be determined easily or obtained in a sequential analysis. Therefore, Equation 3 with an R of 0.76 might be preferable

to Equations 1 or 2 (R = 0.80 to 0.81) which uses values from three systems of analysis.

For temperate grasses, combinations of artificial rumen fermentation values and values from Van Soest's scheme of analysis (Equations 11 and 12, Table 45) were reliable predictors of in vivo DDM with R^2 of 0.84 to 0.94. However, the term TTDMD/Standard as a single predictor was highly correlated with DDM ($r = 0.96$, $P < .01$) suggesting the use of only this laboratory estimate to predict in vivo DDM for grasses. The low simple and partial correlations indicate that digestibility estimated from Van Soest's summative equation ("VS" in Equation 11) had low relationship to in vivo DDM and could be omitted. The inclusion of ADF, CP, ash or 36-hr DMD to TTDMD/Standard (Equations 12, 13, 15, 17) did not improve multiple correlation coefficients or decrease the standard errors when compared with Equation 11, but TTDMD/Standard alone (Equation 19) gave a larger standard error and smaller R^2 than the combinations discussed above. Combinations of chemical components alone (CP, CF, ash, ADF; CP, CF, ADF; ADF, L, CC) as in Equations 14, 16, 18 gave standard errors of 2.2 to 2.8 with R^2 of 0.80 to 0.65. Equations 14 and 16 use terms from both the Van Soest and proximate systems of analysis whereas Equation 18 uses only terms from Van Soest's system. Prediction of DDM from Equation 18 may be accomplished faster but with less precision than that for Equation 14. For grasses, a

combination of an artificial rumen value and some chemical components (Equations 11, 12, 13) gave more accurate prediction of DDM than combinations of chemical components alone. A combination of artificial rumen value and chemical components (TTDMD, CP, ADF) had small R^2 and large SEE and CP did not contribute significantly in this combination (Equation 20).

Digestible dry matter in vivo for silages could be predicted from combinations of CP, CF, ash, ADF or CP, NFE, L/ADF with a standard error of 2.2 and R^2 of 0.73 to 0.68. Most variables used had significant partial correlations with DDM. Artificial rumen values alone or in combination with chemical components did not give satisfactory predictions for silage DDM in this study.

On the whole, multiple regression technique improved the correlations between predictor combinations and DDM and increased precision of prediction for each category of forages. These findings are in agreement with that by Oh et al. (88).

c. Predictions of Dry Matter Intake (DMI) and Digestible Dry Matter Intake (DDMI) for Michigan Forages

Multiple regression equations for predicting DMI and DDMI are presented in Table 46. Multiple regression equations using combinations of chemical components did not satisfactorily predict DMI for temperate legumes. The most satisfactory equation to predict dry matter intake (lb/cwt) of legumes was a combination of DMS, 36-hr DDM and

TABLE 46. Multiple regression equations for estimating dry matter intake and digestible dry matter intake from laboratory analytical values (Michigan forages).

Y1 = Max. DMI (lb/cwt) and Y2 = DDMI ($gm/W_{Kg}^{0.75}$)	n	R	SEE	Corr. coefs. of intake with variables: Simple correlations	Corr. coefs. of intake with variables: Partial correlations
LEGUMES					
1. Y1 = 0.04DMS+0.11(36-hrDMD) -1.32(36 hr-DMD/StndX100)-1.71	32	.82[b]	0.35	0.26, 0.73,[b] 0.56[b]	0.48,[b] 0.80,[b] -.38[a]
2. Y1 = 0.15L+0.04(36-hr DMD)+0.06(TTDMD) -3.66	43	.80[b]	0.36	0.20, 0.68,[b] 0.66[b]	0.46,[b] 0.46,[b] 0.53[b]
3. Y1 = 0.08(TTDMD)-0.02CP-1.55	44	.66[b]	0.45	0.65,[b] 0.12	0.65,[b] -.11
4. Y1 = 0.08(TTDMD)+0.01ADF-0.12CP-1.96	44	.66[b]	0.46	0.65,[b] -.12, 0.12	0.65,[b] 0.05, -.07
GRASSES					
5. Y1 = 0.06(36-hr DMD)-0.15(6-hr DMD) +0.06CW-0.56L+1.08EE-1.99	15	.97[b]	0.17	0.49, 0.55,[a] -.21, -.32 0.72[b]	0.67,[b] -.76,[b] 0.67,[b] -.65[b] 0.93[b]
6. Y1 = 0.07CP+0.07CF+0.58EE-2.54	15	.80[b]	0.37	0.46, -.38, 0.72[b]	0.50, 0.31, 0.74[b]
7. Y1 = 6.53(6hr-DMD/StndX100)-0.17DMS +1.54	12	.75[a]	0.43	0.68,[a] -.02	0.75,[b] -.45

TABLE 46. Continued.

Y1 = Max. DMI (lb/cwt) and Y2 = DDMI ($gm/W_{Kg}^{0.75}$)	n	R	SEE	Corr. coefs. of intake with variables: Simple correlations	Corr. coefs. of intake with variables: Partial correlations
LEGUMES					
8. Y2 = 3.81CP+8.40L+3.63(36-hr DMD) +3.46(TTDMD)-327.21	42	.84[b]	23.4	0.24, 0.06, 0.71,[b] 0.72[b]	0.34,[a] 0.40,[b] 0.55,[b] 0.47[b]
9. Y2 = 6.01(TTDMD)+0.33CP-180.33	43	.72[b]	28.8	0.72,[b] 0.24	0.70,[b] 0.03
GRASSES					
10. Y2 = 253.81(TTDMD/StndX100)+29.37EE -197.63	24	.79[b]	18.7	0.15, 0.60[a]	0.64,[b] 0.72[b]
11. Y2 = 6.41CP+11.86CF+44.16EE-450.29	12	.84[a]	24.9	0.66,[a] -.21, 0.68[a]	0.43, 0.59,[a] 0.68[a]
SILAGES					
12. Y2 = 529.91-6.20ADF-3.53DMS -4.12(6-hr DMD/StndX100)	8	.96[b]	4.5	-.51, 0.06, 0.53	-.92,[b] -.95,[b] -.07
13. Y2 = 5.16CC+1.53ADF+6.66L-209.59	12	.81[a]	11.5	0.69,[a] -.36, 0.05	0.75,[b] 0.30 0.43

DMI = Dry matter intake; DDMI = Digestible dry matter intake; EE = Ether extract. For other abbreviations, see Table 45.

Superscript a = $P < .05$; b = $P < .01$

DMD/Standard with a SEE of 0.35 and R of 0.82. A combination of L, 36-hr DMD, TTDMD (Equation 2, Table 46) could predict DMI just as accurately as Equation 1. Combinations of TTDMD with CP or ADF (Equations 3 or 4) had smaller multiple correlation coefficients and larger standard errors than the first two combinations. Crude protein or ADF had very low partial correlations with DMI indicating that these two variables were not important for predicting intake of legumes. Values from artificial rumen fermentations alone could be used to predict DMI of legumes with r value of about 0.65 to 0.73.

One of the reliable predictors of DMI for temperate grasses was a combination of 36-hr DMD, 6-hr DMD, CW, L, EE with an R of 0.97. Each variable in this combination had significant and high partial correlations with DMI (Equation 5, Table 46). However, this equation involved values from the Van Soest system, the proximate analysis system and two *in vitro* fermentations which are excessive for practical purposes. Combinations such as CP + CF + EE or 6-hr DMD/Standard + DMS might be more convenient but had smaller R^2 and larger standard errors (compare Equations 6 and 7 with No. 5, Table 46).

Dry matter intake of silages could not be satisfactorily predicted from any combinations of laboratory estimates.

Digestible dry matter intake (DDMI) of temperate legumes and grasses could be predicted from combinations

f chemical components alone or chemical components plus
n *vitro* fermentations with R ranging from 0.72 to 0.84
nd standard errors of 18.7 to 28.8. Crude protein in
quation 9 had a very low partial correlation with DDMI
nd could be excluded from TTDMD, CP combination and the
wo-stage DMD alone could be used to predict DDMI of
egumes with an r of 0.72. Two prediction equations for
DMI of silages are given in Table 46 with the equation
sing ADF, DMS, 6-hr DMD/standard having a much greater
and smaller SEE than the equation using CC, ADF, and
ignin.

d. Predictions of Total Digestible Nutrients (TDN) for Michigan Forages

Multiple regression equations for predicting TDN
f legumes are in Table 47 and the best equation utilized
combination of two-stage *in vitro* fermentation value,
P and ash with a standard error of 2.9 and an R of 0.73.
owever, this predictor combination accounted for only
3% (R^2 = 0.53) of the variation in TDN. Combinations
f predictors from the proximate analysis or the Van Soest
ystem (Equations 2 through 5, Table 47) gave still smaller
ultiple correlation coefficients (R = 0.67 to 0.47).

For temperate grasses, TDN could be precisely
redicted from a combination of various *in vitro* rumen
ermentations or a combination of ADF and one *in vitro*
umen fermentation with R's of 0.91 to 0.93 and SEE of
7. All these variables in Equations 6 and 7 had

laboratory analytical values (Michigan forages).

Prediction of Total Digestible Nutrients (Y)	n	R	SEE	Corr. coefs. of TDN with variables: Simple correlations	Corr. coefs. of TDN with variables: Partial correlations
LEGUMES					
1. Y = 0.36(TTDMD)+1.35CP-1.30Ash+23.00	24	.73[b]	2.9	0.43,[a] 0.44,[a] -.01	0.38, 0.64,[b] -.47[a]
2. Y = 1.53CP-1.67Ash+43.91	24	.67[b]	3.1	0.44,[a] -.01	0.67,[b] -.57[b]
3. Y = 0.61CC+1.15ADF-1.77L-4.12	18	.66[a]	3.5	0.35, -.14, -.45	0.52,[a] 0.54,[a] -.43
4. Y = 182.49-1.43CF-2.65Ash-1.44NFE	12	.67	4.0	-.21, 0.04, -.26	-.64,[a] -.54, -.63[a]
5. Y = 108.50-0.53CF-0.85NFE	12	.47	4.4	-.21, -.26	-.41, -.43
GRASSES					
6. Y = 9.64-1.20(36-hr DMD)+0.95(TTDMD) +51.16(36-hr DMD/StndX100)	13	.93[b]	1.7	0.37, 0.68,[a] 0.60[a]	-.86,[b] 0.88,[b] 0.87[b]
7. Y = 73.31-1.03ADF+0.37(36-hr DMD)	18	.91[b]	1.7	-.71,[b] 0.58[a]	-.86,[b] 0.81[b]
8. Y = 37.33+0.19CP-1.14Ash-0.38ADF +40.19(TTDMD/StndX100)	18	.86[b]	2.3	0.35, -.21, -.71,[b] 0.63[b]	0.20, -.51,[a] -.20, 0.62[b]
9. Y = 78.72-0.20CP-1.12ADF +20.76(TTDMD/StndX100)	18	.81[b]	2.5	0.35, -.71,[b] 0.63[b]	-.25, -.58,[a] 0.41

TABLE 47. Continued.

Prediction of Total Digestible Nutrients (Y)	n	R	SEE	Corr. coefs. of TDN with variables: Simple correlations	Corr. coefs. of TDN with variables: Partial correlations
GRASSES					
10. Y = 98.72-0.31CP-1.38ADF+0.18(TTDMD)	18	.79[b]	2.7	0.35, -.71,[b] 0.44	-.38, -.68,[b] 0.27
11. Y = 115.00-0.35CP-1.52ADF	18	.78[b]	2.7	0.35, -.71[b]	-.41, -.73[b]
12. Y = 114.25-0.44CP-1.56ADF+1.11EE	18	.78[b]	2.7	0.35, -.71,[b] 0.22	-.45, -.74,[b] 0.20
13. Y = 113.95-0.32CP-1.47ADF-0.16Ash	18	.77[b]	2.8	0.35, -.71,[b] -.21	-.34, -.69,[b] -.08
SILAGES					
14. Y = 31.98+2.86EE+0.48NFE	7	.98[b]	1.0	0.90,[b] 0.80[a]	0.94,[b] 0.88[b]
15. Y = 67.03-0.04CP-0.40CF+2.28EE	9	.92[a]	1.8	0.43, -.78,[a] 0.81[b]	-.02, -.64, 0.77[a]
16. Y = 92.37-0.64CF-1.02Ash	9	.83[a]	2.3	-.78,[a] -.13	-.82,[b] -.43
17. Y = 83.54-0.61CF	9	.78[a]	2.4	-.78[a]	

For abbreviations, see Tables 45 and 46.

Superscript a = $P < .05$; b = $P < .01$

ignificant partial correlations with TDN. Combinations f rumen fermentation values with other chemical components Equations 8, 9, 10) were less accurate but yet satisfac-ory for predicting TDN with standard errors of 2.3 to .7. As with legumes, combinations of chemical components CP + ADF: CP + ADF + EE: CP + ADF + ash) gave larger tandard errors than did the chemical components plus in itro fermentation values or combinations of the two in itro systems. Yet combinations of chemical components ould predict TDN of grasses with moderate accuracy having tandard errors under 3 percentage units.

Unlike grasses and legumes, TDN of silages could e precisely predicted from EE + NFE or CP + CF + EE with tandard errors of 1.0 to 1.8 and R values of 0.92 to .98 (Equations 14, 15). However, there were only data n 7 to 9 silage samples but both ether extract and NFE ad significant and high partial correlations with TDN nlike CP in the CP, CF, EE combination.

e. Predictions of Digestible Energy (DE) for Michigan Forages

Multiple regression equations for predicting DE re in Table 48. Digestible energy of temperate legumes ɔuld be satisfactorily predicted from a combination of DMD, 6-hr DMD/Standard with a standard error of 2.5 ıd these two variables accounted for 75% of the variation ı DE. Predictor combinations such as CP + EE + ADF or ? + EE + CF could predict DE of legumes with similar

TABLE 46. Multiple regression equations for estimating digestible energy from laboratory analytical values (Michigan forages).

Prediction Equations for Digestible Energy (Y)	n	R	SEE	Corr. coefs. of DE with variables: Simple correlations	Corr. coefs. of DE with variables: Partial correlations
LEGUMES					
1. Y = 2.17(TTDMD)-40.63(6-hr DMD/Stnd X 100) - 34.60	9	.86[a]	2.5	0.76,[a] 0.53	0.81,[a] -.64
2. Y = 81.16+0.43CP-2.19EE-0.68ADF	20	.82[b]	2.6	0.63,[b] 0.22, -.75[b]	0.37, -.42, -.68[b]
3. Y = 71.47+0.66CP-3.30EE-0.58CF	10	.84[b]	2.7	0.68,[a] 0.05, -.66[a]	0.58, -.61, -.61
4. Y = 32.40+0.30CP-0.26ADF +31.64(TTDMD/StndX100)	20	.81[b]	2.7	0.63,[b] -.75,[b] 0.77[b]	0.26, -.26 0.36
5. Y = 5.38+0.39CP+47.82(TTDMD/Stnd x 100)	20	.80[b]	2.7	0.63,[b] 0.77[b]	0.33, 0.63[b]
6. Y = 46.50+0.26CP-0.43ADF+0.40(TTDMD)	20	.81[b]	2.8	0.63,[b] -.75,[b] 0.67[b]	0.22, -.51,[a] 0.34
7. Y = 94.06-2.04EE-0.83ADF	20	.79[b]	2.8	0.22, -.75[b]	-.37, -.78[b]
8. Y = 60.14(TTDMD/StndX100)+0.25	20	.77[b]	2.8	0.77[b]	
9. Y = 72.59+0.37CP-0.56ADF	21	.77[b]	2.9	0.61,[b] -.75[b]	0.29, -.60[b]
10. Y = 83.92-.68ADF	20	.75[b]	2.9	-.75[b]	

Prediction Equations for Digestible Energy (Y)	n	R	SEE	Corr. coefs. of DE with variables: Simple correlations	Corr. coefs. of DE with variables: Partial correlations
11. Y = 0.95(TTDMD)+0.38	9	.75[b]	3.1	0.75[b]	
12. Y = 82.02-0.76CF	9	.72[a]	3.2	-.72	
GRASSES					
13. Y = 59.18(TTDMD/StndX100)-0.68	12	.94[b]	1.2	0.94[b]	
14. Y = 188.24-2.61ADF+5.56L-0.56CC -399.45(L/ADF)	12	.90[b]	1.8	-.46, -.71,[b] 0.27, -.59[a]	-.78,[b] 0.41, -.61,[a] -.65[a]
15. Y = 59.75+0.47CP+1.93CF+1.15Ash -2.22ADF	12	.87[a]	2.0	0.10, 0.28, -.27, -.46	0.47, 0.82,[b] 0.50, -.83[b]
16. Y = 131.93-1.14ADF-4.66L-0.48CC	12	.82[a]	2.2	-.46, -.71,[b] 0.27	-.58,[a] -.76,[b] -.45
17. Y = 67.54+0.44CP+1.48CF-1.74ADF	12	.82[a]	2.2	0.10, 0.28, -.46	0.40, 0.76,[b] -.78[b]
18. Y = 46.05(TTDMD/StndX100)+0.22CP +8.98	24	.78[b]	2.5	0.74,[b] 0.29	0.75,[b] 0.37
19. Y = 60.80-0.13CP-0.81ADF+0.45(TTDMD)	24	.72[b]	2.8	0.29, -.53,[b] 0.55[b]	-.14, -.50,[a] 0.57[b]

TABLE 48. Continued.

Prediction Equations for Digestible Energy (Y)	n	R	SEE	Corr. coefs. of DE with variables: Simple correlations	Corr. coefs. of DE with variables: Partial correlations
SILAGES					
20. Y = 133.62-2.94CP-0.83CF+1.94EE	7	.96[a]	1.6	0.22, -.72, 0.78[a]	-.88,[b] -.90,[b] 0.79[a]
21. Y = 128.98-2.91CP-1.26CF+0.68ADF	7	.96[a]	1.6	0.22, -.72, -.11	-.87,[b] -.96,[b] 0.77[a]
22. Y = 157.45-3.25CP-1.10CF	7	.90[a]	2.2	0.22, -.72	-.78,[a] -.89[b]

For abbreviations, see Tables 45 and 46.

Superscript a = P < .05, b = P < .01

accuracy (R = 0.82 to 0.84, SEE = 2.6 to 2.7). Combination of chemical components with either TTDMD/Standard or TTDMD (Equations 4, 5, 6) could predict DE of legumes with essentially the same accuracy (SEE = 2.7 to 2.8, R = 0.80 to 0.81). Prediction of DE from TTDMD/Standard alone (Equation 8) gave a standard error of 2.8 whereas that from TTDMD had a standard error of 3.1. Addition of EE or CP to ADF as predictors did little to improve accuracy of prediction (Equations 7 and 9 vs. 10) and ADF alone was more accurate than CF (Equations 10 vs. 12).

For temperate grasses, TTDMD/Standard alone seemed to be an excellent predictor of DE (R = 0.94, P < .01, SEE = 1.2). The inclusion of CP with TTDMD/Standard did not improve accuracy of DE prediction (Equations 13 vs. 18, Table 48). Combinations of chemical components such as ADF + L + L/ADF + CC, CP + CF + ash + ADF, ADF + L + CC, CP + CF + ADF could be used to predict DE of grasses with R's ranging from 0.90 to 0.82 and standard errors from 1.8 to 2.2.

There were only data on seven silage samples and digestible energy could be precisely predicted from the three predictor combinations, CP + CF + EE; CP + CF + ADF or CP + CF with R's ranging from 0.96 to 0.90 and standard errors of 1.6 to 2.2. All chemical components used had significant partial correlations with DE indicating that these chemical components contributed significantly to the variation in DE of silages.

f. Predictions of Body Weight Gain for Michigan Forages

Data in Table 49 indicate that body weight gain of sheep consuming temperate legumes could be predicted from a combination of 6-hr DMD, 36-hr DMD, TTDMD and ADF with a standard error of 0.08 and R of 0.84. However, use of this equation would become tedious since it involved three systems of *in vitro* fermentations and one chemical analysis. Combinations of chemical components were not satisfactory ($R \leq 0.52$) to predict weight gain of sheep fed these legumes and none of these equations are presented.

Body weight gain for temperate grasses could be predicted from a combination of ADF, DMS, 6-hr DMD/Standard (Equation 2) with a small standard error (0.07) and large R of 0.85. However, the exclusion of ADF from this combination (Equation 3) did not significantly reduce the R but maintained the same standard error. Combinations of TTDMD or TTDMD/Standard with chemical components such as TTDMD + CP + ADF; TTDMD/Standard + CP + ash + ADF or TTDMD + CP could predict weight gain for grasses with essentially the same accuracy (SEE = 0.08, R = 0.79 to 0.78). Surprisingly, the addition of CP to TTDMD did not improve the correlation over that for TTDMD alone (Equation 6). Combinations of some selected chemical components alone, CP + CF + EE or EE + CW + ADF gave an accuracy similar to that for combination of TTDMD plus chemical components in predicting weight gain for grasses and silages (Equations 7 and 8 vs. 4, 5, 6).

TABLE 49. Multiple regression equations for estimating weight gain from laboratory analytical values (Michigan forages).

Prediction of Weight Gain (Y = lb/d)	n	R	SEE	Corr. coefs. of gain with variables: Simple correlations	Corr. coefs. of gain with variables: Partial correlations
LEGUMES					
1. Y = 0.97(36-hr DMD/StndX100)+0.02ADF +0.04(TTDMD)-0.55(6-hr DMD/Stnd X 100)-2.83	24	.84[b]	0.08	0.54,[b] -.18, 0.59,[b] 0.02	0.56,[b] 0.50,[a] 0.71,[b] -.62[b]
GRASSES					
2. Y = 0.39-0.01ADF-0.04DMS +1.34(6-hr DMD/Stnd X 100)	12	.85[a]	0.07	-.45, -.10, 0.72[b]	-.23, -.52, 0.80[b]
3. Y = 0.10-0.04DMS+1.43(6-hr DMD/Stnd X 100)	12	.84[a]	0.07	-.10, 0.72[b]	-.62,[a] 0.84[b]
4. Y = 0.02(TTDMD)+0.01CP+0.01ADF-1.63	27	.79[b]	0.08	0.77,[b] 0.28 -.17	0.77,[b] 0.24, 0.24
5. Y = 1.04(TTDMD/StndX100)+0.004CP +0.03Ash+0.01ADF-1.51	27	.79[b]	0.08	0.67,[b] 0.28, 0.56,[b] -.17	0.65,[b] 0.14, 0.45,[a] 0.19
6. Y = 0.02(TTDMD)+0.002CP-1.17	27	.78[b]	0.08	0.77,[b] 0.28	0.75,[b] 0.10
7. Y = 0.02CP+0.01CF+0.09EE	15	.77[a]	0.08	0.60,[a] -.47, 0.60[a]	0.57,[a] 0.22, 0.60[a]
SILAGES					
8. Y = 0.11EE+0.03CW-0.03ADF-0.26	12	.77[a]	0.09	0.63,[a] -.34, -.27	0.73,[b] 0.46, -.56

For abbreviations, see Tables 45 and 46. Superscript a = P < .05, b = P < .01

In multiple regression technique, the use of two terms obtained from similar analysis (i.e. 36-hr DMD, TTDMD or CW, ADF, CF) having a high correlation between them is not considered desirable. One of these predictors may contribute little to the predictability of in vivo parameters. Much more precision is usually accomplished by using only one of these two terms plus another term having a low correlation with these two terms.

In order to achieve meaningful improvement in correlations and precision of prediction, several values from many systems of analysis (i.e. chemical, microbiological, enzymatic incubations) would have to be used. Such a practice may be too time-consuming and laborious to be suitable for a routine forage evaluation program. Therefore, use of one good laboratory analytical value from one analysis with an excellent simple correlation coefficient would appear preferable for many forage species where a very high degree of predictability is not needed.

CONCLUSIONS

The studies on forage evaluation using various laboratory techniques yielded the following conclusions:

1. Appropriate buffer, pH level, enzyme concentration and length of incubation for various enzymes used in forage evaluation follow:

Enzyme	Buffer	pH	Quantity of Substrate: Enzyme (mg)	Incub. Time (hrs)
Cellulase	Sodium acetate: Acetic acid	3.85	300:300	60
Amylase	"	5.50	300:200	60
Pepsin	HCl	1.85	300:200	60

2. The solubility of forages incubated with three enzymes, Marschall's cellulase, Clarase 900 (amylase) and pepsin could be used to predict *in vivo* parameters with correlation coefficients (r) of 0.51 to 0.96.
3. Temperate forages had 1.05 times greater *in vitro* dry matter diappearance, 1.28 times greater crude protein (CP), 1.14 times greater lignin (L) than tropical forages but tropical forages were greater than temperate forages by a factor of 1.11, 1.05,

1.21 for cell walls (CW), acid-detergent fiber (ADF) and ash, respectively.

4. Quality of tropical forages was lower than that for temperate forages primarily due to lower levels of CP, digestibility and higher levels of CW, ADF, cellulose (C), hemicellulose (HC) in tropical forages.

5. Values for the two-stage in vitro fermentation (IVDMD), in vitro true dry matter disappearance (IVTDMD) and CP of temperate grasses decreased with advancing maturity at the rates of 0.35, 0.24 and 0.10 percentage unit per day, respectively whereas those for tropical grasses decreased at the lower rates of 0.03, 0.03 and 0.07% unit per day, respectively.

6. Crude protein had a positive correlation with ash but negative correlations with CW, ADF, C, L, silica (Si) and these "fibrous" fractions were mutually and positively correlated.

7. In vitro digestibilities (IVDMD, IVTDMD) were positively correlated with enzymatic incubation values and both sets had positive correlations with CP and ash but negative correlations with fibrous fractions.

8. In vivo dry matter digestibility (DDM), total digestible nutrients (TDN), digestible energy (DE), dry matter intake (DMI), digestible dry matter

intake (DDMI) had positive correlations with CP, ash, *in vitro* fermentation values and enzymatic incubations but negative correlations with CW, ADF, C, HC and lignin.

9. Water-soluble carbohydrates, total nonstructural carbohydrates (TNC), total available carbohydrates after enzymes (TACAE) had low correlations with *in vivo* measurements and these components as well as total ash could not be used as useful single predictors of any *in vivo* parameters.
10. *In vivo* DDM of forages could be predicted by using any of these predictors:
    a. CP, CW, ADF, L with r values of 0.79 to 0.86 for CP and r values of -.70 to -.93 for CW, ADF, L and standard errors of estimate (SEE) of 2.9 to 5.6.
    b. IVDMD or IVOMD with r values of 0.88 to 0.98 and SEE of 1.8 to 4.4.
    c. Cellulase incubation with r values of 0.58 to 0.89 and SEE of 3.8 to 5.4.
    d. Amylase incubation with r values of 0.52 to 0.79 and SEE of 5.4 to 6.1.
    e. Pepsin incubation with r values of 0.74 to 0.90 and SEE of 3.0 to 4.2.
    f. Cellulase plus amylase with r values of 0.77 to 0.89 and SEE of 4.3 to 5.2.

g. Cellulase plus pepsin with r values of 0.63 to 0.93 and SEE of 2.3 to 5.0.

h. Buffer incubation with r values of 0.64 to 0.75 and SEE of 4.5 to 6.9.

11. Dry matter intake ($gm/BW_{Kg}^{0.75}$) of forages could be predicted by using:

a. CW or L with r values of -.46 to -.64 and SEE of 10.2 to 13.4.

b. IVDMD with r values of 0.61 to 0.62 and SEE of 12.0 to 14.3.

c. Cellulase incubation with r values of 0.51 to 0.94 and SEE of 3.2 to 12.0.

d. Amylase incubation with r values of 0.87 to 0.91 and SEE of 4.3 to 5.2.

e. Cellulase plus amylase with r values of 0.92 to 0.96 and SEE of 3.4 to 3.8.

f. Buffer incubation with r values of 0.75 to 0.77 and SEE of 5.7 to 7.4.

12. Total digestible nutrients of forages could be predicted by using:

a. CP or ADF with r values of 0.77 to 0.87 for CP and -.87 to -.98 for ADF with SEE values of 1.8 to 4.4.

b. IVDMD with r values of 0.80 to 0.84 and SEE of 3.3 to 4.9.

c. Cellulase incubation with r values of 0.69 to 0.82 and SEE of 3.2 to 6.5.

d. Pepsin incubation with r value of 0.69 and SEE of 4.0 to 6.5.

e. Cellulase plus pepsin with r values of 0.72 to 0.84 and SEE of 3.0 to 6.3.

13. Digestible energy of grasses could be predicted by using:

a. CP or ADF with r values of 0.70 for CP and -.74 for ADF and SEE of 3.8 to 4.2.

b. IVDMD with r value of 0.85 and SEE of 2.9.

c. Cellulase incubation with r value of 0.87 and SEE of 2.8.

d. Pepsin incubation with r value of 0.68 and SEE of 4.1.

e. Cellulase plus pepsin with r value of 0.87 and SEE of 2.8.

14. Two-stage in vitro fermentation (IVDMD OR TTDMD) was excellent to predict DDM of grasses and legumes whereas cellulase plus pepsin was efficient to predict DDM of grasses.

15. The enzyme cellulase, amylase, cellulase plus amylase were excellent for predicting dry matter intake of grasses whereas cellulase plus pepsin was not acceptable.

16. Regression equations developed from the same seven to eight samples of grasses reveal that DDM of grasses was most accurately predicted from cellulase plus pepsin (r = 0.90, SEE = 2.9); TDN

from ADF (r = -.87, SEE = 2.6); DE from cellulase, cellulase plus pepsin or IVDMD (r = 0.85 to 0.87, SEE = 2.8 to 2.9).

17. Laboratory estimates could predict in vivo parameters much more accurately on a within-species basis than for all forages combined. The best predictors of in vivo parameters for various types of forages were not the same and the prediction equations using the same predictors were different for each forage species.

18. Multiple regression equations using combinations of the chemical components such as CP, CW, ADF, L, crude fiber (CF), ether extract (EE), nitrogen-free extract (NFE) and ash did not significantly improve the precision of predicting DDM or DMI.

19. Multiple regression equations using combinations of the 36-hr DMD or the two-stage in vitro DMD values with various chemical components significantly improved the precision of predicting DDM (SEE = 1.0 to 2.9) and in some cases improved the prediction for DMI.

20. Combinations of the two-stage IVDMD + CP + ash or 36-hr DMD + ADF accurately predicted TDN of legumes (SEE = 2.9) and grasses (SEE = 1.7) whereas combinations of EE + NFE or CP + CF + EE predicted TDN of silages with standard errors of 1.0 and 1.8, respectively.

21. Combinations of chemical components alone such as CP + EE + ADF; ADF + L + L/ADF + cell contents; CP + CF + EE could satisfactorily predict DE of legumes, grasses and silages with standard errors of 2.6, 1.8 and 1.6, respectively.
22. Body weight gain of sheep fed grasses might be predicted from dry matter solubility (DMS) + 6-hr DMD/Standard or two-stage IVDMD + CP + ADF with standard errors of 0.07 to 0.08 lb/d.
23. Some simple and useful prediction equations for different forages were as follows:

Alfalfa

DDM = 1.369 IVDMD - 23.923	(r = 0.88**, SEE = 4.4)
DDM = 1.525 IVOMD - 30.411	(r = 0.96**, SEE = 2.8)
DDM = 1.506 Pepsin + 10.264	(r = 0.90*, SEE = 3.0)
TDN = 269.077 - 5.633 ADF	(r = -.98*, SEE = 1.8)

Bromegrass

DDM = 0.960 IVDMD + 4.579	(r = 0.93**, SEE = 2.8)
DDM = 0.584 (Cell+Pep) + 39.106	(r = 0.87*, SEE = 2.4)
DMI = 0.910 (Cell+Amy) + 21.365	(r = 0.96**, SEE = 3.4)

Reed Canary Grass

DDM = 0.960 (Cell+Pep) + 23.589	(r = 0.93**, SEE = 2.3)
DDM = 87.499 - 8.029 L	(r = -.89*, SEE = 2.9)

Tall Fescue

DDM = 83.038 - 5.115 L	(r = -.93**, SEE = 3.3)
DMI = 30.650 + 0.800 Cell	(r = 0.93**, SEE = 3.2)

Temperate Grasses Combined

DDM = 0.934 IVDMD + 5.716 (r = 0.92**, SEE = 2.6)

DDM = 1.077 IVOMD + 1.621 (r = 0.98**, SEE = 1.8)

DDM = 0.743 (Cell+Pep) + 32.449 (r = 0.88**, SEE = 2.4)

DMI = 0.836 (Cell+Amy) + 26.006 (r = 0.92**, SEE = 3.8)

TDN = 106.305 - 1.506 ADF (r = -.87**, SEE = 2.6)

DE = 0.773 Cell + 33.135 (r = 0.87**, SEE = 2.8)

Crude Protein from Age (X = days of regrowth)

Temp. grass: CP = 19.252 - 0.096 X (r = -.83**, SEE = 2.3)

Trop. grass: CP = 12.024 - 0.073 X (r = -.77**, SEE = 1.4)

In Vitro Dry Matter Disappearance (IVDMD) from Age

Temp. grass: IVDMD = 79.745-0.349 X (r = -.87**, SEE = 7.0)

Trop. grass: IVDMD = 51.133-0.028 X (r = -.07, SEE = 9.0)

In Vitro True Dry Matter Disappearance from IVDMD

Temp. grass:

IVTDMD = 19.760 + 0.700 IVDMD (r = 0.97**, SEE = 2.4)

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APPENDIX

APPENDIX TABLE 1

Terminology of Forage Evaluation

Following are technical terms and abbreviations commonly used in feed and forage evaluation and in animal nutrition.

ADF	Acid-detergent fiber; obtained after boiling for 1 hour and filtering using the acid-detergent solution devised by Van Soest.
ADG	Average daily gain.
ADF-N(ADN)	Acid detergent nitrogen; insoluble nitrogen remaining in the ADF fraction expressed as % of total N or of DM.
ADL	Acid detergent lignin; obtained by dissolving ADF in 72% H_2SO_4 and ashing the residue to determine ADL as weight loss.
AE	Available energy; amount of energy an animal can extract per unit of dry matter consumed.
AI	Availability Index = $100 - \frac{100L}{ADF(100-CWC)}$
Apparent Digestibility	$\frac{\text{Nutrient Intake - Nutrient in Feces}}{\text{Nutrient Intake}} \times 100$
Ash	Mineral residue left after igniting sample at 600 C.
Buffer Extract	Solubility or disappearance of dry matter in a buffer solution.
C	Cellulose, a polymer of β-D- glucose units; β-glucosan.

CC	Cell contents = 100- cell walls.
CD	Cellulose digestibility; amount of C digested, usually expressed as a percentage of total C.
CED	Cupriethylenediamine; chemical used to solubilize dry matter, and supposedly selectively solubilize cellulose.
Cellulase Residue	Residue left after cellulase digestion.
CF	Crude fiber; residue left after boiling sample with dil. acid and dil. alkali by AOAC standard procedure.
CP	Crude protein; calculated from N x 6.25.
CWC or CW	Cell wall constituent; residue left after boiling sample for 1 hour in Van Soest's neutral detergent solution.
CWD	Cell wall digestibility; amount of CWC digested and expressed as percent of total CWC.
Cutin	Acid-detergent cutin; aliphatic cutin composed of polymerized hydroxyl fatty acids, monomeric hydrocarbons, alcohol and aldehydes.
DCW	Digestible cell wall; amount of digested CWC expressed as % of total D.M.
DDMI	Digestible dry matter intake.
DE	Digestible energy; energy in feed minus energy in feces expressed as % of energy intake.
DEI	Digestible energy intake.
Digestibility	The chemical, physical and enzymatic breakdown of feed followed by absorption.
Digestion Coefficient	Amount of digested feed divided by total feed consumed and expressed as a percentage.
Digestible Nutrient	Amount of digested nutrient divided by total nutrient ingested.
DM	Dry matter; moisture free feed.

DMD	Dry matter digestibility $= \frac{\text{DM Intake} - \text{Fecal DM}}{\text{DM Intake}} \times 100$
DDM	Digestible dry matter; same as DMD.
DMD	Dry matter disappearance; term normally used with <u>in vitro</u> system, as dry matter solubilized for a given <u>in vitro</u> system.
DMI	Dry matter intake normally calculated as amount consumed per animal, per unit weight or per unit metabolic weight.
DMNVI	Dry matter nutritive value index = Relative Intake x DMD.
DMS	Dry matter solubility in 1.0 N H_2SO_4.
DOM	Digestible organic matter; digested OM as percentage of total DM.
DP	Digestible crude protein.
EAD	Estimated apparent digestibility = 0.98 CC + CWC (1.81 - .97 log L/ADF x 100) - 3 (Si) - 12.9.
ED	Energy digestibility; same as DE.
EDMD, EDDM	Estimated dry matter digestibility = 0.98CC + CWC (1.81 - .97 log L/ADF x 100).
EE	Ether extract; crude fat obtained from proximate analysis.
ETD	Estimated true digestible dry matter = 0.98CC + CWC (1.81 - .97 log L/ADF 100) - 3.0 (Si).
Forage Quality	Includes voluntary intake, digestibility and output per animal.
GE	Gross energy; obtained from burning sample in bomb calorimeter.
HC	Hemicelluloses; amorphous polysaccharides composed of glucans, polymers of xylose, arabinose, mannose, galactose plus mixed sugar and uronic acid polymers.
Holocellulose	A combination of cellulose plus hemicellulose.

Insoluble Ash	Ash residue containing silica.
In Vitro	In an artificial container, in glass etc.; outside of life.
In Vivo	In, on or with the animal; within life.
Inoculum DMD	Inoculum dry matter disappearance used by Barnes (1969) to check the reliability of the in vitro fermentation.
IVCD	In vitro cellulose digestibility.
IVDMD	In vitro dry matter disappearance; usually that determined by the Tilley and Terry method.
IVTDMD	In vitro true dry matter digestibility; determined by boiling the 48-hr fermentation product with neutral detergent.
IVOMD	In vitro organic matter disappearance.
L	Lignin, an aromatic substance composed of phenylpropane polymers.
Lignocellulose	A complex of lignin, cellulose and hemicellulose.
Maillard Reaction	Non-enzymic browning reaction, a complex condensation of carbonyls and amino acid.
MADF	Modified acid detergent fiber; obtained after boiling sample for 2 hrs. with 1% CTAB in 1 N H_2SO_4 without antifoamant.
MCF	Modified crude fiber; developed by California workers. Includes ash.
ME	Metabolizable energy; DE minus gas and urinary losses.
Metabolic Size	Standardized weight = $BW_{Kg}^{0.75}$
Methoxyl	Chemical radical attached to L molecule; used as a predictor of L content and L complexity.
NC	Nutrient concentration in a feed.
NDF	Neutral-detergent fiber; same as CWC.
NE	Net energy = ME - Heat increment; real energy used for maintenance and production.

NFE	Nitrogen-free Extract; presumably soluble sugars, starch, dextrin.
Non-nutritive Residue	Chemical components of feedstuffs that can not be completely digested.
NPN	Non-protein nitrogen such as urea, amino acids, amines, etc.
Nutritive Value	Quality of feed including chemical composition, voluntary intake and digestibility of feed.
NVI	Nutritive value index = Relative intake x Digestible energy.
NVI (*In Vitro*)	Nutritive value index (*In Vitro*) = 6-hr DMD x 36-hr DMD/100 (Ingalls, 1964).
Nylon Bag Technique	*In vivo* fermentation by suspending sample bags in the rumen of a fistulated animal.
OM	Organic matter; 100 - Total ash.
OMD	Organic matter digestibility; amount of digested OM expressed as % of total OM in feed.
Palatability	The degree to which a food is attractive to animals under defined conditions of choice.
Permanganate Lignin	Lignin obtained from weight loss after oxidizing it from ADF by potassium permanganate.
Prediction Equation	Equation based on laboratory or *in vitro* estimates to predict *in vivo* performances.
RI	Relative Intake = $100 \times \frac{\text{gm daily forage DMI}}{80\ (W_{Kg}^{0.75})}$
Si	Sandy residue composed of SiO_2.
Solubility Test	Mixing or incubating feeds with water, buffer, solvents or enzymes to determine DMD.
Soluble Carbohydrates	Those carbohydrates which are soluble in water or alcohol.
Soluble Ash	Total ash - insoluble ash.

Summative Equation	Scheme for the estimation of *in vivo* digestibility by combining the digestibility of CC, CWC and metabolic fecal losses.
TAC	Total available carbohydrates; plant component hydrolyzable by amylolytic enzyme to simple sugars.
Tannin	Tannic acid ($C_{76}H_{52}O_{46}$); an amorphous polyphenol, strongly astringent substance found in plants.
TDN	Total digestible nutrients = DP + Dig. CF + Dig. NFE + Dig. fat x 2.25
TEE	Total enzyme extract; soluble DM due to enzyme alone.
TNC	Total nonstructural carbohydrates; readily available CHO; sugars, starch, fructosans; similar to TAC.
TRAC	Total residue after cellulase; obtained from incubating sample with cellulase.
TRAE	Total residue after enzyme(s).
TSAE	Total soluble after enzyme(s); same as total enzyme extract.
True Digestibility	Overall digestibility of feeds by taking into account both bacterial and endogenous losses.
TTDMD	Tilley-Terry; *in vitro* rumen fermentation with rumen fluids followed by pepsin digestion.
Turbidity Test	Checking forage quality by measuring opaqueness of a suspension of the sample in water.
VFA	Volatile fatty acids; acids produced in the rumen (acetic, propionic, butyric, valeric acids).
VI	Voluntary intake; unit of intake per unit of body weight normally expressed as gm of feed/$BW_{Kg}^{0.75}$
WS-CHO	Water-soluble carbohydrates, mainly mono-and disaccharides.